



HEALTH RISK RELATED TO WATER SUPPLY AND CONSUMPTION IN A MARGINALISED URBAN AREA

TSHOLOFELO CONFIDENCE BOKAKO

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Department of Environmental Sciences
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Supervisor: Mr P Jagals, M Dip Technology: Public Health
Co-supervisor: Ms B Genthe, MSc: Microbiology

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DECLARATION OF INDEPENDENT WORK



Central University of
Technology, Free State

I TSHOLOFELO CONFIDENCE BOKAKO, do hereby declare that this research project submitted for the degree MAGISTER TECHNOLOGIAE: ENVIRONMENTAL HEALTH, is my own independent work that has not been submitted before to any institution by me or anyone else as part of any qualification.

Bokako
SIGNATURE OF STUDENT

31/01/2000
DATE



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The main aim of this study was to investigate the microbiological and aesthetical water quality used in large low socio-economic urban settlement. Previous studies in the same area indicated that microbiological quality of municipal supply water was good but, once fetched from municipal supplies, the microbiological water quality of container-stored water deteriorated to become unsafe for human consumption. The previous studies revealed biofilm forming inside container walls, possibly adversely affecting the microbiological quality of the stored water. To confirm this assumption (deterioration associated somehow with formation of biofilm in containers) a simple biofilm assessment method was developed.

Epidemiological surveys were conducted simultaneously in the same study area by co-workers studying effects of water quality on human health using diarrhoea as health indicator. From the analyses of data collected, a case-control follow-up study was done of selected households (affected versus not affected by diarrhoea). The quality of water used by the cases and controls was assessed to determine if there were any statistically significant differences in water used by the 2 groups. The effect of distance on the microbiological water quality in containers was investigated as well as the effect of container material of the vessels used by the 2 groups.

Heterotrophic bacteria levels in both the supply water and container water were above the *negligible risk* limits proposed by the *South African Water Quality Guidelines* (DWA, 1996) with heterotrophic bacteria numbers in container water higher. Heterotrophic bacteria numbers in container water containing suspended biofilm were significantly higher than in water before suspension. Heterotrophic bacteria levels in water used by both groups were above *negligible* limits, with the case-group significantly higher than the controls. Turbidity (NTU) in the supply water and container water were below the maximum limits for *significant risk* proposed by DWA (1996). NTU in the container water were slightly higher than in municipal supplied water. NTU levels in the water used by cases were slightly higher than that used by control group. Higher total coliforms (TC) levels were observed in stored water than in supply water. TC levels in the container water were above limits for *negligible risk*. *Escherichia coli* (*E. coli*) were intermittently found in supply and container water, indicating occasional risk of exposure for consumers. *E. coli* numbers in container water were higher than in supply water. *E. coli* before and after suspending biofilm had similar geometric mean values for



cases and controls. *Clostrid* (CP) were present in municipal water supplies and container-stored water with both waters having similar mean values. However, the levels did not exceed risk limits. No somatic coliphages could be detected in the containers water and supply water at any stage.

Water used by the case and control groups were found to be of equally poor microbiological quality. It was found that water that had to be carried over distances of between 10m and 100m contained more total coliforms than water that was carried over distances of less than 10m and more than a 100m. This was probably due to large open-mouthed containers used, which was conducive to environmental contamination. No statistical significant difference was observed in the microbiological water quality in plastic or metal containers.

The microbiological quality of supplied municipal water did not comply with the microbiological limits in terms of DWAF (1996) for heterotrophic bacteria and total coliforms. However, *E. coli* and CP levels did comply with the limits of DWAF (1993) and *Water Quality Criteria* (Aucamp and Vivier, 1990).



Die doelwit van hierdie studie was om die mikrobiologiese en estetiese watergehalte, wat gebruik word in lae sosio- ekonomiese stedelike nedersettings, te bepaal. Vorige studies wat uitgevoer was in dieselfde gebied, het aangedui dat die mikrobiologiese gehalte van die munisipale watervoorsiening goed was, maar dat die gestoorde houerwater, wat vanaf die munisipale voorsiening gehaal word, se mikrobiologiese kwaliteit verswak tot 'n vlak dat die gestoorde water nie meer veilig was vir menslike gebruik nie. Die vorige studies het getoon dat 'n tipe biofilm gevorm word op die binnewande van die waterhouers, wat moontlik die mikrobiologiese gehalte van die water in hierdie houers nadelig kon beïnvloed. Om die aanname te bevestig (vorming van biofilm tot verswakking van die watergehalte in die houers lei), was 'n eenvoudige biofilm ondersoek tegniek ontwikkel.

Epidemiologiese opnames was uitgevoer in dieselfde gebied deur medewerkers om die effek van die watergehalte op menslike gesondheid met die gebruik van diarree as gesondheids indikator, te bestudeer. Vanuit die analises van die data wat versamel was, was 'n opvolg gevalle- kontrole studie gedoen op geselekteerde huishoudings (beïnvloed deur teenoor geen invloed deur diarree). Die gehalte van water wat deur die gevalle – kontrole gebruik word was bepaal om vas te stel of daar enige statistiese betekenisvolle verskille was in die water wat deur die 2 groepe gebruik word. Die effek van afstande op die mikrobiologiese watergehalte in die houers, asook die effek van houermateriaal wat gebruik word deur die 2 groepe, was ondersoek.

Heterotrofiese bakteriële vlakke in beide die voorsiene en houerwater was bokant die *onbeduidende risiko* perke soos voorgestel deur die *South African Water Quality Guidelines* (DWAF, 1996) met die heterotrofiese bakteriële vlakke in die houerwater hoër as in die voorsiene water. Heterotrofiese bakterie hoeveelhede in die houerwater met gesuspendeerde biofilm was betekenisvol hoër as die water voor die suspensie. Heterotrofiese bakterie vlakke in water, wat gebruik word deur beide groepe, was bokant die *onbeduidende risiko* perke met die gevalle groep betekenisvol hoër as die kontrole groep. Turbiditeitsvlakke (NTE) in die voorsiene water en die houerwater was laer as die maksimum perk vir *betekenisvolle risiko* soos voorgestel deur die DWAF (1996). NTE vlakke in die houerwater was gering hoër as in die van die munisipale voorsiene water. NTE vlakke in die water, wat



gebruik word deur die gevalle- en kontrole groepe. Hoër vlakke van totale kolivorme (TK) was waargeneem in die gestoorde water as in die voorsiende water. TK vlakke in die houerwater was ook bokant die perke vir *onbeduidende risiko*. *Escherichia coli* (*E. coli*) wat wel met tye gevind was in die voorsiende water en houerwater het risiko blootstelling van verbruikers aandui. *E. coli* hoeveelhede in die houerwater was hoër as die voorsiende water. *E. coli* voor en na die suspendeerde biofilm het soortgelyke geometriese gemiddelde waardes gehad vir gevalle en kontrole groepe. *Clostridium perfringens* (CP) was opgespoor by munisipale watervoorsieningspunte en die houerwater met beide water wat soortgelyke geometriese gemiddelde waardes gehad het. CP vlakke het nie die risiko perke oorskry nie. Geen somatiese kolifage kon by enige stadium opgespoor word in die houerwater of voorsiende water nie.

Water wat gebruik is deur deur gevalle-kontrole groepe het getoon dat die water mikrobiologies van ewe slegte gehalte was. Meer totale kolivorme was gevind in water wat oor afstande van tussen 10m en 100m gedra word as in water wat oor afstande van minder as 10m en meer as 100m gedra word. Dit kan moontlik wees as gevolg van die oop mond houers wat gebruik word, wat blootgestel word aan omgewings kontaminasie. Geen statistiese beduidende verskille was opgemerk in die mikrobiologiese gehalte van die water in die plastiek of metaal houers nie.

Die mikrobiologiese kwaliteit van die water met verwysing na heterotrofiese bakterie en totale kolivorme vlakke het nie voldoen aan die voorgestelde DWAF (1996) nie. *E. coli* en CP vlakke het voldoen aan die voorgestelde DWAF (1993) en die *Water Quality Criteria in South Africa* (Aucamp en Vivier, 1990).

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CHAPTER 1

INTRODUCTION

Chapter 1: INTRODUCTION

1.1 HEALTH EFFECTS OF DRINKING WATER QUALITY: Historical Background

The expression “Water is life” is found in many cultures around the world. It underscores the fact that clean water is pre-requisite for healthy living (World Health Organisation (WHO), 1997b). Nevondo and Cloete (1999) stated that water intended for human consumption should be safe, palatable and aesthetically pleasing.

According to the South African Government’s Reconstruction and Development Programme (African National Congress (ANC), 1994) water is a natural resource and should be made available in a sustainable manner to all South Africans. Nevertheless, less than half the South African rural population has a safe and accessible water supply, and only one person in seven has access to adequate sanitation. Access to water resources is dominated by a privileged minority while the majority of the population enjoy little or no water security. The need to provide adequate water supplies to communities is a well-recognised central component of the Constitution of the Republic of South Africa (Government Gazette, 1996). South Africa is a water-scarce country. The existing limited water resources are also unevenly distributed with 70% of the country receiving 11 % of the rainfall (ANC, 1994).

It is well understood and documented that the health of a community is significantly influenced by drinking water quality. Dramatic statistics to emphasise this fact are available (Mackintosh and Linde, 1997; Schalecamp, 1990):

- ◆ In South Africa, 20% of all deaths in infants from 1-5 years can be attributed to diarrhoea.
- ◆ 80% of all enteric diseases worldwide are attributed to lack of drinking water quality.
- ◆ 50 000 people worldwide die daily because of water-borne and water-related diseases.



In developing countries, communicable diseases related to defects in water supply and sanitation are the single most important group of diseases. They are responsible for some 25 million deaths world-wide each year and around 80% of all morbidity (Mara and Alabaster, 1994).

Failure to provide adequate protection and effective treatment of potable water supplies will expose a community to the risk of outbreaks of intestinal and other infectious diseases (WHO, 1997b). Poor water quality together with inadequate sanitation, are rated by the World Health Organisation (1997a), as the leading causes of death in poorer communities.

1.2 WATER-RELATED DISEASES

Infectious diseases are frequently transmitted through contaminated drinking water (American Water Works Association (AWWA), 1990). A water-related disease is one which is in some gross way related to water in the environment or impurities within water (Genthe and Seager, 1996). In the discussion to follow, the health impacts of water supply as well as water-related diseases, in particular diarrhoea, are reviewed.

Water-related diseases may be divided into those that are caused by a biological agent of disease (a pathogen), or those that are caused by some chemical substance in water. The water-related infections are so described because their transmission depends in part upon water (Craun, 1986). There are four categories of water-related diseases. These are (AWWA, 1990; Genthe and Seager, 1996; Craun, 1986; Tchobanoglous and Schroeder, 1987; Mason, 1991; Wibowo and Tisdell, 1993):

1.2.1 WATER-BORNE DISEASE: Are the most important concern about the quality of water. These are diseases transmitted through the ingestion of contaminated water, usually faecal contamination. The water acts as the passive carrier of the infectious or chemical agent. Classic water-borne diseases are cholera and typhoid fever, but also include a wide range of other diseases such as infectious hepatitis and some diarrhoea and dysentery. Diarrhoeal illness is an important cause of infant mortality and morbidity in developing countries.

1.2.2 WATER-WASHED DISEASE: These diseases are related to poor hygiene habits and sanitation. Unavailability of water for washing and bathing



contributes to diseases of the eyes and skin. These may be significantly reduced following improvements in domestic and personal hygiene. These improvements in hygiene often depend upon increased availability of water and the use for hygiene purposes of increased volume of water and achieving a high bacteriological quality.

1.2.3 WATER-BASED DISEASES: These are diseases caused by helminths (parasitic flukes and flatworms) in which the pathogen spends an essential part of its life in water. Water quality and cultural social behaviour play a role in the transmission of these diseases.

1.2.4 WATER-VECTOR DISEASES: These diseases are transmitted by insects, which either breed in water (malaria-carrying mosquito's), or insects which bite near water (riverine tsetse fly)

Grabow (1996) reported that little information is available on water-borne diseases in South Africa. This is probably due to the absence of an infrastructure for the detection and recording of such infections. The lack of information tends to create a false sense of security. In terms of escalating demands and pollution of the limited water sources, particularly in rural and developing communities, the risk may even be relatively high.

Although there is a wide range of water-related diseases, the emphasis on diarrhoea in this review stems from the fact that diarrhoea was taken as an health indicator. Traditionally, we have been given to understand that, because many of the disease agents are water-borne, we fall ill because of the water we drink. Based on this premise, we assume that if we can improve water quality for drinking purposes, we can then minimise the occurrence of diarrhoea.

1.3 DIARRHOEA AS AN INDICATOR OF HEALTH

All humans, whatever their stage of development and their social and economic conditions, must have access to drinking water in quantities which satisfy their basic needs (United Nations, 1977). Improvement of water supplies and sanitation has been the subject of intervention studies worldwide (Huttly et al., 1997). Gorter et al., (1991) agree to the above statement by reporting that in 1982, the Diarrhoeal Diseases Control Programme of the WHO commenced a systematic study of the strategies that might play a role in the control of diarrhoea. One of the seven

strategies considered to be improvement of water supplies and sanitation. However, the relationship between improvements in water supply or sanitation and diarrhoeal disease is still not completely understood (Gorter et al., 1991; Esrey et al., 1991).

It is often said that good sanitation improves the quality of life by reducing communicable diseases like childhood diarrhoea. However, good sanitation facilities alone are unlikely to decrease transmission unless accompanied by good hygiene practices (Victora et al., 1988). Moe et al. (1991) also reported that improvements in water quality alone caused a median reduction of 16% in diarrhoea morbidity (range, 0-90%) and that improvements in both water quality and availability resulted in a median reduction of 37% (range, 0-82%). In addition to safe water and sanitation, a number of other factors may affect diarrhoea mortality. Water quality is a significant determinant of diarrhoea as well as socio-economic conditions such as income, occupation and literacy rate. Levels of formal education can also influence the incidence of diarrhoea, although a specific designed education programme for personal hygiene and diarrhoea prevention seems to be more effective in this respect (Wibowo and Tisdell, 1993). A survey carried out by the Lesotho Ministry of Health shows that in a suburb of Maseru, the capital city, where a clean water supply and good sanitation facilities have been constructed, there was still a high incidence of childhood diarrhoea. This is attributed to poor hygiene practices of residents, particularly of those responsible for the care of children (Aquatech, 1988). A recent analysis of data from demographic and health surveys conducted in eight countries demonstrated that improvements in sanitation had a greater impact on diarrhoea prevalence than improvements in water supply (Huttly et al., 1997).

It is estimated that there are more than 800 million cases of diarrhoea every year in developing countries, causing up to 4.5 million deaths (Pegram et al., 1998). Diarrhoea was taken as a health indicator since diarrhoea-causing agents may be transmitted by poor water quality, either directly in water or through contact with contaminated environmental water resources. Esrey et al. (1990) (cited in Pegram et al., 1998); used the World Health Organisation definition of diarrhoea which is three or more watery stools passed in 24 hours. Diarrhoea among children continues to be a major public health problem in most developing countries and it may be on the increase (Bukonya and Nwokolo, 1991; Molbak et al., 1989).



Factors contributing to the re among young children include poor sanitation, contaminated drinking water and poor hygiene (Bukonya and Nwokolo, 1991; Henry et al., 1990). Several routes for diarrhoeal disease were important in the study environment. Few households had tap in yard and that water was usually stored in large containers. Excreta disposal facilities-generally pit latrines-were usually shared by large numbers of people. Moe et al. (1991) reported that many households in their area of concern did not have access to any such facilities and use open fields. The selected area for this study also had similar situation. According to Victora et al. (1988) results estimated that improved water supply, excreta disposal and hygiene education might reduce diarrhoea mortality rates by 35-50% and larger reduction might be expected in diarrhoea mortality in some circumstances.

Von Schirnding et al. (1993) reported that diarrhoea mortality rates have declined over the last one-and half decades in South Africa but there are still major discrepancies by race and age. In general, the rates are lowest in the settled urban parts of South Africa, followed by peri-urban areas, with the rural areas still experiencing the highest mortality rates. Using the available mortality data in South Africa as well as internationally comparable information on the risks of contracting disease among children, it is possible to estimate that there are around 1.5 million cases of diarrhoea in children under the age of 5 years, per annum in South Africa (Von Schirnding et al., 1993).

South Africa is no exception, with an estimated 12 million without access to adequate supply of potable water and about 21 million lack basic sanitation (Genthe et al., 1995; Genthe and Seager, 1996; Pegram et al., 1998). The provision of these basic services was planned as a part of coherent social development strategy (Department of Water Affairs and Forestry (DWAF), 1994, 1996). Preventive strategies for morbidity are needed, however, if the estimated annual thousand million episodes of diarrhoea are to be reduced and further decreases in the 3.3 million diarrhoea-related deaths are to be achieved (Huttly et al., 1997).

Studies in developing countries have shown 14-48% decrease in diarrhoeal disease incidence as a result of hand washing or hygiene interventions. Improved hygiene and provision of adequate quantities of water have been showed to reduce diarrhoeal disease morbidity in developing countries (Peterson et al., 1998; Pinfold 1990).



Most of the pathogens that cause diarrhoea and all the pathogens that are known to be major causes of diarrhoea in many countries, are transmitted primarily or exclusively by the faecal-oral route. Faecal-oral transmission may be water-borne, food-borne, or via direct contact (Feachem, 1984). In areas with inadequate water supplies, diarrhoea can be an indication of the breakdown of any number of social habits or some unknown etiologic agent resulting from areas such as food and water supplies, as well as personal hygiene patterns (Jagals et al., 1997; Von Schirnding et al., 1993). It is believed that if transmission routes are such that if good quality water is provided in adequate quantities, personal hygiene standards can be expected to improve, thus minimising the occurrence of diarrhoea (Aquatech, 1988).

Feachem (1984) stated that relevant literature generally contains many observations that confirm that diarrhoea rates are highest in families with the lowest levels of educational attainment. Hygiene and literacy may be closely related. Such observations in themselves are not useful because families with the lowest education attainment will tend to be those with the lowest income, poorest housing, crowding and worst sanitary facilities. These confounding variables will also promote the transmission of enteric pathogens (Feachem, 1984).

1.4 DOMESTIC WATER QUALITY

As possible factors in the transmission of diarrhoea, two attributes of water, namely quantity and quality are important role players. The former impacts directly on factors of personal and environmental hygiene, because of issues such as inadequate maintenance of latrine facilities, as well as personal hygiene (Herbert, 1983). As mentioned before water is a basic necessity of life. It is nevertheless essential to recognise that both the quantity and the health-related quality of water supplies are of equal importance (Water Research Commission (WRC), 1998).

Improved water supply and sanitation may improve the quality of life, facilitate other development activity, save the time spent in carrying water over distances, but the foremost benefit anticipated is improved health (Genthe and Seager, 1996).

In South Africa, consumers in cities generally receive a constant supply of conventionally treated water. However, many communities in under-developed urban area as well as in rural areas receive partially treated water, or use untreated water directly from rivers, streams or boreholes and therefore are exposed to a variety of water-related diseases. A primary goal of the South African Government



therefore is to ensure that all have access to essential basic water supply as well as sanitation services, which are affordable, both to the household and to the country as a whole (WRC, 1998).

The concept “basic water” supply includes National Sanitation Task Team, 1996:

- ◆ **Quantity:** 25 litres per person per day is considered the minimum requirement for direct consumption for the preparation of food and for personal hygiene.
- ◆ **Transport and distance:** the maximum distance a person should have to transport water to their dwellings is 200 m.
- ◆ **Availability:** the flow rate of water from the outlet should not be less than 10 litres a minutes and the water should be available on a regular daily basis.
- ◆ **Assurance of supply:** the supply should provide water security for the community. This means water will always be available at public standpipes.
- ◆ **Quality:** health-related water quality is as important as quantity in achieving the goal of an adequate water supply. The quality of water provided should be in accordance with currently accepted minimum limits (in the South African Water Quality Guidelines) with respect to health-related chemical and microbial contaminants. It should also be acceptable to consumers in terms of its potability (taste, odour and appearance).

1.5 DOMESTIC WATER QUALITY PROBLEMS

1.5.1 GENERAL

Adequate supply of safe drinking water (water free from chemical substances and microorganisms in concentrations that could cause illness in any form) is universally recognised as a basic human need. Yet more than 1 000 million people world-wide do not have ready access to an adequate and safe water supply and a variety of physical, chemical and biological agents render many water sources less than wholesome and healthy (WHO, 1997a).

In conditions of poverty and water shortage, all the potentially water-borne diseases can also pass from person to person in a number of different ways. Contaminated water is one route, but contaminated fingers, plates and food can be just as common and even more dangerous (Aquatech, 1988). For many of the world’s poor, the first health requirement is not for cleaner water but for more water

(Aquatech, 1988).

In developing countries, public health control over water supplied to standpipes (pumps or taps) is often not sufficient to protect communities against water-borne infectious diseases (UNEP, 1991). If contaminated water penetrates distribution mains, water that has already been treated and disinfected becomes re-contaminated (WHO, 1997a). Factors such as treated piped water being contaminated due to low pipe pressure and breakdown in the network system may contribute to diarrhoeal infections (Herbert, 1983). Growth of bacteria in drinking water distribution and storage systems can lead to the deterioration of water quality, violation of water quality standards and increasing operating costs (Schaule and Flemming, 1997 and Schaule et al., 1996).

To supply a community with treated piped water in a trouble free system does not necessarily mean that water-related health risks would be totally eliminated (Jagals et al., 1997; 1999). Pinfold (1990) and Jagals et al., (1997) reported that water fetched from a supply point and transported home becomes contaminated during haulage, storage and handling at home by consumers, therefore contributing to risk of microbiological infection. These additional aspects of water haulage, storage and handling must therefore, be taken into consideration in any water supply programme to ensure that the consumer is provided with safe drinking water at the point of consumption (Forsyth, 1993; Jagals et al., 1997; 1999).

1.5.2 SOUTHERN AFRICA

In South Africa, the availability of safe and clean water is a serious problem, especially in rural areas (Nevondo and Cloete, 1999). In this instance “safe” means that there are no negative health effect either on the short term or on the long term.

Simply telling people to use more water is unlikely to change their habits. The most effective way to increase a poor community’s water consumption is to provide the water closer to their homes. Jagals et al. (1997) reported that certain urban communities with supplied water had to travel up to 300m to collect water. The collected water may have been contaminated by en-route environmental input such as dust.

Although provision of improved supplies is undertaken on a vast scale by water utilities, is unlikely that high-quality water will be provided to the majority of such people in the immediate future. Another limiting factor is that in other areas where



such water supplies have been found to be insufficient and residents may often have to revert to traditional unprotected sources until the supply is restored (Nevondo and Cloete, 1999). These water sources should therefore be examined for indicators of pollution and when the inspection shows that they are subjected to contamination, remedial action should then be taken. This will result in the decline in infectious and other communicable diseases and ultimately improve the health standards of rural communities (Nevondo and Cloete, 1999).

1.5.3 SUPPLY WATER PROBLEMS

Source protection is almost invariably the best method of ensuring safe drinking water (AWWA, 1990), and is to be preferred to treating a contaminated water supply to render it suitable for consumption. As far as possible, raw water sources must be protected from contamination by human and animal waste, which can contain a variety of bacterial, viral and protozoan pathogens. However, with growing urban areas, pollution impacts on surface source waters are increasing (Jagals et al., 1997), making treatment inevitable. Failure to provide adequate protection and effective treatment will expose the community to the risk of outbreaks of internal and other infectious diseases (WHO, 1993).

Protection of treated water supplies from contamination had become the first line of defence. The WHO (1993) reported that every effort should be made to supply drinking water quality as far as practicable to all people in protected systems.

However, water supplied in systems is not always that safe. Herbert (1983) stated that it is common knowledge that water supply systems in the developing countries are often prone to contamination. Provision of piped water will not necessarily ensure that the water will be protected from faecal or other sort of contamination. This is due mainly to factors such as low line pressure, pipe breaks in badly fouled environments or a combination of all especially when work had been done on broken pipelines (Herbert, 1983).

South African studies have shown that even when safe water is supplied, high degrees of contamination occur because of the distances water has to be fetched from the supplied source to be used at home (Daniels et al., 1990; Genthe and Seager, 1996; Jagals et al., 1997; 1999).

These studies had shown that drinking water became contaminated during the



many stages of collection and furthermore, the inefficient disposal of excreta contributes to a fouled domestic environment from which various forms of environmental dispersion mechanisms (such as wind and dust) can lead to stored water contamination, exposing the community to pathogens.

Even when safe water is supplied to communities, the only way to prevent this type of contamination is to shorten the tap-to-glass sequence by supplying people with water inside their houses. In South Africa, this is unlikely to happen for various reasons - one of which would be the lack of water-borne sanitation in many areas. Storage containers could still be used in communities for a long time to come.

1.5.4 STORAGE PROBLEMS

The provision of a good drinking water supply alone is insufficient to ensure health WHO (1997c). It is now evident that the way in which water is stored at home presents a problem. Jagals et al. (1997; 1999) reported that the microbiological water quality deteriorated once water was fetched and stored in containers at home. They conducted studies in the same area as this study - a large low socio-economic urban area in Botshabelo, South Africa. Their studies concentrated on the relationship between water quality, water availability and water accessibility. It was found that the particular communities were exposed to water-related risk of infection because water-use patterns that developed around fetching and storing water from a safe water supply led to microbiological contamination of stored supply.

The results indicated that, although the public supplied water at the standpipe was of a good quality, the stored water at the households, once fetched from the standpipes and stored in containers deteriorated to a microbiological water quality not safe for human consumption. The exact reason for the deterioration was not clear. The quality of the water deteriorated in the container regardless whether the storage containers were protected (closed) or unprotected (open-topped) or the various distances people had to travel to fetch water.

These findings had important consequences for water consumption in the study area because to improve hygiene, priority was given by the local authority to improve the situation by moving water supply points that were not close to individual households right into the individual yards (Jagals et al., 1999).

They concluded that the closer proximity of the standpipes immediately had an effect on the water-fetching and -storing activities of this particular community.



Smaller open-topped vessels

Central University of
Technology, Free State

ter to carry when filled, immediately replaced the closed container types used by the community up to the new service provision.

Although the smaller containers led to more regular replenishment of the stored supply, the expected positive impact of improving the overall microbiological water quality in such containers was not evident (Jagals et al., 1999). This also related to recent findings by Genthe and Seager (1996) that seem to suggest that it is difficult to detect a health impact even where there are substantial improvements to drinking water quality.

This implied that providing a closer (geographically) supply to households does not seem to improve the microbiological quality of the water consumers eventually get to drink which again means that the expected more positive health outcomes are not achieved. Water quality seems to deteriorate even in containers that are filled regularly with fresh water. Although the exact cause for the deterioration was not established and is still not known, some mechanism seems to exist that causes this.

During one of these previous studies Jagals et al. (1997), used a dry-swab method cited in Jay (1992), to remove the biofilm on the inside of container walls. Microbiological analysis of this substance revealed substantial levels of heterotrophic bacteria, indicating that the contaminant layer forming on the inside of container walls was probably a type of biological film similar to that found in distribution lines (Kastl and Fisher, 1997).

Biofilm can develop even under oligotrophic conditions, such as those prevailing in drinking water-purified systems. Floating bacteria that might be introduced into the water volume can attach to the surface and become part of biofilm. Such bacteria can replicate if biodegradable dissolved organic carbon is available as a food source. Water in open containers is subjected to contamination from the outside environment. Domestic environments such as the study area, where substantial volumes of fossil fuels are inefficiently burnt in open drums in and around houses for heating and cooking, could provide sources of aerosolised carbon.

Such biofilm can adversely affect the quality of the water stored in these containers. They not only represent potential contamination sources for heterotrophic bacteria, but they also provide habitats protecting pathogenic organisms against disinfection (Schaule and Flemming, 1997).



Excessive heterotrophic bacteria are caused by factors such as bacteria, which migrate from a biofilm layer to a water body and vice-versa. Film activity destroys residual chlorine, allowing greater survival of bacteria especially on the surface of the film (Kastl and Fisher, 1997).

To confirm the assumption that the deterioration in water quality is linked to the biofilm inside container sidewalls, appropriate sampling was required (Schaule and Flemming, 1997). They suggested that, if biofilms are to be investigated, it is absolutely necessary to sample on surfaces in contact with water. Such a sampling technique had to be developed for this study.

1.6 LINKING DOMESTIC WATER QUALITY TO INFECTION RISK

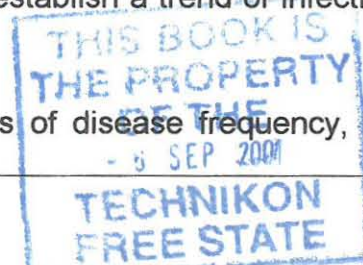
To determine the risk of infection to consumers of the supply water in the study area, the domestic water quality needed to be assessed and compared with health-related water quality guidelines such as the South African Water Quality Guidelines (1996) as well as the Water Research Commission: Assessment Guide: Quality of Domestic Water Supplies (WRC, 1998). Vol. 1, 2nd edition.

An epidemiological survey was conducted at the same time in the same study area (Section K, Botshabelo) by co-workers as part of a larger programme studying the effects of water quality on human health (Theron, 2000). As epidemiological studies are designed to identify predominant risk factors that play a role in the health status of populations (Genthe and Seager, 1996) the abovementioned survey was conducted to determine the environmental health status of the study community. Apart from water quality, accessibility and availability, this survey included other variables such as sanitation and domestic hygiene.

Studies are increasingly being conducted to determine health risks that may be associated with environmental exposure, especially drinking water (Gunther et al., 1996). Often epidemiological evidence linking drinking water contaminants to health effects in a population is lacking and very costly to obtain (Joan and Charles, 1991).

To achieve this in this study with the limited resources available, this study aimed to compare the quality of water used by households excessively affected by diarrhoea to that of unaffected households to try and establish a trend of infection and water quality.

As epidemiology requires several measures of disease frequency, a meaningful





measure of disease frequency and an accurate compilation of cases and an estimate of the susceptible population or population at risks (Gunther et al., 1996). In the survey by Theron (2000), 300 households were selected and interviewed as well as observed out of an area population of 3 000 households.

From the analyses of the data collected by Theron (2000), Nala and Jagals (1999) did a case-control follow-up study of the same selected households. A case-control design can be considered an efficient sampling technique to measure exposure disease associations in a study base (Katzenellenbogen et al., 1997).

From the households previously surveyed by Theron (2000), 75 households were identified as cases. These households experienced diarrhoea for one or more of the members during the 12 months gone by. For the purposes of this study, they were followed up for 12 more months during which time the water these households used was sampled and the microbiological as well as aesthetical quality assessed. The possibility of biofilm that may have formed on the sidewalls of containers was also investigated.

Another 75 households were identified that had NOT experienced diarrhoea for one or more of the members during the 12 months gone by (as controls) and also followed up for the 12 months as for the cases.

The risk of infection to consumers of the supply water in the study area was determined by comparing the water quality with various health-related water quality guidelines. The following guidelines were used:

- ◆ South African Water Quality Guidelines: Vol. 1: Domestic Water (Department of Water Affairs & Forestry (DWAF), 1993; 1996).
- ◆ Proposed Water Quality Criteria in South Africa of the National Department of Health (Aucamp & Vivier, 1990)
- ◆ Water Research Commission: Assessment Guide: Quality of Domestic Water Supplies (WRC, 1998). Vol. 1, 2nd edition.

1.7 ASSESSING DOMESTIC WATER QUALITY

Pathogenic organisms of health concern include bacteria, viruses and protozoan parasites. The diseases they cause may vary in severity from mild gastro-enteritis to severe and sometimes fatal diarrhoea (Genthe and Kfir, 1995). Microbiological



infection agents are regarded as an important water quality aspect of water safety for supplied water for whatever purpose of human use (Grabow, 1996; Jagals et al., 1999).

Evaluation of water quality by microbiological indicators provides a useful method of comparing different types of water sources and of assessing the risk of contamination (Genthe and Seager, 1996).

Genthe and Kfir (1995) reported that the main objectives of monitoring water microbial quality are to ensure protection of public health and at the same time to evaluate the effectiveness of water treatment processes.

1.7.1 ASSESSING MICROBIOLOGICAL WATER QUALITY

Various pathogenic bacteria, viruses and invertebrate parasites that are transmitted through faecal contamination present a potential public health hazard. If water is to be used for drinking, it must be free of pathogens (Mason, 1991).

To assess whether water is microbiologically safe to drink - that is "does not contain any pathogenic micro-organisms" - the presence of indicators of pollution should be detected (Genthe and Kfir, 1995). It is impractical to routinely test the water supply for all pathogens related to water-borne disease, because of the complexity of the testing and the time and cost related to it. It is therefore preferable to use indicator organisms that are able to indicate the presence of pathogens, and related health risks in water (Grabow, 1996).

The ideal indicator is used in the place of the real pathogen. Such an indicator should therefore fulfil a number of criteria, namely (Genthe and Kfir, 1995; Genthe and Seager, 1996):

- ◆ It should be present when the pathogen is present and should be absent in unpolluted water.
- ◆ It should be present in numbers greater than the pathogen it indicates.
- ◆ Its survival in the environment and resistance to treatment processes should be comparable to that of pathogens.
- ◆ It should not be harmful to human health.
- ◆ It should be easy to identify and isolate.

The indicator organism groups selected for this study had to indicate the presence



of pathogenic bacteria, viruses and resistant microorganisms such as protozoan spores.

1.7.1.1 Bacterial indicators

Commonly used indicators are excreted or released consistently by virtually all humans because they are members of the normal microbial flora of humans. Warm-blooded animals also consistently excrete some of these indicators (Cabelli, 1977; DWAF, 1996; Grabow, 1990, 1996).

The following bacterial indicators were selected to use in this study:

- *Heterotrophic bacteria*: primarily used as a practical indicator of microbiological water quality (Standard Methods, 1998). Heterotrophic bacteria indicate the general microbiological quality of water (Grabow, 1996).
- *Total coliforms*: primarily used as a practical indicator of organic pollution of water (Grabow, 1996; Standard Methods, 1998).
- *Escherichia coli (E. coli)*: highly specific indicator of faecal pollution that originates from human and warm-blooded animals (DWAF, 1996; Grabow, 1996).

1.7.1.2 Viruses

Enteropathogenic viruses in water are very costly and complicated to assess. Certain species of bacteriophages are used instead (Grabow, 1996). Bacteriophages are viruses that infect bacteria. The survival and incidence of bacterial viruses such as somatic coliphages in water means that organic pollution, possibly of virus-containing faecal origin, had taken place of the water. Somatic coliphages resemble human viruses more closely than most other indicators of faecal pollution (DWAF, 1996; Grabow et al., 1993; 1996; Payment, 1991).

1.7.1.3 Resistant spores

Although *Clostridium perfringens* are bacteria, they are primarily used as indicators of resistant faecal pollution as well as protozoan spores because they are also spore forming and can survive adverse conditions such as the water treatment processes (Payment and Franco, 1993). *Clostridium perfringens* are, like *E. Coli*, highly specific for faecal pollution, which is a very good reason why any members of this group should not be detected in drinking water supplies.



1.7.2 INVESTIGATING BIC

To confirm the assumption that the deterioration in water quality found by Jagals et al (1997; 1999) is linked to biofilm, appropriate sampling was required (Schaule and Flemming, 1997). They suggested that, if biofilms were to be investigated, it was absolutely necessary to sample on surfaces in contact with water. Standard Methods (1998) recommends that the levels of assimilable organic carbon (AOC) in a suspected water body could be used as a gross measure for the potential of the water to sustain bacterial regrowth. Such levels could then be an indication of the potential for biofilm to develop on surfaces that are in contact with such water.

Measuring AOC is a sensitive, complex and costly biochemical procedure beyond the scope and resources of this study. Standard Methods (1998) also mentions bioassays using a bacterial inoculum as alternatives. However, the methods for these are still in the development phases and were not considered within the scope of this study.

It was decided to assess the physical quality of the water instead of complex biochemical and biological studies.

A simple technique was developed to determine whether the perceived film contributed to the deterioration of the quality of the water as well as whether the film can influence the aesthetic acceptability (defined by the clarity) of the water for human consumption. Brushing the inside walls of filled containers simulated the effect of the organic layer / biofilm being loosened every time an unwashed or unrinsed container was filled by a household member. The brushing technique merely accelerated this process and also maximised the contamination that could take place with filling as well as handling.

Increases in *turbidity* as a gross quality measure were then used in addition to the microbiological indicators to assess the outcomes of applying this technique (DWAF, 1996; Tchobanoglous and Schroeder, 1987).

- *Turbidity*: The turbidity of water is also related to clarity, a measure of the transparency of water and suspended matter in suspension (such as loosened biofilm). Turbidity is important because it affects both the acceptability of water to consumers and the selection and efficiency of treatment processes (WHO, 1997c). However, for this study, the concept was applied that microorganisms are often associated with turbidity, hence

1.8 SUMMARISING THE AIMS AND OBJECTIVES OF THIS STUDY

Water quality of case and controls (as described in Section 1.6) was assessed to determine if there was any significant difference in their water quality. To further investigate possible reasons for the significant differences between the water quality used by both cases and controls, the effect of distance in the microbiological water quality in containers was investigated. Further investigation to determine the effect of water storage container material used by both the case and control groups.

The main aim of this study therefore was to assess the microbiological as well as aesthetical quality of the water that the people of the study community consumed in order to describe the risk of infection to such consumers.

To achieve this aim, the following objectives had to be met:

- ◆ To determine any significant difference in the water quality from the supply and the general quality of water samples in all the containers.
 - To determine the health-related quality of the supplied water at various supply points used by the study community.
 - To determine the health-related quality of stored water.
- ◆ To assess any significant differences between the quality of the water used by case and control groups:
 - To assess any significant differences between the quality of the water used by case and control groups in the areas serviced by:
 - Yard taps – to determine whether the closer taps had any effect on the stored water quality.
 - Areas serviced by remote taps - to determine whether the longer distance for taps had any effect on the stored water quality.
 - This assessment will also be done on a basis of comparing the microbiological and aesthetically water quality before and after the suspending biofilm.
- ◆ To determine whether biofilm formed on the sidewalls of the water storage

containers had any effect:

- Assess whether this affected the water quality of the case and control groups respectively.
- Investigate whether the possible presence of biofilm contributed to the deterioration of water quality in containers.
- Assess whether container materials had different effects on the water quality.
- ◆ Describe the risk of infection to consumers posed by the water quality.

CHAPTER 2

METHODOLOGY

2.1 STUDY SITE

The area chosen for the study was Section K, a suburb of the city of Botshabelo (Figure 2.1) in the Free State Province of South Africa. Botshabelo is a low socio-economic urban development located 55km east of Bloemfontein. Botshabelo's population is estimated to be 220 000 (WRC, 1993).

Water and sanitation systems vary between the residential blocks. Section K is one of the lesser-developed and poorer sections of Botshabelo and has only limited sanitary facilities (no water-borne sewage). Houses in general do not have in-house running water.

2.1.1 WATER SUPPLY

- *Yard taps* – for this study, these taps were considered as the taps in the yard with an approximate maximum distance of 10m. Not all families have taps in their yards - only the ones who could afford to pay the municipality to install the tap in their yards.
- *Communal taps* – These were the public street taps initially installed by the municipality for community use. In the study by Theron (2000) 82% of the people of Section K that used community taps lived between yard tap distance (maximum 10 meters) and 100 meters from their nearest communal tap. People living between 100 and 200 meters away amounted 15% while only 3% of the people lived further than the RDP (ANC, 1994) distance of 200 meters. For this study, the effect of distance on the container-stored water was also investigated. The selected distances were:
 - More than 10m but less than 100m (The distance of the majority).
 - More than 100m (The distance of the minority).

2.1.2 SANITATION

Sanitation was provided through individual inadequately constructed pit-latrines (too shallow with inadequate fly proofing) and is not effectively maintained. In vacant areas between the formal housing sectors in the section, informal settlements

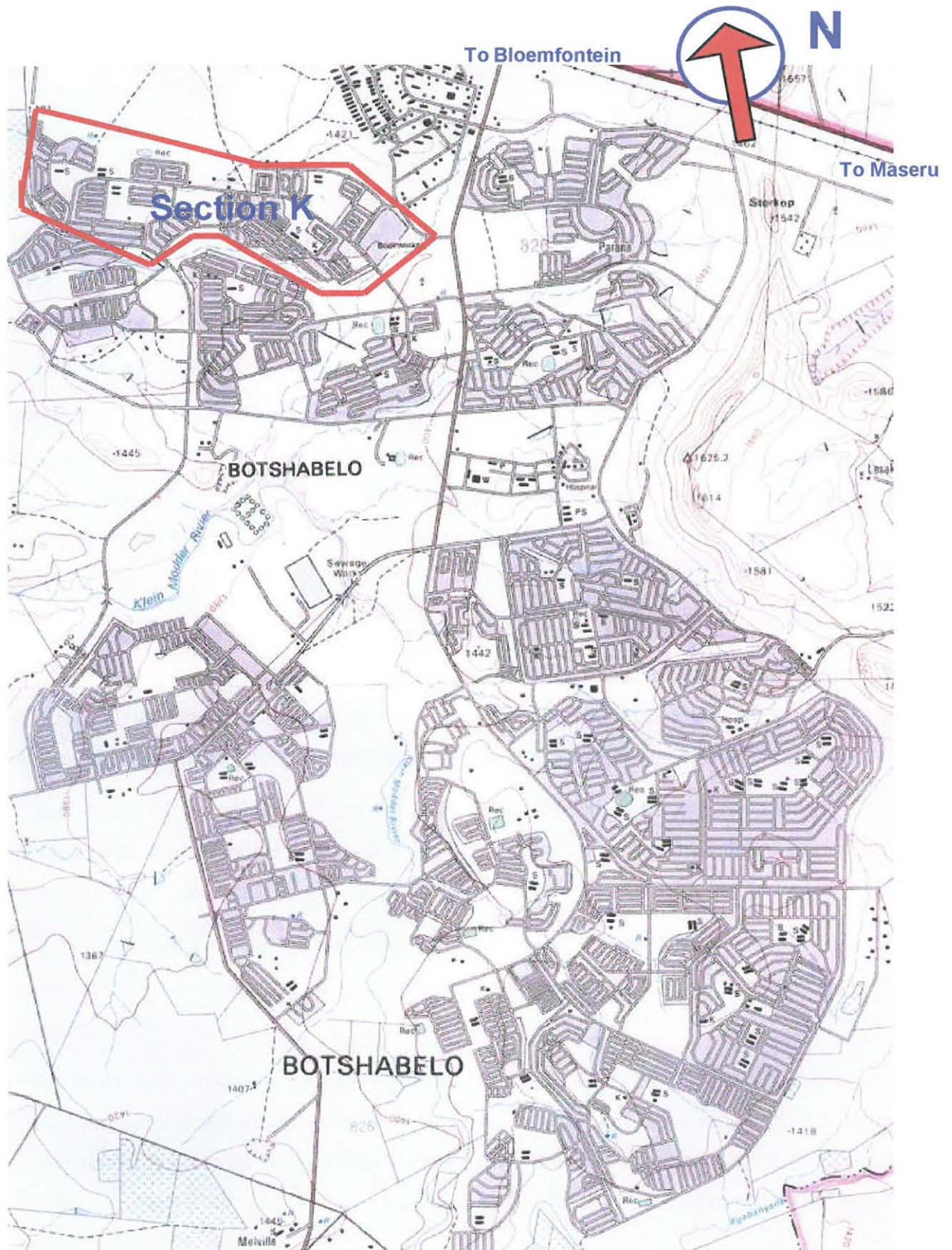


FIGURE 2.1: A MAP OF BOTSHABELO, SHOWING THE LAYOUT OF THE VARIOUS SECTIONS AS WELL AS THE STUDY AREA OF SECTION K.

generally developed without s

The members of these sectors of the community used pit-latrines or neighbouring facilities or the surrounding environment for latrine.

2.2 CASE CONTROL SELECTION

Other related studies are presently and were also previously conducted in the same area. A descriptive cross-sectional epidemiological survey was conducted during August 1998 by a co-worker (Theron, 2000) from Technikon Free State. The survey was designed to try and find a link between water use patterns of selected households and incidences of diarrhoea experienced by some of its members.

Section K had a household population of approximately 3100 households. Theron (2000) with the help of a bio-statistician, randomly selected 10% of this population as a sample to represent the whole area. This meant that 300 households were selected randomly - 270 from the residential plots and 30 households from informal settlements.

A follow-up observational analytical case-control study (Nala and Jagals, 1999) was also conducted in the same Section K Botshabelo based on the study by Theron (2000). This study was an in-depth case-control follow-up study of some of the same households previously selected and surveyed by Theron (2000).

From these households, 75 households were identified as cases. These case households experienced diarrhoea for one or more of the members during the preceding 12 months (January – December 1998).

Another 75 households were identified as controls. These households had not experienced diarrhoea for one or more of the members during the preceding 12 months.

This study was linked to the case-control follow-up study for 12 months. Both the case and control groups were followed up with the co-workers during which time the water these households used was sampled and the microbiological and aesthetical quality assessed as well as the potential biofilm forming on containers sidewalls investigated.

2.3 WATER SAMPLING

2.3.1 CONSUMER WATER SAMPLING

Drinking water samples were collected on a weekly basis.

The water samples were collected aseptically in 900 ml sterile Whirlpacks® from:

- Containers (plastic and metal) kept inside households to assess the microbiological and aesthetical domestic water quality.
- Communal (remote) taps and yards taps to assess the microbiological and aesthetical quality of municipal water supply. The mouths of the taps were disinfected with a flame and the water run for 1 minute before the samples were taken.

Samples were placed in cooler bags (7°C – 10°C) and transported to laboratories at the Technikon Free State. The samples were analysed within 6 hours of collection. Water samples for coliphage assessments were transported to the University of Pretoria (Department of Medical Virology) and analysed within 24 – 30 hours from collection.

2.3.2 SAMPLING WATER WITH SUSPENDED BIOFILM

It was decided to loosen any possible biofilm from the sidewalls of the containers with a simple methodology (Figure 2.2) based on the swabbing technique used by Jagals et al. 1997. The philosophy behind using this method was simply to accelerate the process of biofilm loosening that would be brought about by the filling action of running tapwater into the container. This would then maximise the contamination that could take place with filling as well as handling the container during storage at home.

The method involved the following:

- A water sample was taken from the container while care was taken not to disturb the contents (hereafter referred to as the Undisturbed Water Sample - UWS).
- The inside walls of the same container were then scrubbed with a sterile long handled brush to loosen any film that may have developed on the container walls. Care was taken not to introduce any substance from the outside

environment such as 1 er by the analyst or creating floating dust in the dwelling.

- The container was then shaken to suspend the loosened film.
- A follow-up water sample was then taken from the mixed suspension (Mixed-suspension Container Water - MCW).



FIGURE 2.3: PLASTIC CONTAINERS USED TO COLLECT AND STORE DRINKING WATER AND ALSO THE APPLICATION OF BRUSHING TECHNIQUE.

2.4 WATER QUALITY INDICATORS

The Membrane Filtration Technique (Appendix B) was used to enumerate Total coliforms, *Escherichia coli* and *Clostridium perfringens*. The membrane filtration technique is highly reproducible (greater accuracy) and yields reliable numerical results more rapidly than some of the other techniques such as the multiple-tube procedure (Standard Methods, 1998). The Spread Plate Method (Appendix B) was used to enumerate heterotrophic bacteria. The Double-layer Plaque Assay method (Appendix E) was used to enumerate somatic coliphages.

A sterile phosphate buffer was used (Appendix A) for diluting samples and rinsing funnels during and after filtration (Millipore, 1992). This was done to minimise cross



contamination during filtration
sample.

sterilised by boiling between each

2.4.1 BACTERIAL INDICATORS

2.4.1.1 Heterotrophic plate count (HPC) (Appendix C)

Heterotrophic bacteria were used as a practical indicator of general microbiological water quality (Standard Methods, 1998).

HPC were enumerated using a spread plate method with glucose yeast agar inside a laminar flow cabinet.

Incubation: The prepared plates were inverted and incubated aerobically in an incubator at 37°C for 48hr.

Identification: Heterotrophic bacterial colonies were all the visible colonies on the plates.

2.4.1.2 Total coliforms (TC) (Appendix C)

Total coliforms were used as indicators for organic pollution of water (Grabow, 1996; Standard methods, 1998).

TC were enumerated on Chromocult® Coliform Agar for the simultaneous detection of coliforms and *Escherichia coli* in the same water samples (Merck, 1996) with the membrane filtration technique in triplicate on 90-mm petri dishes.

Incubation: The prepared plates were inverted and incubated in an incubator at 35°C-37°C for 24hours.

Identification: Total Coliform colonies appeared in various shades of salmon to red (Merck, 1996).

Confirmation: API® 20E (bioMérieux® test kits) (Appendix D)

2.4.1.3 *Escherichia coli* (*E. coli*) (Appendix C)

E. coli were used as indicators for faecal pollution and other pathogenic enterobacteria, which may pollute water (Grabow, 1996; Standard Methods, 1998).

E. coli were enumerated on Chromocult® Coliform Agar used for the simultaneous detection of total coliforms and *E. coli* in water samples (Merck, 1996) with the membrane filtration technique in triplicate on 90-mm petri dishes.



- Incubation:** The prepared membranes were inverted and incubated in an incubator at 35°C-37°C for 24 hours.
- Identification:** *E. coli* colonies appeared in various shades of dark blue-to-violet (Merck, 1996).
- Confirmation:** API® 20E (bioMérieux® test kits) (Appendix D).

Both TC and *E. coli* were enumerated on the same membranes on the Chromocult® Coliform Agar. The *E. coli* were easily distinguishable from the rest of the TC's by their coloration (Appendix C) and were also counted separately. When the TC's were counted from the same membrane, the *E. coli* were included in the final calculations because they are part of the TC group.

2.4.2 VIRUSES

Because tests for enteropathogenic viruses in water are very costly and complicated – certain species of bacteriophages are used as indicators of enteric viruses (Grabow, 1996).

2.4.2.1 Somatic coliphages (SC) (Appendix E)

Possible enteric virus contamination was assessed using somatic coliphages as virus indicators (Grabow et al., 1993; 1996; Payment, 1991).

SC were enumerated by the Plaque Assay method for somatic coliphages with Double Agar Layer technique (Grabow et al., 1997). The method is based on conventional plaque assay for SC (Grabow et al., 1993) in small volumes of water (generally 10 ml) using petri dishes (90 mm diameter).

Incubation: The prepared plates were inverted and incubated overnight at 35-37 °C.

Identification: Plaques that formed in the matted host overgrowth on the plates.

2.4.3 RESISTANT SPORES

Clostridium perfringens are spore-forming and can survive adverse conditions as the water treatment process (Payment and Franco, 1993).

2.4.3.1 *Clostridium perfringens* (*C. perfringens*) (Appendix C)

C. perfringens were used to indicate remote faecal pollution as well as the possible



Cryptosporidium (Payment and Franco, 1993).

C. perfringens was enumerated with the membrane filtration technique using supplemented Perfringens Agar (Oxoid, 1990). Enumeration was done in triplicate on 90-mm petri dishes.

Pasteurisation: The samples were pasteurised prior to enumeration (Appendix C) to knock out background flora that might interfere with the process. The pasteurisation process does not damage the vegetative spores of *C. perfringens*.

Incubation: The prepared plates were inverted and incubated anaerobically in an incubator at 37⁰C for 48 hours. Oxoid[®] gas generating kits producing atmospheres of 95% hydrogen and 5% carbon dioxide were used.

Identification: *C. perfringens* colonies appeared as partially or fully discoloured dark brown to black colonies.

Confirmation: Cultured isolates confirmed on Rapid ID[®] 32A galleries (bioMérieux[®]) (Appendix D).

2.4.4 PHYSICAL WATER QUALITY

It was decided to use a gross physical quality measure in addition to the microbiological indicators to assess the aesthetic quality of the container water (whether the water becomes cloudy or "dirty") after applying the simple scrubbing technique. It was decided to use water clarity as the parameter with the level of turbidity as the indicator.

2.4.4.1 Turbidity

Turbidity was used for indication of the concentration of suspended matter in water, which is also related to the clarity. Turbidity is important because it affects both the acceptability of water to consumers and the selection and efficiency of treatment processes (WHO, 1997c). However, for this study, the concept was applied that microorganisms are often associated with turbidity, hence low turbidity minimises the potential for transmission of infectious diseases. (DWAF, 1996; Tchobanoglous and Schroeder, 1987).



A HACH 2100 turbidity meter was used to measure turbidity level. The measurements were recorded as Nephelometric Turbidity Units (NTU's).

2.5 COLONY VERIFICATION (Appendix D)

Confirmation of indicator colonies was done using analytical profile index (API) testing kits of bioMérieux®. This was done to establish the levels of false positive indicators in order to accurately calculate the detected indicator levels as well as to establish the selectivity of the various media for detecting the selected indicators (Standard Methods, 1998).

API® 20E Multi-test Galleries (bioMérieux®) were used for the confirmation of total coliform and *Escherichia coli* colonies.

Rapid ID® 32 A Multi-test Galleries (bioMérieux®) were used for the colony confirmation of *Clostridium perfringens*.

2.6 COUNTING OF COLONIES (Appendix B)

After incubation for prescribed periods of time, colonies were counted according to the prescriptions (from the respective manufacturers' manuals) for each group of organisms.

2.7 STATISTICAL AND MATHEMATICAL ANALYSES OF DATA

For this component of the study, the study population was stratified in two categories depending on the different levels of water supply namely:

- Yard tap as the main water supply for individual households.
- Remote taps as main water supply for individual households collectively (inclusive of the selected distances, which were used in the investigations in areas where effect of distances were suspected).

Data were placed in Microsoft Excel® 97 spreadsheets, which were set up to do the required descriptive statistical analyses such as the sample size, range, geometric mean, median, and the 95% confidence intervals.

The statistical programme SigmaStat Version 2.0 (1997) was used to calculate and test for sample size, normality and statistical significant differences using ANOVA.

of the data in graphs. This was done to enable visual appraisal of the data. All the graphs were displayed using boxplots to visualise data more effectively.

2.8 STATISTICAL CONSIDERATIONS

2.8.1 DEVELOPING HYPOTHESES (Appendix F)

Statistical tests are the most quantitative ways to determine whether hypotheses can be substantiated or whether they must be modified or rejected outright. Hypotheses were formulated for each of the various Sections contained in the Results Chapter.

2.8.2 DETERMINING THE SAMPLE SIZE (Appendix F)

The minimum sample sizes for statistical significance were determined before each series of experiments commenced at the various levels and approaches of this study. As the microbiological data in the sets used for this study were expected to be generally non-parametric (Standard Methods, 1998; Helsel and Hirsch, 1995) the sample sizes were determined accordingly (Appendix F).

After assessing the first 15 samples (Standard Methods, 1998), the median differences and the standard deviation of the residuals expected for each ($n = 15$) data set were used to estimate the final minimum size and to confirm whether the initial sample sizes were big enough (Appendix F: Section 5).

- *Heterotrophic bacteria*: The minimum detectable differences in the means were calculated at 1.76 and expected standard deviation at 0.82. The formula returned a minimum sample size of 5.
- *Turbidity*: The minimum detectable differences in the means were calculated at 0.19 and expected standard deviation at 0.3. The formula returned a minimum sample size of 39.
- *Total coliforms*: The minimum detectable differences in the means were calculated at 0.71 and expected standard deviation at 0.58. The formula returned a minimum sample size of 12.
- *E. coli*: The minimum detectable differences in the means were calculated at 0.12 and expected standard deviation at 0.22, The formula returned a minimum

sample size of 52.

- *C. perfringens*: The minimum detectable differences in the means were calculated at 0.08 and the expected standard deviation at 0.08. The formula returned a minimum sample size of 17.

2.8.3 TESTING FOR DIFFERENCES (ANOVA)

The One Way ANOVA tests the hypothesis of no differences between the several treatment groups, but does not determine which of the groups are different, or the sizes of these differences. Multiple comparison tests were used to isolate these further differences by doing further comparisons between the experimental groups (Appendix F).

2.8.4 STATISTICAL ANALYSES - SECTION BY SECTION

The section numbers used in this description are the numbers used in Chapter 3: Results.

SECTION 3.1 GENERAL WATER QUALITY FROM THE PUBLIC STANDPIPES AND CONTAINER-STORED WATER.

This approach was followed to assess whether water from the supply point was of a better quality than that of water stored in containers by individual households as found by Jagals et al. (1997; 1999).

Fifty water samples from the municipal supply as well as 150 water samples from the container-stored water (plastic and metal) kept in selected individual households were collected and analysed. These sample sizes were larger than what was required in the calculations in 2.8.2 above.

To determine whether there were any statistical significant differences between the water quality of supply water and container-stored water, the non-parametric Mann-Whitney Rank Sum Test was applied since the data was not paired or was unequal.

The following hypothesis was developed.

Null hypothesis (H_0): there will be no statistically significant difference between the water quality of the municipal supply from the standpipes and water stored in household containers.



SECTION 3.2: **GENERAL** WATER QUALITY BEFORE AND AFTER SUSPENDING BIOFILM.

As mentioned in the above paragraph, the same approach of suspending biofilm was used to determine if indeed biofilm contributed to the deterioration of water quality.

Hundred-and-fifty water samples before and after suspending biofilm were collected and analysed. This sample size was larger than what was required in the calculations in 2.8.2 above.

To determine any significant difference in the water quality, the non-parametric Wilcoxon Signed Rank Test was applied on the paired data “before” and “after” the suspension of biofilm.

The following hypothesis was developed.

Null hypothesis (H_0): there will be no statistically significant differences in the microbiological and aesthetical containers water quality before and after suspending biofilm.

SECTION 3.3: UNDISTURBED WATER QUALITY USED BY CASES AND CONTROLS.

The approach was followed since it was expected that more contamination would be found in container water used by cases as compared to water used by control groups.

Twenty-nine undisturbed water samples in containers fetched from the yard taps used by cases and 25 undisturbed water samples in containers from the yard taps used by controls were collected and analysed. Thirty-eight undisturbed water samples in containers fetched from the communal taps used by cases and 46 undisturbed water samples in containers from the controls were also collected and analysed. These sample sizes were larger than what was required in the calculations in 2.8.2 above.

The non-parametric Mann-Whitney Rank Sum Test was applied to the undisturbed water quality used by both the cases and controls to determine the significant differences in their water quality. Mann-Whitney Rank Sum Test was also used because the data was not paired and the data sets were of unequal size.

The following hypothesis was c

Null hypothesis (H_0): there will be no statistical significant difference in the microbiological and aesthetical quality of undisturbed water used by cases and controls.

Statistical significant differences in the undisturbed water quality used by both the case and control groups were investigated further. Distance was one of the variables investigated to determine the water quality differences by comparing the distances people had to travel to fetch water.

SECTION 3.4: MIXED SUSPENSION CONTAINER WATER QUALITY USED BY CASES AND CONTROLS.

The approach was followed since it was expected that the quality of water used by cases would indicate more deterioration than the water quality used by controls after suspending the biofilm.

Twenty-nine mixed suspension container water samples fetched from the yard taps used by cases and 25 mixed suspension container water samples fetched from the yard taps used by controls were collected and analysed. Thirty-eight mixed suspension container water samples fetched from the communal taps used by cases and 46 mixed suspension container water samples fetched from the controls were also collected and analysed. These sample sizes were more than what was required in the calculations in 2.8.2 above.

The non-parametric Mann-Whitney Rank Sum Test was applied to the mixed suspension container water quality used by both the cases and controls to determine the significant differences in their water quality.

The following hypothesis was developed.

Null hypothesis (H_0): there will be no statistical significant difference in the microbiological and aesthetical mixed suspension water quality used by cases and controls.

A further investigation was done to determine any statistical significant difference in the mixed suspension container water quality used by both study groups. The Kruskal-Wallis ANOVA and Tukey multiple comparison tests on ranks were used. The Kruskal-Wallis ANOVA test was the method used to compare results from three



and more different experiments

(ie various distances people have to carry water) that may be affected by a single factor. The Tukey multiple comparison test was used to identify the different variable/s amongst the experimental groups were different – a feature that the Kruskal-Wallis ANOVA test does not offer.

SECTION 3.5: CONTAINER WATER QUALITY BEFORE AND AFTER SUSPENDING BIOFILM (CASES vs. CONTROLS)

The approach was followed to determine more specifically the quality of water used by both study groups before and after suspending the biofilm and placing the findings in one table for a broader perspective.

Seventy paired water samples before and after suspending biofilm in containers used by cases and controls were collected and analysed. These sample sizes were larger than what was required in the calculations in 2.8.2 above.

The non-parametric Wilcoxon Signed Rank Test was applied to both water qualities used by cases and controls to determine any significant difference in the water quality after the suspension of biofilm.

The following hypothesis was developed.

Null hypothesis (H_0): there will be no statistical significant difference in the microbiological and aesthetical container waters quality after suspending biofilm in the containers used by cases and controls.

SECTION 3.6: PLASTIC AND METAL CONTAINER WATER QUALITY (General)

The approach followed was to determine generally the differences in the water quality in the different container types. This was to find out which type of containers contributed more to the deterioration of water quality, if any.

Two-hundred and twenty-four water samples from the container water and 76 water samples from the metal containers were collected and analysed. These sample sizes were larger than what was required in the calculations in 2.8.2 above. The non-parametric Mann-Whitney Rank Sum Test was applied to both water qualities from different container types.

The following hypothesis was developed.



Null hypothesis (H₀): the statistical significant difference in the microbiological and aesthetical water quality in plastic and metal containers used by both study groups.

2.9 PARAMETERS FOR INFECTION RISK

The risk of infection to consumers of the supply water in the study area was determined by comparing the water quality with various health-related water quality guidelines. The following guidelines were used:

- South African Water Quality Guidelines: Vol. 1: Domestic Water (DWAf, 1993; 1996).
- Proposed Water Quality Criteria in South Africa of the National Department of Health (Aucamp and Vivier, 1990).
- Water Research Commission: Assessment Guide: Quality of Domestic Water Supplies (WRC, 1998) Vol. 1, Second Edition.

The South African Water Quality Guidelines: Vol. 1: Domestic Water (DWAf, 1993) was used for estimating *E. coli* risk limits. As no other guideline was published between the period 1993 – 1999, about the *E. coli* risk limits.

Table 2: Summary for the guidelines value

| Parameters investigated and Targeted Water Quality Range | Effects on human health | Guidelines |
|--|---|--|
| Heterotrophic Bacteria ≤100 organisms / 1ml | Negligible risk of microbial infection | South African Water Quality Guidelines (DWAf, 1996) |
| Turbidity 0-1 NTU's | Significant risk of infectious microorganisms | South African Water Quality Guidelines (DWAf, 1996) |
| Total coliforms 1. 10-5 organisms / 100ml 2. 10 organisms / 100ml | 1. Negligible risk of microbial infection 2. Insignificant chance of infection | 1. South African Water Quality Guidelines (DWAf, 1996) 2. Assessment Guide: Quality of Domestic Water Supplies (1998) |

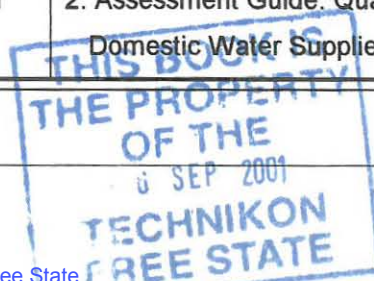
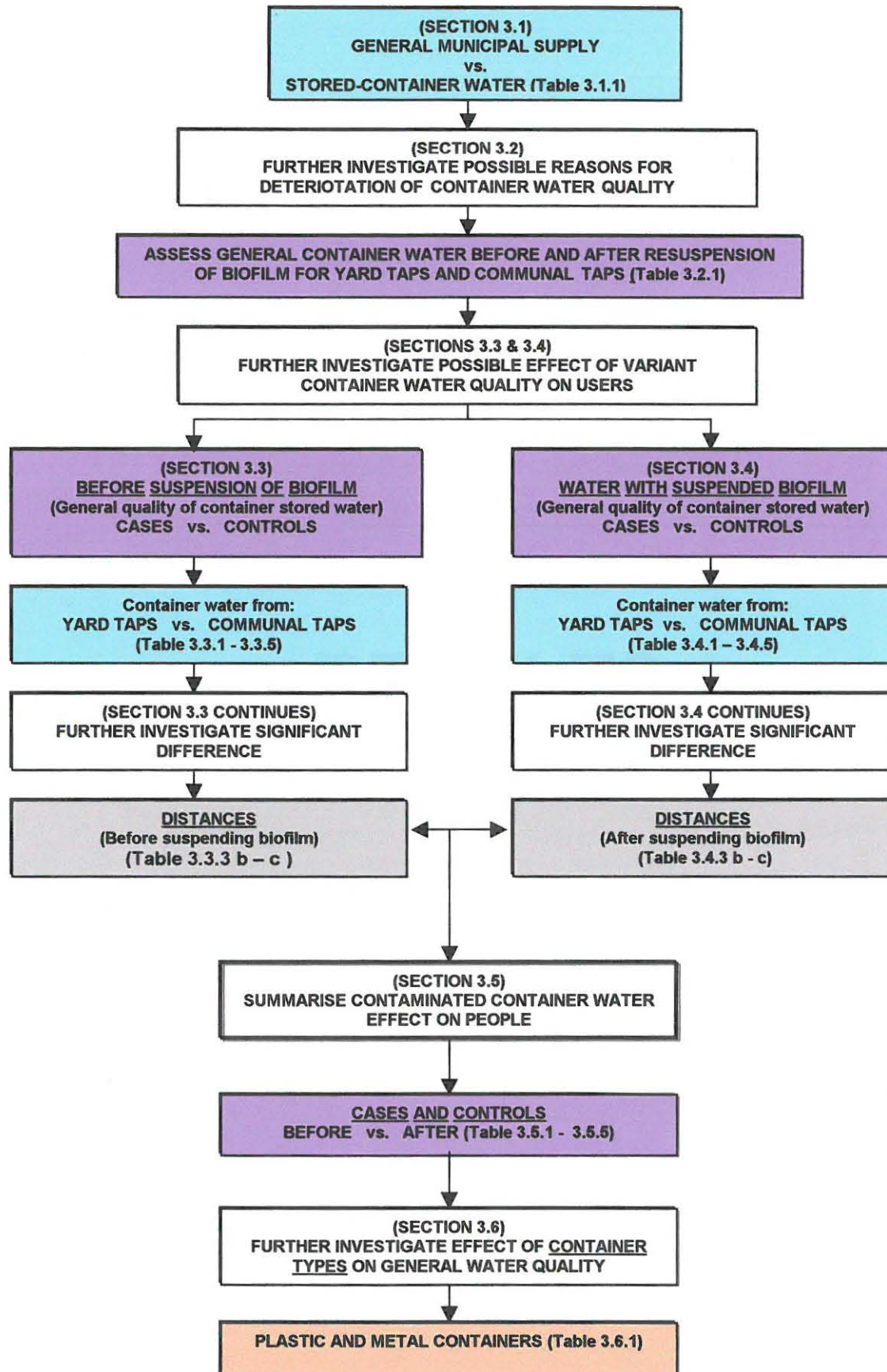


Table 2 (continues): Summary for the guidelines value

| Parameters investigated and Targeted Water Quality Range | Effects on human health | Guidelines |
|---|---|---|
| <i>Escherichia coli</i> 0 organism / 100ml | Negligible risk of microbial infection | South African Water Quality Guidelines (DWAF, 1993) |
| <i>Clostridium perfringens</i> 1 organisms / 100ml | Insignificant risk limits | Water Quality Criteria in South Africa (Aucamp and Vivier 1990) |
| Somatic coliphages 0-1 organism / 100ml | Negligible risk of viral infection is indicated | South African Water Quality Guidelines (DWAF, 1996) |

The following Figure provides an outline of the results format presented in Chapter 3.

OUTLINE FOR RESULTS FORMAT (For Chapter 3)



CHAPTER 3

RESULTS

3.1 GENERAL WATER QUALITY FROM THE STANDPIPES AND CONTAINER WATER.

The emphasis in this section was the supply water quality at standpipes as well as the quality of water in stored container in individual households.

Table 3.1.1: Levels of indicators in general water supply and container water.

| | Supply water | Container water | Comparing groups for supply types |
|-------------------------------------|--|--|---|
| Heterotrophic bacteria / 1ml | <p>n = 48</p> <p>Geometric Mean = 1.39×10^4</p> <p>Min = 50</p> <p>Max = 5.77×10^6</p> <p>(log-transformed data)</p> <p>Median = 4.01</p> <p>25th Percentile limit = 3.10</p> <p>75th Percentile limit = 5.19</p> <p>95 % Confidence Intervals = ± 0.41</p> | <p>n = 140</p> <p>Geometric Mean = 4.39×10^5</p> <p>Min = 167</p> <p>Max = 9.60×10^7</p> <p>(log-transformed data)</p> <p>Median = 5.77</p> <p>25th Percentile limit = 4.32</p> <p>75th Percentile limit = 6.88</p> <p>95 % Confidence Intervals = ± 0.24</p> | <p>Significant difference</p> <p>$P \leq 0.001$</p> <p>H₀ rejected</p> <p>Normality: Failed ($P \leq 0.001$)</p> <p>Mann-Whitney Rank Sum</p> |
| Turbidity NTU's | <p>n = 50</p> <p>Arithmetic Mean = 0.78</p> <p>Min = 0.11</p> <p>Max = 3.5</p> <p>(log-transformed data)</p> <p>Median = 0.62</p> <p>25th Percentile limit = 0.50</p> <p>75th Percentile limit = 0.91</p> <p>95 % Confidence Intervals = ± 0.15</p> | <p>n = 145</p> <p>Arithmetic Mean = 1</p> <p>Min = 0.11</p> <p>Max = 4.2</p> <p>(log-transformed data)</p> <p>Median = 0.81</p> <p>25th Percentile limit = 0.53</p> <p>75th Percentile limit = 1.33</p> <p>95 % Confidence Intervals = ± 0.12</p> | <p>Significant difference</p> <p>$P = 0.022$</p> <p>H₀ rejected</p> <p>Normality: Failed ($P \leq 0.001$)</p> <p>Mann-Whitney Rank Sum</p> |
| Total coliforms / 100ml | <p>n = 52</p> <p>Geometric Mean = 10</p> <p>Min = 0</p> <p>Max = 2.46×10^3</p> <p>(log-transformed data)</p> <p>Median = 0.98</p> <p>25th Percentile limit = 0</p> <p>75th Percentile limit = 1.85</p> <p>95 % Confidence Intervals = ± 0.29</p> | <p>n = 150</p> <p>Geometric Mean = 92</p> <p>Min = 0</p> <p>Max = 6.18×10^5</p> <p>(log-transformed data)</p> <p>Median = 1.69</p> <p>25th Percentile limit = 0.60</p> <p>75th Percentile limit = 3.15</p> <p>95 % Confidence Intervals = ± 0.26</p> | <p>Significant difference</p> <p>$P \leq 0.001$</p> <p>H₀ rejected</p> <p>Normality: Failed ($P \leq 0.001$)</p> <p>Mann-Whitney Rank Sum</p> |

| | Supply water | Container water | Comparing groups for supply types |
|---|---|--|--|
| <i>Escherichia coli</i> / 100ml | n = 52 Geometric Mean = 1 Min = 0 Max = 49 (log-transformed data) Median = 0 25 th Percentile limit = 0 75 th Percentile limit = 0 95 % Confidence Intervals = ± 0.07 | n = 150 Geometric Mean = 2 Min = 0 Max = 4.87×10^4 (log-transformed data) Median = 0 25 th Percentile limit = 0 75 th Percentile limit = 0 95 % Confidence Intervals = ± 0.11 | No significant difference $P = 0.547$ H₀ accepted Normality: Failed ($P \leq 0.001$) Mann-Whitney Rank Sum |
| <i>Clostridium perfringens</i> / 100ml | n = 52 Geometric Mean = 1 Min = 0 Max = 0.90 (log-transformed data) Median = 0 25 th Percentile limit = 0 75 th Percentile limit = 0 95 % Confidence Intervals = ± 0.01 | n = 150 Geometric Mean = 1 Min = 0 Max = 43 (log-transformed data) Median = 0 25 th Percentile limit = 0 75 th Percentile limit = 0.10 95 % Confidence Intervals = ± 0.04 | Significant difference $P = 0.019$ H₀ rejected Normality: Failed ($P \leq 0.001$) Mann-Whitney Rank Sum |

Somatic coliphages were not detected in any water samples and will therefore not be reported on.

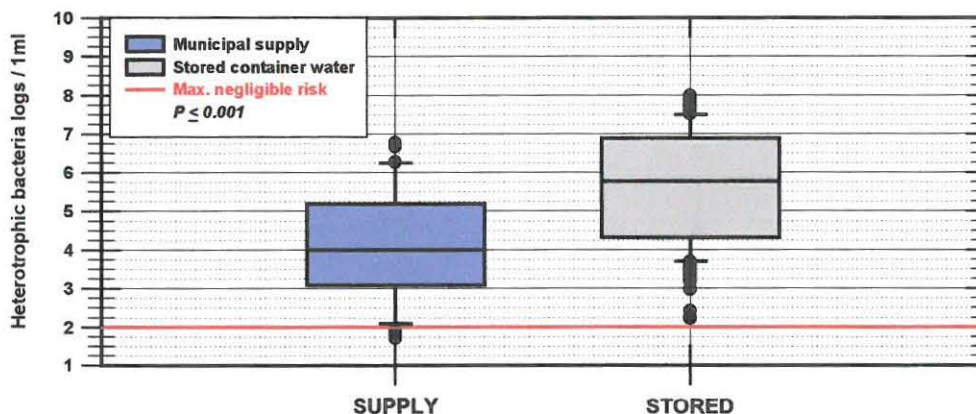


FIGURE 3.1.1 (a): Heterotrophic bacteria in municipal supply and container-stored water

In general the results indicated that there were statistically significant difference in the water quality from the standpipes and container water with the exception of *E. coli*. The null hypothesis was therefore rejected ($P \leq 0.001$). The results clearly showed high heterotrophic bacterial counts in both the supply water and container

water. These counts for both did not comply with the *negligible risk* (≤ 100 organisms / 1ml) limits proposed by the DWAF (1996). Although both types of water at the standpipes and container water posed an increased risk of microbial infection disease transmission (DWAF, 1996), the stored water in the containers posed a greater risk due to higher heterotrophic bacterial counts.

Figure 3.1.1 (b) illustrates the results of the turbidity levels in water samples from the supply point and the stored container water.

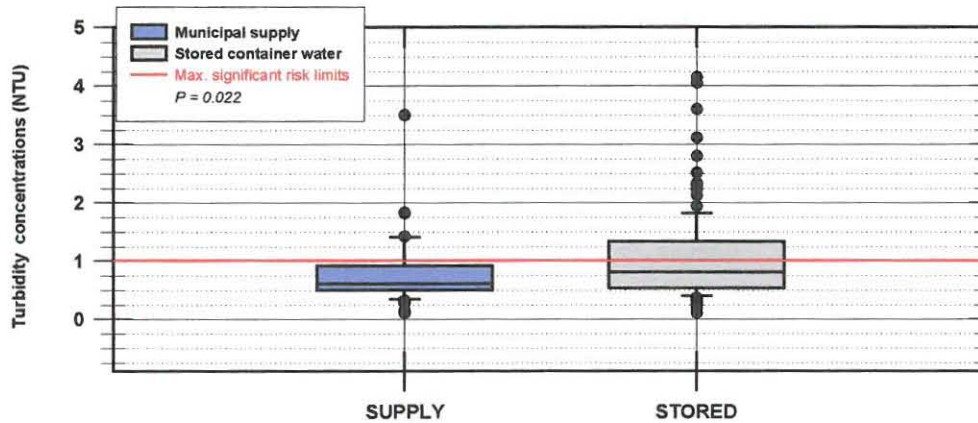


FIGURE 3.1.1 (b): Turbidity levels in municipal supply and container-stored water

The mean turbidity level in the supply water as well as in the stored container water was lower than the maximum limits for *significant risk* (0-1 NTU) proposed by the DWAF (1996).

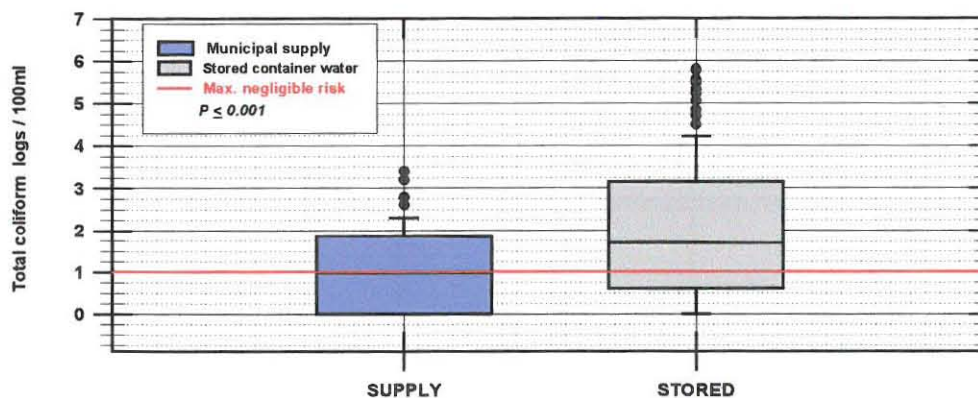


FIGURE 3.1.1 (c): Total coliform in municipal supply and container-stored water

Judging by Figure 3.1.1 (c), the results indicated a statistically significant increase in the median value for total coliforms in the stored container water as compared to the

supply water ($P \leq 0.001$). T
ner water used by the community did not comply with the limits for *negligible risk* (10 organisms / 100ml) of microbial infection proposed by DWAF (1996) as well as the Assessment Guide: Quality of Domestic Water Supplies (1998) (0-5 organisms / 100ml).

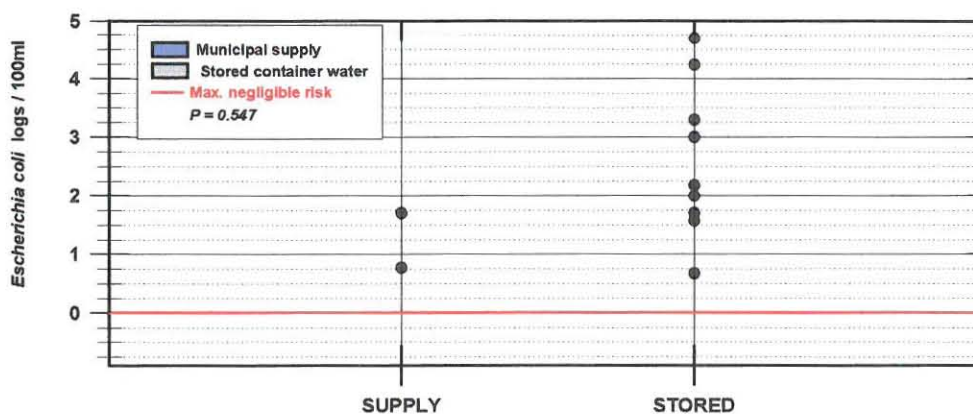


FIGURE 3.1.1 (d): *Escherichia coli* In municipal supply and container-stored water

Although the results in Figure 3.1.1 (d) indicated that there were no statistical significant differences observed between data from two sets. However, the results did indicate a trend of more positive samples in stored water observed. Although the median values were zero, the geometric mean values indicated that *Escherichia coli* was intermittently found in the samples.

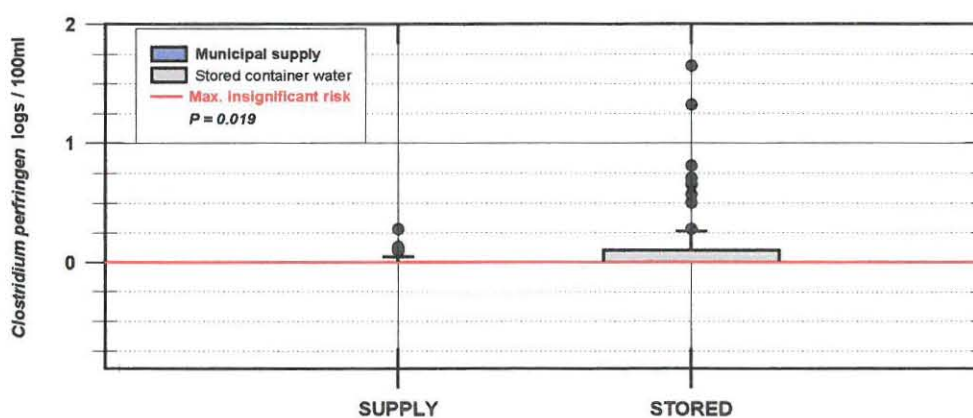


FIGURE 3.1.1(e): *Clostridium perfringens* in municipal supply and container-stored water

Figure 3.1.1 (e) illustrates the log-transformed results of the two data sets. The data

shown on the graph between sets indicated that there was indeed a statistically significant difference in the two data sets ($P = 0.019$).

The mean values for both sets of water were within the limits of *insignificant risk* (1 organism / 100ml) according to the *Water Quality Criteria in South Africa* (Aucamp and Vivier, 1990).

3.2 GENERAL WATER QUALITY BEFORE AND AFTER SUSPENDING BIOFILM.

In an effort to establish what may have caused the significant differences between the container water and the supply water, the effect of the biofilm, as discussed in Chapter 2, was assessed. This section describes the water quality before and after suspension of biofilm and immediately after the loosening process.

In this section, “before” refers to the “*undisturbed water*” as discussed in Chapter 2 in the container before suspending biofilm and “after” refers to the “*mixed suspension container*” as discussed in Chapter 2 after the sidewalls had been brushed.

Table 3.2.1: Levels of indicators in the container water quality.

| | Before | After | Comparing pairs for supply types |
|-------------------------------------|--|---|--|
| Heterotrophic bacteria / 1ml | n = 140 Geometric Mean = 4.39×10^5 Min = 167 Max = 9.60×10^7 (log-transformed data) Median = 5.77 25 th Percentile limit = 4.32 75 th Percentile limit = 6.88 95 % Confidence Intervals = ± 0.24 | n = 140 Geometric Mean = 8.59×10^5 Min = 400 Max = 1×10^8 (log-transformed data) Median = 6.15 25 th Percentile limit = 4.91 75 th Percentile limit = 7.01 95 % Confidence Intervals = ± 0.21 | Significant difference $P \leq 0.001$ H ₀ rejected Normality: Failed (P \leq 0.001) Wilcoxon Signed Rank |
| Turbidity NTU's | n = 145 Arithmetic Mean = 1 Min = 0.11 Max = 4.2 (log-transformed data) Median = 0.81 25 th Percentile limit = 0.53 75 th Percentile limit = 1.33 95 % Confidence Intervals = ± 0.12 | n = 145 Arithmetic Mean = 3 Min = 0.31 Max = 30 (log-transformed data) Median = 1.87 25 th Percentile limit = 1.31 75 th Percentile limit = 3.1295 % Confidence Intervals = ± 0.15 | Significant difference $P \leq 0.001$ H ₀ rejected Normality: Failed (P \leq 0.001) Wilcoxon Signed Rank |

Table 3.2.1 (continues): Levels of ir... inner water quality.

| | Before | After | Comparing pairs for supply types |
|--|--|---|--|
| Total coliforms / 100 mL | n = 150 Geometric Mean = 92 Min = 0 Max = 6.18×10^5 (log-transformed data) Median = 1.69 25 th Percentile limit = 0.60 75 th Percentile limit = 3.15 95 % Confidence Intervals = ± 0.28 | n = 150 Geometric Mean = 230 Min = 0 Max = 7.20×10^5 (log-transformed data) Median = 2.25 25 th Percentile limit = 1.20 75 th Percentile limit = 3.55 95 % Confidence Intervals = ± 0.26 | Significant difference $P \leq 0.001$ H ₀ rejected Normality: Failed (P \leq 0.001) Wilcoxon Signed Rank |
| Escherichia coli / 100ml | n = 150 Geometric Mean = 2 Min = 0 Max = 4.87×10^4 (log-transformed data) Median = 0 25 th Percentile limit = 0 75 th Percentile limit = 0 95 % Confidence Intervals = ± 0.11 | n = 150 Geometric Mean = 2 Min = 0 Max = 4.87×10^4 (log-transformed data) Median = 0 25 th Percentile limit = 0 75 th Percentile limit = 0 95 % Confidence Intervals = ± 0.14 | Significant difference $P \leq 0.001$ H ₀ rejected Normality: Failed (P \leq 0.001) Wilcoxon Signed Rank |
| Clostridium perfringens / 100ml | n = 150 Geometric Mean = 1 Min = 0 Max = 43 (log-transformed data) Median = 0 25 th Percentile limit = 0 75 th Percentile limit = 0.10 95 % Confidence Intervals = ± 0.04 | n = 150 Geometric Mean = 2 Min = 0 Max = 133 (log-transformed data) Median = 0.13 25 th Percentile limit = 0 75 th Percentile limit = 0.32 95 % Confidence Intervals = ± 0.05 | Significant difference $P \leq 0.001$ H ₀ rejected Normality: Failed (P \leq 0.001) Wilcoxon Signed Rank |

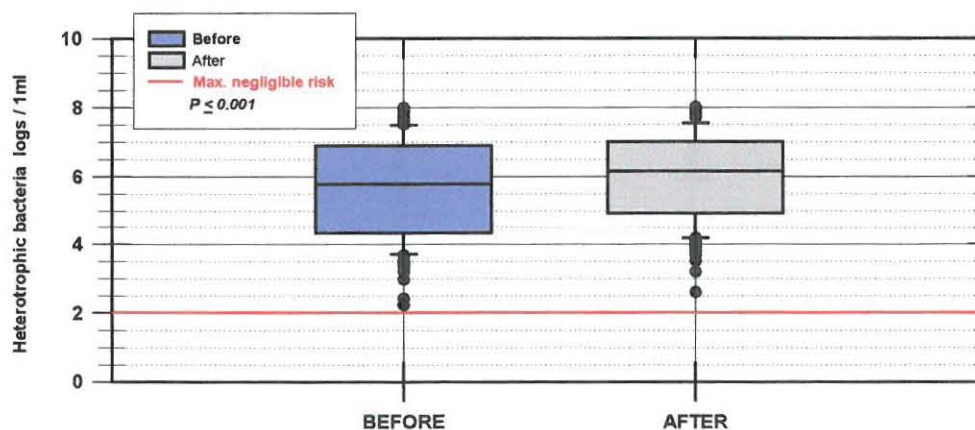


FIGURE 3.2.1 (a): Heterotrophic bacteria in container-stored water before and after suspending biofilm

Statistically significant difference

observed for all the parameters investigated before and after brushing. In some instances, the microbiological counts were highest after suspending biofilm.

Even though the significant differences were marginal, statistically there was a significant difference in water quality before and after suspending biofilm ($P \leq 0.001$). Both data sets did not comply with the limits for *negligible risk* (≤ 100 organisms / 1ml) of microbial infection proposed by DWAF (1996).

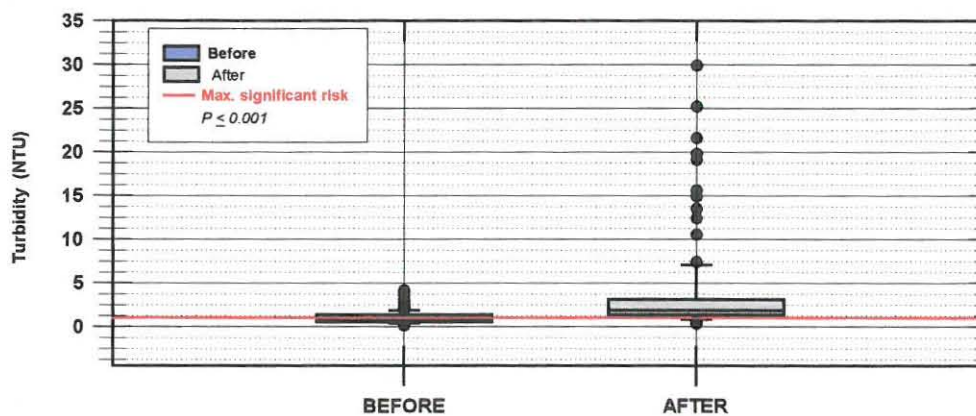


FIGURE 3.2.1 (b): Turbidity in container-stored water before and after suspending biofilm

Figure 3.2.1 (b) illustrates turbidity before and after suspending the biofilm. The water quality after suspending biofilm did not comply with the *significant risk* (0-1 NTU) limits proposed by DWAF (1996). Before suspending the biofilm, turbidity was within the risk limits for infectious disease as proposed by (DWAF, 1996).

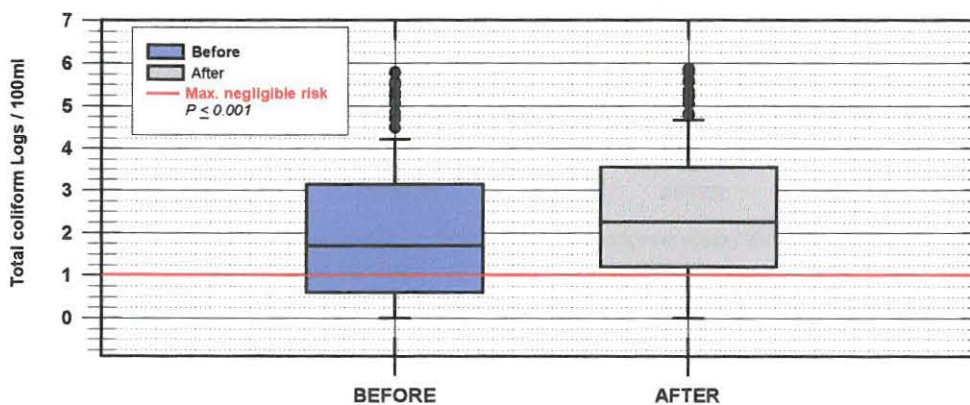


FIGURE 3.2.1 (c): Total coliform in container-stored water before and after suspending biofilm

Figure 3.2.1 (c) indicates that the water quality of the water tap did not comply with *negligible risk* (10 organisms / 100ml) limits for total coliforms proposed by the DWAF (1996) as well as Assessment Guide: *Quality of Domestic Water Suppliers* (1998).

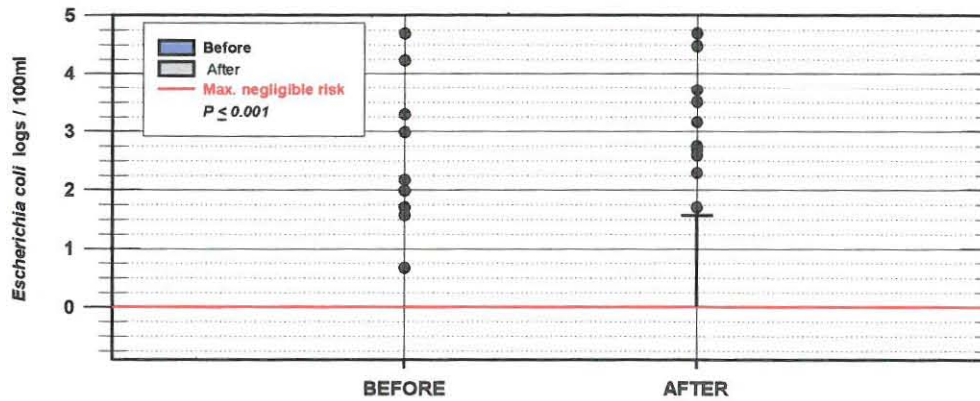


FIGURE 3.2.1 (d): *Escherichia coli* in container-stored water before and after suspending biofilm

Judging by Figure 3.2.1 (d), both water qualities did not comply with the *negligible risk* (0 organism / 100ml) of microbial infection limits proposed by the DWAF (1993). Although the median values indicate a zero presence, the geometric mean values indicated that *Escherichia coli* was intermittently found.

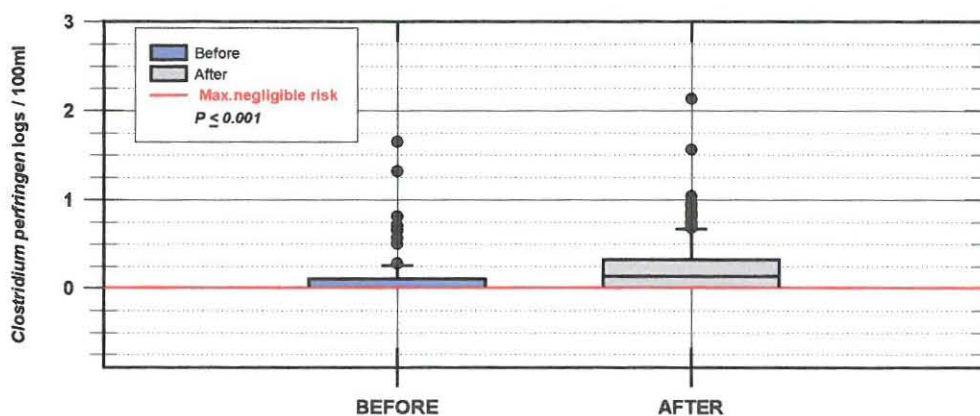


FIGURE 3.2.1 (e): *Clostridium perfringens* in container-stored water before and after suspending biofilm

Figure 3.2.1 (e) illustrates the log-transformed results for *C. perfringens* in both sets of data before and after suspending biofilm.

The results showed that, before and after suspending biofilm, there was a statistical

significant difference in the median values for water quality before and after suspending biofilm were within the limits of *insignificant risk* (1 organism / 100ml) according to the proposed *Water Quality Criteria in South Africa* (Aucamp and Vivier, 1990).

3.3 UNDISTURBED WATER QUALITY: CASES AND CONTROLS

Microbiological and aesthetical undisturbed water quality used by cases and controls were compared to determine if there was any statistical significant difference in their water quality in containers.

3.3.1 HETEROTROPHIC BACTERIA

The data from both sets of samples (cases and controls) are shown in Table 3.3.1.

Table 3.3.1: Heterotrophic bacteria in undisturbed water used by cases and controls.

| | Cases | Controls | Comparing groups for supply types |
|---|---|--|--|
| Yard Taps | n = 29 Geometric Mean = 5.17×10^5 Min = 167 Max = 7.02×10^7 (log-transformed data) Median = 6.00 25 th Percentile limit = 4.07 75 th Percentile limit = 7.08 95 % Confidence Intervals = ± 0.56 | n = 25 Geometric Mean = 5.40×10^5 Min = 4.4×10^3 Max = 5.17×10^7 (log-transformed data) Median = 5.91 25 th Percentile limit = 4.62 75 th Percentile limit = 6.93 95 % Confidence Intervals = ± 0.52 | No significant difference $P = 0.92$ H₀ accepted Normality: Passed (P = 0.12) Variance: Passed (P = 0.47) Mann-Whitney Rank Sum |
| Communal Taps | n = 38 Geometric Mean = 4.45×10^5 Min = 2.67×10^3 Max = 9.60×10^7 (log-transformed data) Median = 5.83 25 th Percentile limit = 4.12 75 th Percentile limit = 6.80 95 % Confidence Intervals = ± 0.43 | n = 46 Geometric Mean = 3.52×10^5 Min = 256 Max = 5.42×10^7 (log-transformed data) Median = 5.39 25 th Percentile limit = 4.37 75 th Percentile limit = 7.00 95 % Confidence Intervals = ± 0.43 | No significant difference $P = 0.86$ H₀ accepted Normality: Passed (P = 0.02) Variance: Passed (P = 0.45) Mann-Whitney Rank Sum |
| Comparing supply type for groups | No significant difference $P = 0.85$ H₀ accepted Normality: Passed (P = 0.01) Variance: Passed (P = 0.51) Mann-Whitney Rank Sum | No significant difference $P = 0.61$ H₀ accepted Normality: Passed (P = 0.035) Variance: Passed (P = 0.404) Mann-Whitney Rank Sum | |

The results showed no statistically significant differences between case and control water quality for both yard and communal taps ($P = 0.92$ and $P = 0.86$ respectively).

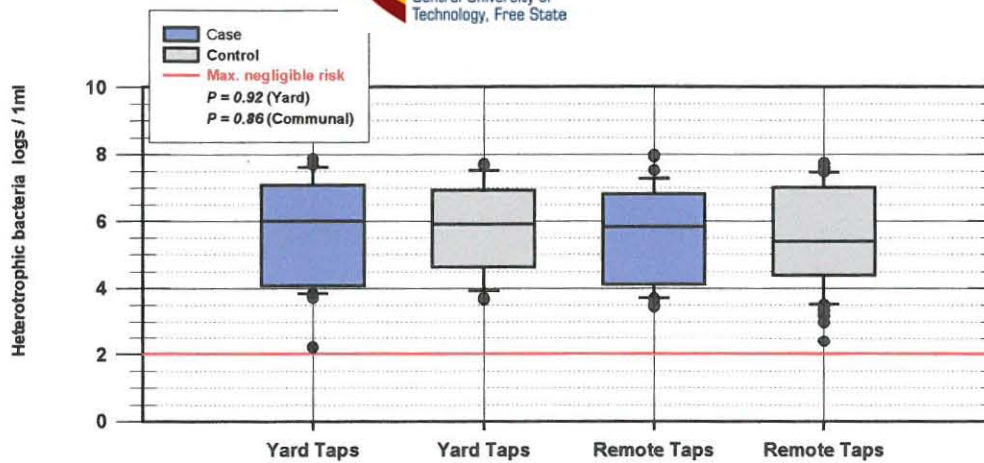


FIGURE 3.3.1 (a): Heterotrophic bacteria in container-stored water used by cases and controls

Figure 3.3.1 (a) illustrates that the heterotrophic bacteria levels were above the maximum limits for *negligible risk* proposed DWAF (1996).

3.3.2 TURBIDITY

The results from both yard and communal taps are shown in Table 3.3.2. The arithmetic mean values were used to compare the difference in both sets.

Table 3.3.2: Turbidity levels detected in the undisturbed water quality used by two groups.

| | Cases | Controls | Comparing groups for supply types |
|---|---|--|---|
| Yard Taps | n = 29 Arithmetic Mean = 1 Min = 0.37 Max = 9 Median = 0.82 25 th Percentile limit = 0.544 75 th Percentile limit = 1.485 95 % Confidence Intervals = ±0.60 | n = 25 Arithmetic Mean = 1 Min = 0.11 Max = 4 Median = 0.81 25 th Percentile limit = 0.544 75 th Percentile limit = 1.485 95 % Confidence Intervals = ±0.32 | No significant difference P = 0.654 H₀ accepted Normality: Failed (P ≤ 0.001) Mann-Whitney Rank Sum |
| Communal Taps | n = 40 Arithmetic Mean = 1 Min = 0.19 Max = 4 Median = 0.92 25 th Percentile limit = 0.547 75 th Percentile limit = 1.450 95 % Confidence Intervals = ±0.24 | n = 46 Arithmetic Mean = 0.94 Min = 0.25 Max = 4 Median = 0.74 25 th Percentile limit = 0.52 75 th Percentile limit = 1.21 95 % Confidence Intervals = ±0.19 | No significant difference P = 0.211 H₀ accepted Normality: Failed (P ≤ 0.001) Mann-Whitney Rank Sum |
| Comparing supply type for groups | No significant difference P = 0.898 H₀ accepted Normality: Failed (P ≤ 0.001) Mann-Whitney Rank Sum | No significant difference P = 0.622 H₀ accepted Normality: Failed (P ≤ 0.001) Mann-Whitney Rank Sum | |

The results indicated that in the median values between the undisturbed water qualities used by both groups was not great enough to exclude the possibility that the difference was due to random sampling variability. This implied that there was not a statistical significant difference in the undisturbed water quality used by case and controls

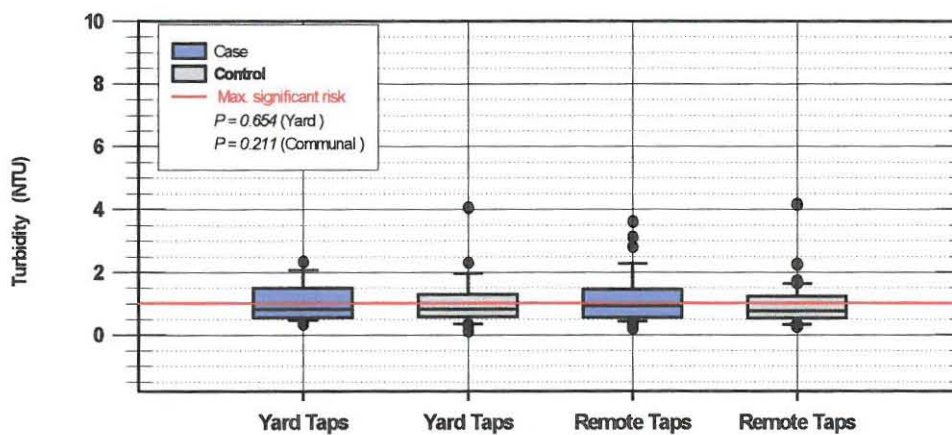


FIGURE 3.3.2: Turbidity in undisturbed container water used by cases and controls

Figure 3.3.2 illustrates the turbidity level in the undisturbed water quality used by cases and controls.

The results indicated that the undisturbed container water quality used by cases and controls were within the limit for *significant risk* (0-5 NTU) proposed by the DWAF (1996).

3.3.3 TOTAL COLIFORMS

Table 3.3.3 (a) shows that the difference in water quality in undisturbed container water quality from the yard taps and communal taps used by cases and controls was not great enough to exclude the possibility that the difference was due to random sampling variability.

The results indicated that there was no statistical significant difference between the sets of water. However, differences were observed in the undisturbed water quality fetched from yard taps and communal taps used by cases, with water from remote taps having higher total coliform number than from yard taps (Figure 3.3.3 a).

Table 3.3.3 (a): Total coliforms in undisturbed container water quality used by case and controls.

| | Cases | Controls | Comparing groups for supply types |
|---|--|--|--|
| Yard Taps | n = 29 Geometric Mean = 16 Min = 0 Max = 3.34×10^3 (log-transformed data) Median = 1.11 25 th Percentile limit = 0 75 th Percentile limit = 2.33 95 % Confidence Intervals = ± 0.48 | n = 25 Geometric Mean = 46 Min = 0 Max = 7.02×10^4 (log-transformed data) Median = 1.34 25 th Percentile limit = 0 75 th Percentile limit = 2.76 95 % Confidence Intervals = ± 0.59 | No significant difference $P = 0.256$ H₀ accepted Normality: Failed ($P \leq 0.001$) Mann-Whitney Rank Sum |
| Communal Taps | n = 43 Geometric Mean = 207 Min = 0 Max = 6.18×10^5 (log-transformed data) Median = 2.04 25 th Percentile limit = 0.63 75 th Percentile limit = 3.82 95 % Confidence Intervals = ± 0.57 | n = 51 Geometric Mean = 188 Min = 0 Max = 3.18×10^5 (log-transformed data) Median = 2.35 25 th Percentile limit = 1.140 75 th Percentile limit = 3.172 95 % Confidence Intervals = ± 0.42 | No significant difference $P = 0.906$ H₀ accepted Normality: Passed ($P = 0.09$) Variance: Passed ($P = 0.120$) Mann-Whitney Rank Sum |
| Comparing supply type for groups | Significant difference $P = 0.012$ H₀ rejected Normality: Failed ($P \leq 0.001$) Mann-Whitney Rank Sum | No significant difference $P = 0.105$ H₀ accepted Normality: Passed ($P > 0.200$) Variance: Passed ($P = 0.774$) Mann-Whitney Rank Sum | |

Figure 3.3.3 illustrates the log-transformed results of the undisturbed container water quality used by cases and controls.

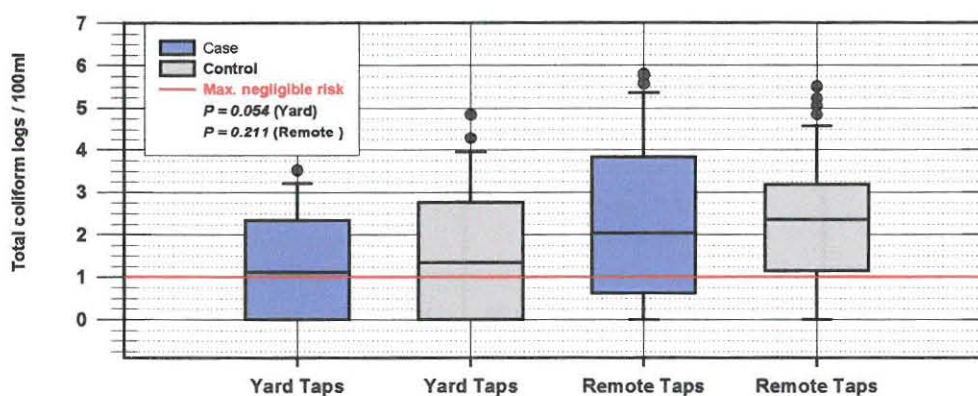


FIGURE 3.3.3: Total coliform In undisturbed container water used by cases and controls

The results indicated that although there was no statistically significant difference observed in undisturbed water quality fetched from the yard taps and communal taps used by both cases and controls, both groups' water quality did not comply with the *negligible risk* (10 organisms / 100ml) of microbial infection limits proposed by the DWAF (1996) as well as the Assessment Guide: *Quality of Domestic Water Supplies* (1998) (0-5 organisms / 100ml). The median for total coliforms in the communal taps was slightly higher than that of the yard taps' water quality used by the cases.

Distance was investigated to determine statistical significant differences in water quality by comparing distances the case and control groups travelled to fetch water.

Table 3.3.3 (b): Effect of distance on undisturbed water quality used by cases and controls

| Distance in meters | Cases | Controls | Comparing groups for supply types |
|-----------------------------------|---|--|---|
| Yard Taps Max 10 m distance | n = 29 Geometric Mean = 16 Min = 0 Max = 3.34×10^3 (log-transformed data) Median = 1.11 25 th Percentile limit = 0 75 th Percentile limit = 2.33 95 % Confidence Intervals = ± 0.48 | n = 25 Geometric Mean = 46 Min = 0 Max = 7.02×10^4 (log-transformed data) Median = 1.34 25 th Percentile limit = 0 75 th Percentile limit = 2.76 95 % Confidence Intervals = ± 0.59 | No significant difference $P = 0.256$ H₀ accepted Normality: Failed ($P \leq 0.001$) Mann-Whitney Rank Sum |
| Communal taps >10≤100 | n = 25 Geometric Mean = 786 Min = 0 Max = 6.18×10^5 (log-transformed data) Median = 3.00 25 th Percentile limit = 1.60 75 th Percentile limit = 4.74 95 % Confidence Intervals = ± 0.75 | n = 35 Geometric Mean = 157 Min = 0 Max = 3.18×10^5 (log-transformed data) Median = 2.34 25 th Percentile limit = 1.11 75 th Percentile limit = 3.14 95 % Confidence Intervals = ± 0.51 | No significant difference $P = 0.150$ H₀ accepted Normality: Passed ($P > 0.200$) Variance: Passed ($P = 0.220$) Mann-Whitney Rank Sum |
| Communal taps >100 | n = 18 Geometric Mean = 32 Min = 0 Max = 5.94×10^5 (log-transformed data) Median = 1.25 25 th Percentile limit = 0 75 th Percentile limit = 2.04 95 % Confidence Intervals = ± 0.74 | n = 17 Geometric Mean = 278 Min = 0 Max = 1.14×10^5 (log-transformed data) Median = 2.43 25 th Percentile limit = 1.438 75 th Percentile limit = 3.32 95 % Confidence Intervals = ± 0.69 | No significant difference $P = 0.056$ H₀ accepted Normality: Passed ($P > 0.200$) Variance: Passed ($P = 0.813$) Mann-Whitney Rank Sum |
| | Significant difference $P = \leq 0.001$ H₀ rejected Normality: Passed ($P = 0.046$) Variance: Passed ($P = 0.170$) Kruskal-Wallis One Way ANOVA | No significant difference $P = 0.341$ H₀ accepted Normality: Passed ($P = 0.105$) Variance: Passed ($P = 0.953$) Kruskal-Wallis One Way ANOVA | |

The Tukey multiple comparison identify the different variable/s amongst the experimental groups were different – a feature that the Kruskal-Wallis ANOVA test does not offer.

Table 3.3.3 (c): Results of the Tukey multiple comparison tests for the supply types.

| | Difference of the Means | q | $P < 0.05?$ |
|--|-------------------------|------|-------------|
| Remote Case >10<100 vs. Yard Case | 1.68 | 5.48 | Yes |
| Remote Case >10<100 vs. Remote Case >100 | 1.37 | 3.94 | Yes |
| Remote Case >100 vs. Yard Case | 0.31 | 0.92 | No |

The difference of the Means is a gauge of the size of the difference the 3 groups compared. The larger the figure, the larger the span of differences (Remote Case >10<100m versus the other two distances people have to travel to fetch water).

The q test statistic indicates the number of means spanned. The larger the values of q , the more acceptable the conclusion that the difference of two or more groups being compared is statistically significant.

Statistical significant differences were found in the distance (Remote Case >10<100m versus Yard Case) and (Remote Case >10m<100m versus Remote Case>100m). Although the differences between the two groups were not prominent, statistically there was a significant difference. No significant difference was found in the distance Remote Case >100m versus Yard Case.

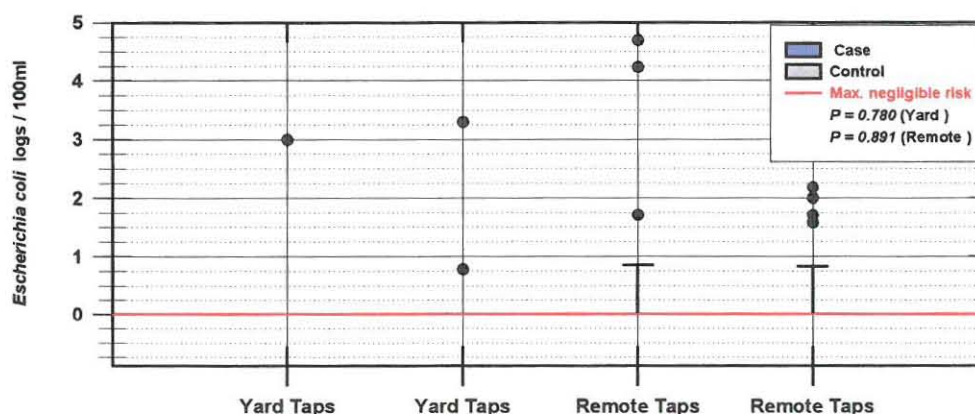
The results in Table 3.3.3 (c) show that the Remote Case >10m<100m distance was involved in both comparisons that showed the significant differences. The Remote Case >100 m comparison showed no significant difference of their means. Therefore it can be concluded that 10 and 100m distance category is the one differs from the other two distances. The reasons for the differences in the distances will be discussed in Chapter 4.

3.3.4 *Escherichia coli*

Figure 3.3.4 and Table 3.3.4 shows the levels of *Escherichia coli* in undisturbed container water used by cases and controls.

Table 3.3.4: *Escherichia coli* lev water used by cases and controls.

| | Cases | Controls | Comparing groups for supply types |
|---|---|---|--|
| Yard Taps | n = 29 Geometric Mean = 1 Min = 0 Max = 971 (log-transformed data) Median = 0 25 th Percentile limit = 0 75 th Percentile limit = 0 95 % Confidence Intervals = ± 0.20 | n = 25 Geometric Mean = 1 Min = 0 Max = 1.95×10^3 (log-transformed data) Median = 0 25 th Percentile limit = 0 75 th Percentile limit = 0 95 % Confidence Intervals = ± 0.26 | No significant difference $P = 0.780$ H ₀ accepted Normality: Failed ($P \leq 0.001$) Mann-Whitney Rank Sum |
| Communal Taps | n = 43 Geometric Mean = 2 Min = 0 Max = 4.87×10^4 (log-transformed data) Median = 0 25 th Percentile limit = 0 75 th Percentile limit = 0 95 % Confidence Intervals = ± 0.30 | n = 51 Geometric Mean = 2 Min = 0 Max = 146 (log-transformed data) Median = 0 25 th Percentile limit = 0 75 th Percentile limit = 0 95 % Confidence Intervals = ± 0.15 | No significant difference $P = 0.891$ H ₀ accepted Normality: Failed ($P \leq 0.001$) Mann-Whitney Rank Sum |
| Comparing supply type for groups | No significant difference $P = 0.226$ H ₀ accepted Normality: Failed ($P \leq 0.001$) Mann-Whitney Rank Sum | No significant difference $P = 0.698$ H ₀ accepted Normality: Failed ($P \leq 0.001$) Mann-Whitney Rank Sum | |


 FIGURE 3.3.4: *Escherichia coli* in undisturbed container water used by cases and controls

Undisturbed container water quality fetched from the yard and communal taps used by cases and controls did not comply with the *negligible risk* (zero organism / 100ml) limits proposed by the DWAF (1993). Although the median values indicate a zero



presence, the geometric r
intermittently found.

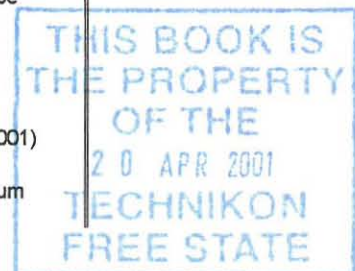
indicated that *Escherichia coli* was

3.3.5 *Clostridium perfringens*

The difference in water quality in the undisturbed water quality used by cases and controls was not great enough to exclude the possibility that the difference was due to random sampling variability. The results implied that there was no statistically significant difference between the undisturbed water quality fetched from the yard and communal taps ($P = 0.638$ and $P = 0.2000$ respectively).

Table 3.3.5: *Clostridium perfringens* levels in the undisturbed water used by cases and controls.

| | Cases | Controls | Comparing groups for supply types |
|---|--|---|--|
| Yard Taps | n = 29 Geometric Mean = 1 Min = 0 Max = 1.54 (log-transformed data) Median = 0 25 th Percentile limit = 0 75 th Percentile limit = 0.02 95 % Confidence Intervals = ±0.02 | n = 25 Geometric Mean = 1 Min = 0 Max = 5 (log-transformed data) Median = 0 25 th Percentile limit = 0 75 th Percentile limit = 0.11 95 % Confidence Intervals = ±0.08 | No significant difference $P = 0.638$ H₀ accepted Normality: Failed ($P \leq 0.001$) Mann-Whitney Rank Sum |
| Communal Taps | n = 43 Geometric Mean = 2 Min = 0 Max = 43 (log-transformed data) Median = 0 25 th Percentile limit = 0 75 th Percentile limit = 0.13 95 % Confidence Intervals = ±0.11 | n = 51 Geometric Mean = 1 Min = 0 Max = 4 (log-transformed data) Median = 0 25 th Percentile limit = 0 75 th Percentile limit = 0.10 95 % Confidence Intervals = ±0.04 | No significant difference $P = 0.200$ H₀ accepted Normality: Failed ($P \leq 0.001$) Mann-Whitney Rank Sum |
| Comparing supply type for groups | No significant difference $P = 0.107$ H₀ accepted Normality: Failed ($P \leq 0.001$) Mann-Whitney Rank Sum | No significant difference $P = 0.947$ H₀ accepted Normality: Failed ($P \leq 0.001$) Mann-Whitney Rank Sum | |



The results indicated that the median values of the two types of water posed an *insignificant risk* (1 organism / 100ml) of infection proposed by the *Water Quality Criteria in South Africa* (Aucamp and Vivier, 1990).

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The results further indicated that with the groups were within the limits in terms of *Water Quality Criteria in South Africa (1990)*.

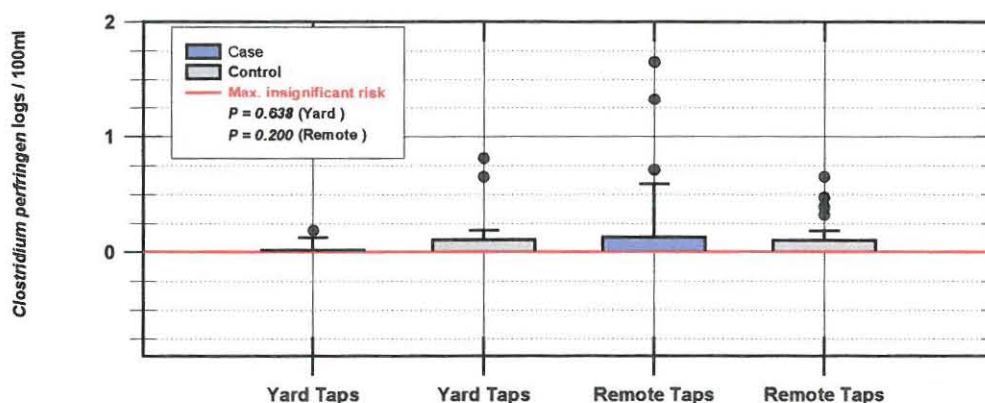


FIGURE 3.3.5: *Clostridium perfringens* in undisturbed container water used by case and controls

3.4 MIXED SUSPENSION CONTAINER WATER QUALITY: CASES AND CONTROLS

The brushing technique was applied to container sidewalls to suspend biofilm and water samples were collected from the water used by both cases and controls.

The emphasis in this section is the hygienic water quality after the suspension of biofilm.

3.4.1 HETEROTROPHIC BACTERIA

The data from both case and control groups are shown in Table 3.4.1. There was no statistically significant difference in the “mixed suspension container” water quality used by both groups based on the presence of heterotrophic bacteria.

Figure 3.4.1 shows that the median levels for heterotrophic bacteria in both water qualities were far above the *negligible risk* (≤ 100 organisms / 1ml) limits proposed by the DWAF (1996). However, the occurrence of heterotrophic bacteria in the mixed suspension container water used by cases was generally higher than in the mixed suspension container water used by control group in yard taps and communal taps.

Table 3.4.1: Levels of heterotroph and suspension container water used by cases and controls.

| | Cases | Controls | Comparing groups for supply types |
|---|---|---|---|
| Yard Taps | n = 29 Geometric Mean = 8.81×10^5 Min = 400 Max = 8.43×10^7 (log-transformed data) Median = 6.33 25 th Percentile limit = 4.61 75 th Percentile limit = 7.25 95 % Confidence Intervals = ± 0.53 | n = 25 Geometric Mean = 1.03×10^6 Min = 5×10^3 Max = 6.62×10^7 (log-transformed data) Median = 6.22 25 th Percentile limit = 4.91 75 th Percentile limit = 6.97 95 % Confidence Intervals = ± 0.47 | No significant difference P = 0.952 H ₀ accepted Normality: Passed (P = 0.10) Variance: Passed (P = 0.39) Mann-Whitney Rank Sum |
| Communal Taps | n = 38 Geometric Mean = 9.81×10^5 Min = 3.22×10^3 Max = 1×10^8 (log-transformed data) Median = 6.22 25 th Percentile limit = 5.18 75 th Percentile limit = 6.99 95 % Confidence Intervals = ± 0.37 | n = 46 Geometric Mean = 2.89×10^5 Min = 0.67 Max = 9.23×10^7 (log-transformed data) Median = 5.60 25 th Percentile limit = 4.41 75 th Percentile limit = 7.05 95 % Confidence Intervals = ± 0.55 | No significant difference P = 0.348 H ₀ accepted Normality: Passed (P > 0.20) Variance: Passed (P = 0.04) Mann-Whitney Rank Sum |
| Comparing supply type for groups | No significant difference P = 0.960 H ₀ accepted Normality: Passed (P = 0.05) Variance: Passed (P = 0.27) Mann-Whitney Rank Sum | No significant difference P = 0.386 H ₀ accepted Normality: Passed (P = 0.18) Variance: Passed (P = 0.10) Mann-Whitney Rank Sum | |

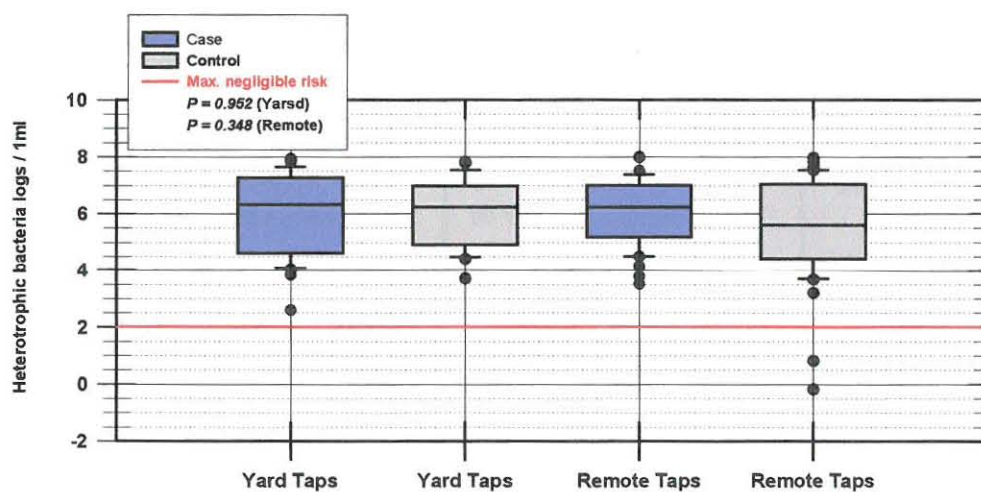


FIGURE 3.4.1: Heterotrophic bacteria in mixed suspension container water used by cases and controls

3.4.2 TURBIDITY

Turbidity levels were determined in mixed suspension container water quality from the yard taps and communal taps used by cases and controls.

The graph shows a high median value in the mixed suspension container water in the yard taps and communal taps used by cases as compared to the controls.

Both data sets did not comply with the *significant risk* (0-1 NTU) limits proposed by the DWAF (1996) but no statistical significant differences between either cases and controls or yard and communal taps were observed.

The results from both sets are shown in Table 3.4.2. The mean values were used to compare the difference in water quality after suspending biofilm.

Table 3.4.2: Turbidity levels in mixed suspension container water used by cases and controls.

| | Cases | Controls | Comparing groups for supply types |
|---|--|--|---|
| Yard Taps | <p>n = 29</p> <p>Arithmetic Mean = 3</p> <p>Min = 0.41</p> <p>Max = 20</p> <p>Median = 2.04</p> <p>25th Percentile limit = 1.15</p> <p>75th Percentile limit = 2.69</p> <p>95 % Confidence Intervals = ± 1.3</p> | <p>n = 25</p> <p>Arithmetic Mean = 3</p> <p>Min = 0.34</p> <p>Max = 16</p> <p>Median = 1.82</p> <p>25th Percentile limit = 1.44</p> <p>75th Percentile limit = 3.30</p> <p>95 % Confidence Intervals = ± 1.5</p> | <p>No significant difference</p> <p>$P = 0.931$</p> <p>H₀ accepted</p> <p>Normality: Failed ($P \leq 0.001$)</p> <p>Mann-Whitney Rank Sum</p> |
| Communal Taps | <p>n = 40</p> <p>Arithmetic Mean = 5</p> <p>Min = 0.35</p> <p>Max = 30</p> <p>Median = 2.48</p> <p>25th Percentile limit = 1.42</p> <p>75th Percentile limit = 5.46</p> <p>95 % Confidence Intervals = ± 2.2</p> | <p>n = 46</p> <p>Arithmetic Mean = 2</p> <p>Min = 0.36</p> <p>Max = 14</p> <p>Median = 1.87</p> <p>25th Percentile limit = 1.41</p> <p>75th Percentile limit = 2.65</p> <p>95 % Confidence Intervals = ± 0.7</p> | <p>No significant difference</p> <p>$P = 0.091$</p> <p>H₀ accepted</p> <p>Normality: Failed ($P \leq 0.001$)</p> <p>Mann-Whitney Rank Sum</p> |
| Comparing supply type for groups | <p>No significant difference</p> <p>$P = 0.129$</p> <p>H₀ accepted</p> <p>Normality: Failed ($P \leq 0.001$)</p> <p>Mann-Whitney Rank Sum</p> | <p>No significant difference</p> <p>$P = 0.933$</p> <p>H₀ accepted</p> <p>Normality: Failed ($P \leq 0.001$)</p> <p>Mann-Whitney Rank Sum</p> | |

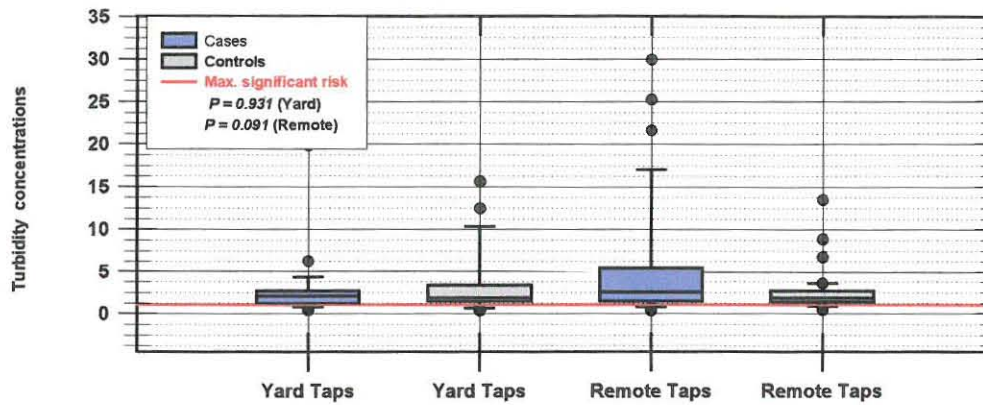


FIGURE 3.4.2: Turbidity in mixed suspension container water used by cases and controls

3.4.3 TOTAL COLIFORMS

The differences in the water quality used by cases and controls were not great enough to exclude the possibility that the difference was due to random sampling variability (Table 3.4.3 (a)).

Table 3.4.3 (a): Total coliforms in mixed suspension container water used by cases and controls.

| | Cases | Controls | Comparing groups for supply types |
|---|--|--|---|
| Yard Taps | n = 29 Geometric Mean = 66 Min = 0 Max = 5.91×10^5 (log-transformed data) Median = 1.49 25 th Percentile limit = 0 75 th Percentile limit = 3.31 95 % Confidence Intervals = ± 0.60 | n = 25 Geometric Mean = 90 Min = 0 Max = 1.60×10^5 (log-transformed data) Median = 1.77 25 th Percentile limit = 0.78 75 th Percentile limit = 2.92 95 % Confidence Intervals = ± 0.62 | No significant difference $P = 0.742$ H₀ accepted Normality: Passed ($P = 0.02$) Variance: Passed ($P = 0.51$) Mann-Whitney Rank Sum |
| Communal Taps | n = 43 Geometric Mean = 480 Min = 0 Max = 7.20×10^5 (log-transformed data) Median = 2.09 25 th Percentile limit = 1.49 75 th Percentile limit = 4.15 95 % Confidence Intervals = ± 0.52 | n = 51 Geometric Mean = 383 Min = 0 Max = 3.90×10^5 (log-transformed data) Median = 2.73 25 th Percentile limit = 1.42 75 th Percentile limit = 3.42 95 % Confidence Intervals = ± 0.39 | No significant difference $P = 0.970$ H₀ accepted Normality: Passed ($P > 0.20$) Variance: Passed ($P = 0.13$) Mann-Whitney Rank Sum |
| Comparing supply type for groups | Significant difference $P = 0.04$ H₀ rejected Normality: Failed ($P = 0.029$) Variance: Passed ($P = 0.760$) Mann-Whitney Rank Sum | No significant difference $P = 0.076$ H₀ accepted Normality: Passed ($P > 0.200$) Variance: Passed ($P = 0.774$) Mann-Whitney Rank Sum | |

The results indicated that there was a statistically significant difference in water quality fetched from the yard taps and communal taps used by both cases and controls. The only significant difference was between the yard and communal taps used by the cases.

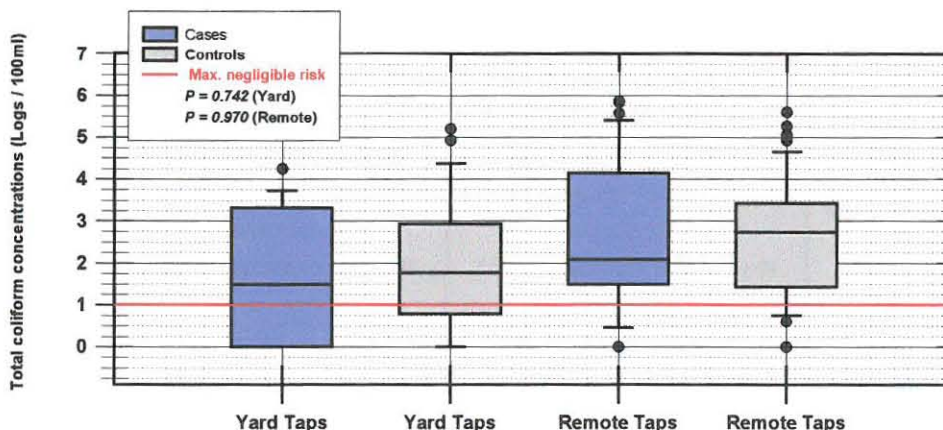


FIGURE 3.4.3: Total coliform in mixed suspension container water used by cases and controls

Figure 3.4.3 shows that the results from both sets of data did not comply with the limits for *negligible risk* (10 organisms / 100ml) of microbial infection proposed by the DWAF (1996) as well as the Assessment Guide: *Quality of Domestic Water Supplies* (1998) (0-5 organisms / 100ml).

The effect of distance on water quality was investigated by comparing the distances the case and control groups had to travel to fetch water.

Table 3.4.3 (b): Total coliforms in mixed suspension container water used by cases and controls.

| Distance in metres | Cases | Controls | |
|-----------------------------------|---|--|---|
| Yard Taps Max 10 m distance | n = 29 Geometric Mean = 66 Min = 0 Max = 5.91 X 10 ⁵ (log-transformed data) Median = 1.49 25 th Percentile limit = 0 75 th Percentile limit = 3.31 95 % Confidence Intervals = ±0.60 | n = 25 Geometric Mean = 90 Min = 0 Max = 1.60 X 10 ⁵ (log-transformed data) Median = 1.77 25 th Percentile limit = 0.78 75 th Percentile limit = 2.92 95 % Confidence Intervals = ±0.62 | No significant difference P = 0.742 H ₀ accepted Normality: Passed (P = 0.02) Variance: Passed (P = 0.51) Mann-Whitney Rank Sum |



Table 3.4.3 (b) (continues): Total coliform suspension container water used by cases and controls.

| Distance in metres | Cases | Controls | |
|--------------------|---|---|--|
| >10≤100 | <p>n = 25</p> <p>Geometric Mean = 2.14×10^3</p> <p>Min = 0</p> <p>Max = 6.60×10^5</p> <p>(log-transformed data)</p> <p>Median = 3.18</p> <p>25th Percentile limit = 2.15</p> <p>75th Percentile limit = 4.79</p> <p>95 % Confidence Intervals = ± 0.65</p> | <p>n = 35</p> <p>Geometric Mean = 255</p> <p>Min = 0</p> <p>Max = 3.90×10^5</p> <p>(log-transformed data)</p> <p>Median = 2.50</p> <p>25th Percentile limit = 1.29</p> <p>75th Percentile limit = 3.34</p> <p>95 % Confidence Intervals = ± 49</p> | <p>Significant difference</p> <p>$P = 0.043$</p> <p>H₀ rejected</p> <p>Normality: Passed</p> <p>($P > 0.200$)</p> <p>Variance: Passed ($P = 0.410$)</p> <p>Mann-Whitney Rank Sum</p> |
| >100 | <p>n = 18</p> <p>Geometric Mean = 81</p> <p>Min = 0</p> <p>Max = 7.20×10^5</p> <p>(log-transformed data)</p> <p>Median = 1.65</p> <p>25th Percentile limit = 1.28</p> <p>75th Percentile limit = 2.09</p> <p>95 % Confidence Intervals = ± 0.68</p> | <p>n = 17</p> <p>Geometric Mean = 857</p> <p>Min = 0</p> <p>Max = 1.12×10^5</p> <p>(log-transformed data)</p> <p>Median = 3.07</p> <p>25th Percentile limit = 2.26</p> <p>75th Percentile limit = 3.68</p> <p>95 % Confidence Intervals = ± 0.57</p> | <p>Significant difference</p> <p>$P = 0.009$</p> <p>H₀ rejected</p> <p>Normality: Passed</p> <p>($P > 0.200$)</p> <p>Variance: Passed ($P = 0.911$)</p> <p>Mann-Whitney Rank Sum</p> |
| | <p>Significant difference</p> <p>$P = 0.002$</p> <p>H₀ rejected</p> <p>Normality: Passed</p> <p>($P = 0.117$)</p> <p>Variance: Passed</p> <p>$P = 0.298$)</p> <p>Kruskal-Wallis One Way ANOVA</p> | <p>No significant difference</p> <p>$P = 0.110$</p> <p>H₀ accepted</p> <p>Normality: Passed</p> <p>($P > 0.200$)</p> <p>Variance: Passed</p> <p>($P = 0.569$)</p> <p>Kruskal-Wallis One Way ANOVA</p> | |

Table 3.4.3 (c): Results of the Tukey multiple comparison tests for the supply types for detecting total coliforms after suspending biofilm.

| | Difference of the Means | q | P < 0.05? |
|--|-------------------------|------|-----------|
| Remote Case >10<100 vs. Yard Case | 1.51 | 4.87 | Yes |
| Remote Case >10<100 vs. Remote Case >100 | 1.42 | 4.05 | Yes |
| Remote Case >100 vs. Yard Case | 0.09 | 0.26 | No |

The results in Table 3.4.3 (c) Remote Case >10<100m distance was involved in both comparisons that showed the significant differences. The Remote Case >10 m comparison showed no significant difference of their means. Therefore it can be concluded that 10 and 100m distances differs significantly from the other two distances. The reasons for the differences in the distances will be discussed in Chapter 4.

3.4.4 *Escherichia coli*

Escherichia coli levels in mixed suspension container water quality used by cases and controls were assessed. After suspending the biofilm through mixing, water samples were taken from the container water used by cases and controls. The difference in the mixed suspension container water samples observed was not great enough to exclude the possibility that the difference was due to random sampling variability. This implied that there was no statistically significant difference in the median value displayed by *Escherichia coli* levels ($P = 0.767$ - Yard and $P = 0.681$ - Communal taps respectively).

Table 3.4.4: *Escherichia coli* in mixed suspension container water used by cases and controls.

| | Cases | Controls | Comparing groups for supply types |
|----------------------|---|---|--|
| Yard Taps | n = 29 Geometric Mean = 2 Min = 0 Max = 1.46×10^3 (log-transformed data) Median = 0 25 th Percentile limit = 0 75 th Percentile limit = 0 95 % Confidence Intervals = ± 0.28 | n = 25 Geometric Mean = 2 Min = 0 Max = 5.11×10^3 (log-transformed data) Median = 0 25 th Percentile limit = 0 75 th Percentile limit = 0 95 % Confidence Intervals = ± 0.30 | No significant difference $P = 0.767$ H ₀ accepted Normality: Failed ($P \leq 0.001$) Mann-Whitney Rank Sum |
| Communal Taps | n = 43 Geometric Mean = 3 Min = 0 Max = 4.87×10^4 (log-transformed data) Median = 0 25 th Percentile limit = 0 75 th Percentile limit = 0 95 % Confidence Intervals = ± 0.35 | n = 51 Geometric Mean = 3 Min = 0 Max = 560 (log-transformed data) Median = 0 25 th Percentile limit = 0 75 th Percentile limit = 0.77 95 % Confidence Intervals = ± 0.21 | No significant difference $P = 0.681$ H ₀ accepted Normality: Failed ($P \leq 0.001$) Mann-Whitney Rank Sum |
| | No significant difference $P = 0.747$ H ₀ accepted Normality: Failed ($P \leq 0.001$) Mann-Whitney Rank Sum | No significant difference $P = 0.280$ H ₀ accepted Normality: Failed ($P \leq 0.001$) Mann-Whitney Rank Sum | |

Figure 3.4.4 illustrates the results of *Escherichia coli* levels in the mixed suspension container water quality used by cases and controls. The data represented in Figure 3.4.4 indicated that both sets of water did not comply with the negligible risk (0 organism / 100ml) limits proposed by the DWAF (1993) for drinking water. Although the median value indicates a zero presence, the geometric mean values indicated that *Escherichia coli* was intermittently found. There were no statistically significant differences between yard and communal taps or between cases and controls water qualities.

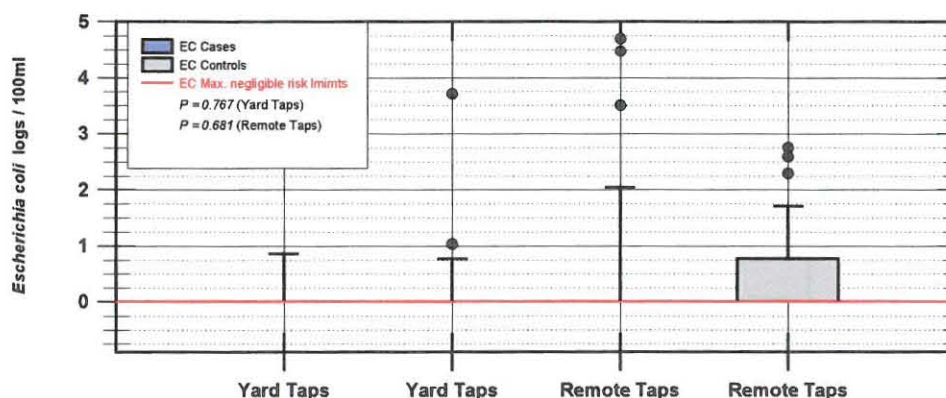


FIGURE 3.4.4: *Escherichia coli* in mixed suspension container water used by cases and controls

3.4.5 *Clostridium perfringens*

Clostridium perfringens level was assessed in the mixed suspension container water samples. Mixed suspension container water used by case and controls were analysed.

Table 3.4.5 results from both sets failed the Kolmogorov-Smirnov test for normality. The Mann-Whitney Sum Rank Test was used to test the differences in water quality for both groups.

The difference water quality samples fetched from the yard taps and communal taps were not great enough to exclude the possibility that the difference was due to random sampling variability. The results implied that there was no statistical significant difference between the two sets of data from both groups.

Table 3.4.5: *Clostridium perfringens* suspension container water used by cases and controls.

| | Cases | Controls | Comparing groups for supply types |
|----------------------|--|--|--|
| Yard Taps | n = 29 Geometric Mean = 2 Min = 0 Max = 10 (log-transformed data) Median = 0.10 25 th Percentile limit = 0 75 th Percentile limit = 0.29 95 % Confidence Intervals = ±0.09 | n = 25 Geometric Mean = 2 Min = 0 Max = 35 (log-transformed data) Median = 0 25 th Percentile limit = 0 75 th Percentile limit = 0.29 95 % Confidence Intervals = ±0.15 | No significant difference $P = 0.821$ H₀ accepted Normality: Failed ($P \leq 0.001$) Mann-Whitney Rank Sum |
| Communal Taps | n = 43 Geometric Mean = 2 Min = 0 Max = 43 (log-transformed data) Median = 0.13 25 th Percentile limit = 0 75 th Percentile limit = 0.55 95 % Confidence Intervals = ±0.11 | n = 51 Geometric Mean = 2 Min = 0 Max = 2.40×10^3 (log-transformed data) Median = 0.19 25 th Percentile limit = 0 75 th Percentile limit = 0.32 95 % Confidence Intervals = ±0.16 | No significant difference $P = 0.970$ H₀ accepted Normality: Failed ($P \leq 0.001$) Mann-Whitney Rank Sum |
| | No significant difference $P = 0.304$ H₀ accepted Normality: Failed ($P \leq 0.001$) Mann-Whitney Rank Sum | No significant difference $P = 0.257$ H₀ accepted Normality: Failed ($P \leq 0.001$) Mann-Whitney Rank Sum | |

Judging by Figure 3.4.5, the results indicated that both sets of data from the suspending biofilm on sidewalls container water did not comply with the limits for *insignificant risk* (1 organism / 100ml) proposed by the *Water Quality Criteria in South Africa* (Aucamp and Vivier, 1990). However, the results further indicated that both sets of data had similar levels of *Clostridium perfringens* except the communal taps used by cases which was slightly higher than the risk limits proposed (Aucamp and Vivier, 1990).

Although the median values indicated a zero presence, the geometric mean values indicated that *Clostridium perfringens* was intermittent found. No statistically significant differences in yard and communal taps observed in both case and control groups ($P = 0.821$ and $P = 0.970$ respectively).

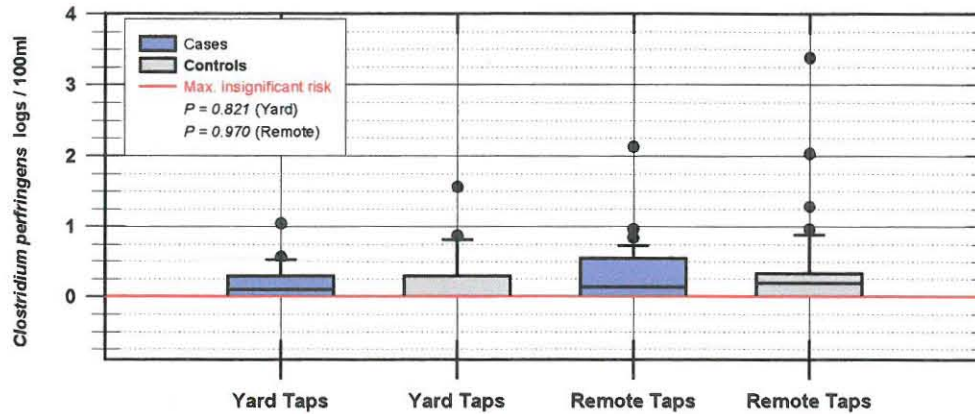


FIGURE 3.4.5: *Clostridium perfringens* in mixed suspension container water used by cases and controls

3.5 CONTAINER WATER QUALITY BEFORE AND AFTER SUSPENDING BIOFILM (CASES VS. CONTROLS)

The general water quality of container water used by both the case and control groups was compared in this section. The data for both groups included the data sets for water from the undisturbed water quality sample routine as well as data of the mixed suspension container water.

3.5.1 HETEROTROPHIC BACTERIA

The emphasis in this section is the comparison of the hygienic water quality indicated by heterotrophic bacteria of container-stored water used by cases and controls.

The data generated by both input groups are showed in Table 3.5.1. The results indicated that the difference in the water quality used by both groups was great enough to exclude that the difference was due to random sampling variability.

There was a statistically significant difference between the water quality in containers before and after suspending biofilm, the situation that was not observed in water quality before and after suspending biofilm in water in vertical data sets in Table 3.5.1 ($P \leq 0.001$). Heterotrophic bacteria levels were higher after suspending the biofilm.

Table 3.5.1: Heterotrophic bacteria before and after suspending biofilm.

| | Before | After | Comparing pairs for supply types |
|---|--|---|---|
| Cases | n = 70 Geometric Mean = 4.82×10^5 Min = 167 Max = 9.60×10^7 (log-transformed data) Median = 5.99 25 th Percentile limit = 4.12 75 th Percentile limit = 6.86 95 % Confidence Intervals = ± 0.33 | n = 70 Geometric Mean = 9.93×10^5 Min = 400 Max = 1×10^8 (log-transformed data) Median = 6.27 25 th Percentile limit = 5.02 75 th Percentile limit = 7.00 95 % Confidence Intervals = ± 0.30 | Significant difference $P \leq 0.001$ H ₀ rejected Normality: Failed ($P \leq 0.001$) Wilcoxon Signed Rank |
| Controls | n = 70 Geometric Mean = 3.99×10^5 Min = 256 Max = 5.42×10^7 (log-transformed data) Median = 5.44 25 th Percentile limit = 4.45 75 th Percentile limit = 6.89 95 % Confidence Intervals = ± 0.34 | n = 70 Geometric Mean = 7.37×10^5 Min = 1.59×10^3 Max = 9.23×10^7 (log-transformed data) Median = 5.85 25 th Percentile limit = 4.86 75 th Percentile limit = 6.94 95 % Confidence Intervals = ± 0.30 | Significant difference $P \leq 0.001$ H ₀ rejected Normality: Failed ($P \leq 0.001$) Wilcoxon Signed Rank |
| Comparing pairs for supply types | No significant difference $P = 0.777$ H ₀ accepted Normality: Failed ($P \leq 0.001$) Mann-Whitney Rank Sum Test | No significant difference $P = 0.532$ H ₀ accepted Normality: Failed ($P = 0.005$) Mann-Whitney Rank Sum Test | |

Figure 3.5.1 results shows that the quality of water did not comply with the limits for negligible risk ($\leq 100 / 100\text{ml}$) of infection to consumers proposed by the DWAF (1996).

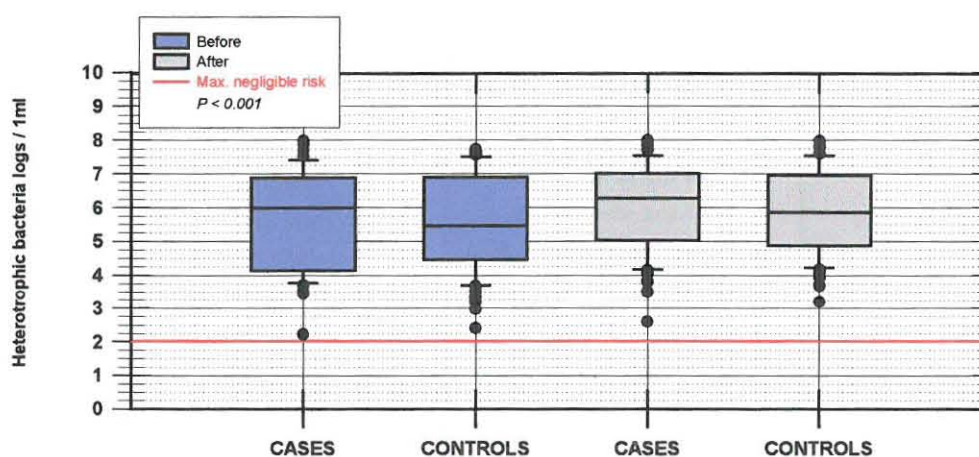


FIGURE 3.5.1: Heterotrophic bacteria in container-stored water before and after suspending biofilm (cases and controls)

3.5.2 TURBIDITY

The clarity of the container-stored water used by cases and controls before and after suspending biofilm was assessed. Turbidity measurement was used to assess the clarity of the container water used by both groups.

The results from both sets of data are shown in Table 3.5.2. The mean values were used to compare the water quality differences in both inputs groups. The results indicated that the difference in the median values between the two groups was great enough to exclude the possibility that the difference was due to random sampling variability. This implied that there was a statistically significant difference in the clarity of water used by both groups when comparing data horizontally ($P < 0.001$), but there was no statistically significant difference in water quality before and after suspending biofilm when comparing data sets vertically ($P = 0.394$ – Before and $P = 0.454$ After).

Table 3.5.2: Turbidity in container water before and after suspending biofilm.

| | Before | After | Comparing pairs for supply types |
|---|---|---|--|
| Cases | <p>n = 73</p> <p>Arithmetic Mean = 1</p> <p>Min = 0.19</p> <p>Max = 4</p> <p>Median = 0.87</p> <p>25th Percentile limit = 0.53</p> <p>75th Percentile limit = 1.39</p> <p>95 % Confidence Intervals = ± 0.16</p> | <p>n = 73</p> <p>Arithmetic Mean = 4</p> <p>Min = 0.31</p> <p>Max = 30</p> <p>Median = 2.06</p> <p>25th Percentile limit = 1.26</p> <p>75th Percentile limit = 3.67</p> <p>95 % Confidence Intervals = ± 1.34</p> | <p>Significant difference</p> <p>$P \leq 0.001$</p> <p>H₀ rejected</p> <p>Normality: Failed ($P \leq 0.001$)</p> <p>Wilcoxon Signed Rank</p> |
| Controls | <p>n = 72</p> <p>Arithmetic Mean = 0.98</p> <p>Min = 0.11</p> <p>Max = 4.2</p> <p>Median = 0.75</p> <p>25th Percentile limit = 0.53</p> <p>75th Percentile limit = 1.22</p> <p>95 % Confidence Intervals = ± 0.17</p> | <p>n = 72</p> <p>Arithmetic Mean = 3</p> <p>Min = 0.34</p> <p>Max = 16</p> <p>Median = 1.85</p> <p>25th Percentile limit = 1.38</p> <p>75th Percentile limit = 2.68</p> <p>95 % Confidence Intervals = ± 0.67</p> | <p>Significant difference</p> <p>$P \leq 0.001$</p> <p>H₀ rejected</p> <p>Normality: Failed ($P \leq 0.001$)</p> <p>Wilcoxon Signed Rank</p> |
| Comparing pairs for supply types | <p>No significant difference</p> <p>$P = 0.394$</p> <p>H₀ accepted</p> <p>Normality: Failed ($P \leq 0.001$)</p> <p>Mann-Whitney Rank Sum</p> | <p>No significant difference</p> <p>$P = 0.454$</p> <p>H₀ accepted</p> <p>Normality: Failed ($P \leq 0.001$)</p> <p>Mann-Whitney Rank Sum</p> | |

Figure 3.5.2 shows that the median level for turbidity before suspending biofilm in water quality used by both the cases and controls was within the risk limits (0-1

NTU), while both groups after suspending biofilm was slightly above the limit for significant risk proposed by the DWAF (1996).

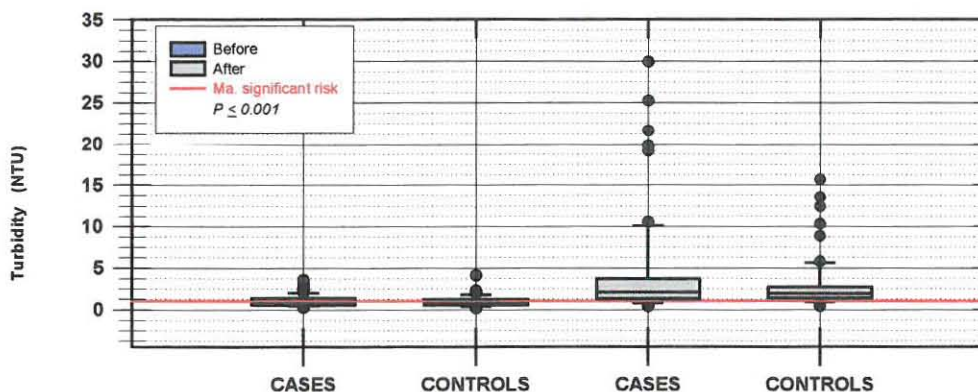


FIGURE 3.5.2: Turbidity in container-stored water before and after suspending biofilm (cases and controls)

3.5.3 TOTAL COLIFORMS

Total coliform levels in the container-stored water used by the case and controls were assessed (Table 3.5.3).

Table 3.5.3: Total coliforms in container water before and after suspending biofilm.

| | Before | After | Comparing pairs for supply types |
|---|--|--|---|
| Cases | n = 75 Geometric Mean = 75 Min = 0 Max = 6.18×10^5 (log-transformed data) Median = 1.61 25 th Percentile limit = 0 75 th Percentile limit = 3.06 95 % Confidence Intervals = ± 0.39 | n = 75 Geometric Mean = 230 Min = 0 Max = 7.20×10^5 (log-transformed data) Median = 2.20 25 th Percentile limit = 1.06 75 th Percentile limit = 3.71 95 % Confidence Intervals = ± 0.39 | Significant difference $P \leq 0.001$ H₀ rejected Normality: Failed ($P \leq 0.001$) Wilcoxon Signed Rank |
| Controls | n = 75 Geometric Mean = 113 Min = 0 Max = 3.18×10^5 (log-transformed data) Median = 1.75 25 th Percentile limit = 1.04 75 th Percentile limit = 3.15 95 % Confidence Intervals = ± 0.34 | n = 75 Geometric Mean = 231 Min = 0 Max = 3.90×10^5 (log-transformed data) Median = 2.26 25 th Percentile limit = 1.34 75 th Percentile limit = 3.37 95 % Confidence Intervals = ± 0.34 | Significant difference $P \leq 0.001$ H₀ rejected Normality: Failed ($P \leq 0.001$) Wilcoxon Signed Rank |
| Comparing pairs for supply types | Significant difference $P = 0.354$ H₀ rejected Normality: Failed ($P \leq 0.001$) Mann-Whitney Rank Sum | No significant difference $P = 0.878$ H₀ accepted Normality: Passed ($P = 0.034$) Variance: Passed ($P = 0.219$) Mann-Whitney Rank Sum | |

The difference in the water quality groups was great enough to exclude the possibility that the difference was due to random sampling variability. There was no statistically significant difference between total coliforms level of container-stored water used by cases and controls. A difference occurred after suspending the biofilm, although this was not statistically significant.

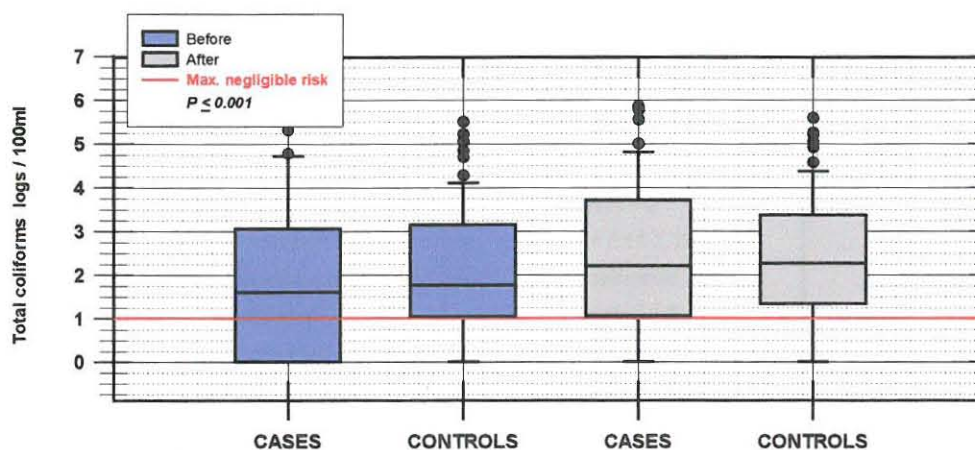


FIGURE 3.5.3 : Total coliform in container-stored water before and after suspending biofilm

Figure 3.5.3 illustrates the log-transformed results of the container-stored water used by selected cases and controls. The total coliform values had been adapted to exclude false positives.

The results below indicated that the median levels for total coliforms in both the water quality in stored container water used by cases and controls did not comply with limits for the *negligible risk* (10 org /100ml) of microbial infection proposed by the DWAF (1996) as well as the Assessment Guide: *Quality of Domestic Water Supplies* (1998) (0-5 org / 100ml). More total coliform counts were observed after suspension of biofilm in the container water.

3.5.4 Escherichia coli

Escherichia coli was used to indicate possible faecal pollution in container-stored water used by cases and controls before and after suspending biofilm.

The results indicated that the differences in the water quality used by both groups were greater enough to exclude the possibility that the difference was due to random sampling variability. There was a statistically significant difference between the water quality in containers before and after suspending biofilm ($P \leq 0.001$).



Table 3.5.4: *Escherichia coli* in c

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Technology, Free State

e and after suspending biofilm.

| | Before | After | Comparing pairs for supply types |
|---|--|--|---|
| Cases | n = 75 Geometric Mean = 2 Min = 0 Max = 4.87×10^4 (log-transformed data) Median = 0 25 th Percentile limit = 0 75 th Percentile limit = 0 95 % Confidence Intervals = ± 0.19 | n = 75 Geometric Mean = 2 Min = 0 Max = 4.87×10^4 (log-transformed data) Median = 0 25 th Percentile limit = 0 75 th Percentile limit = 0 95 % Confidence Intervals = ± 0.23 | Significant difference $P \leq 0.001$ H₀ rejected Normality: Failed ($P \leq 0.001$) Wilcoxon Signed Rank |
| Controls | n = 75 Geometric Mean = 2 Min = 0 Max = 1.95×10^3 (log-transformed data) Median = 0 25 th Percentile limit = 0 75 th Percentile limit = 0 95 % Confidence Intervals = ± 0.13 | n = 75 Geometric Mean = 2 Min = 0 Max = 5.11×10^3 (log-transformed data) Median = 0 25 th Percentile limit = 0 75 th Percentile limit = 0 95 % Confidence Intervals = ± 0.17 | Significant difference $P \leq 0.001$ H₀ rejected Normality: Failed ($P \leq 0.001$) Wilcoxon Signed Rank |
| Comparing pairs for supply types | No significant difference $P = 0.799$ H₀ accepted Normality: Failed ($P \leq 0.001$) Mann-Whitney Rank Sum | No significant difference $P = 0.829$ H₀ accepted Normality: Failed ($P \leq 0.001$) Mann-Whitney Rank Sum | |

The results indicated a presence of faecal pollution in the container water used by both groups.

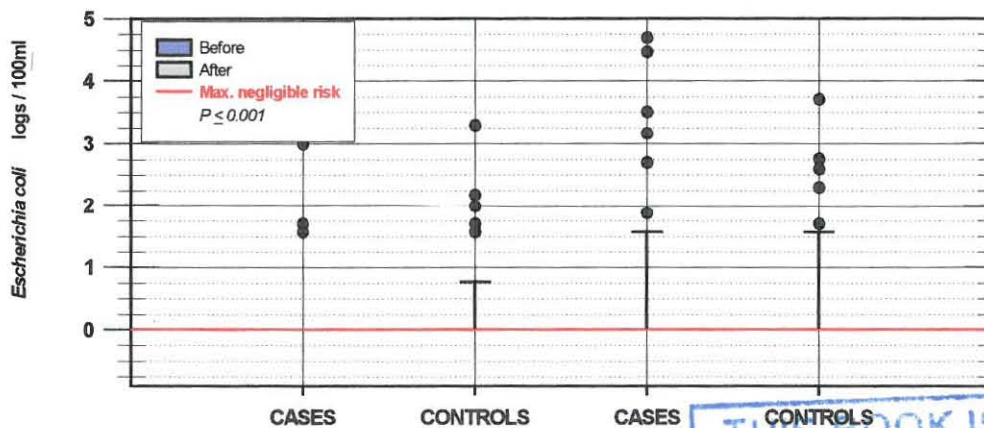
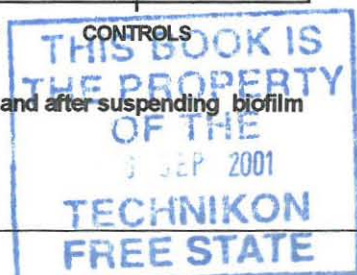


FIGURE 3.5.4: *Escherichia coli* in container-stored water before and after suspending biofilm



Both data sets had similar values for *Escherichia coli* levels (Figure 3.5.4), although the median values indicated a zero presence, the geometric mean value showed that *Escherichia coli* was intermittently found.

3.5.5 *Clostridium perfringens*

Clostridium perfringens were used as an indication of resistant faecal pollution in container-stored water used by cases and controls.

Table 3.5.5: *Clostridium perfringens* in container water before and after suspending biofilm.

| | Before | After | Comparing pairs for supply types |
|---|---|---|---|
| Cases | n = 75 Geometric Mean = 1 Min = 0 Max = 43 (log-transformed data) Median = 0 25 th Percentile limit = 0 75 th Percentile limit = 0.10 95 % Confidence Intervals = ±0.06 | n = 75 Geometric Mean = 2 Min = 0 Max = 133 (log-transformed data) Median = 1.34 25 th Percentile limit = 0 75 th Percentile limit = 0.42 95 % Confidence Intervals = ±0.08 | Significant difference $P \leq 0.001$ H₀ rejected Normality: Failed ($P \leq 0.001$) Wilcoxon Signed Rank |
| Controls | n = 75 Geometric Mean = 1 Min = 0 Max = 5 (log-transformed data) Median = 0 25 th Percentile limit = 0 75 th Percentile limit = 0.13 95 % Confidence Intervals = ±0.04 | n = 75 Geometric Mean = 2 Min = 0 Max = 35 (log-transformed data) Median = 0.13 25 th Percentile limit = 0 75 th Percentile limit = 0.28 95 % Confidence Intervals = ±0.07 | Significant difference $P \leq 0.001$ H₀ rejected Normality: Failed ($P \leq 0.001$) Wilcoxon Signed Rank |
| Comparing pairs for supply types | No significant difference $P = 0.761$ H₀ accepted Normality: Failed ($P \leq 0.001$) Mann-Whitney Rank Sum | No significant difference $P = 0.813$ H₀ accepted Normality: Failed ($P \leq 0.001$) Mann-Whitney Rank Sum | |

The results indicated that the difference in the water quality used by both groups was great enough to exclude the possibility that the difference was due to random sampling variability. There was a statistically significant difference between the water quality in containers before and after suspending biofilm ($P < 0.001$). There was no statistically significant difference between the water quality in containers before and after suspending biofilm in vertical data sets.

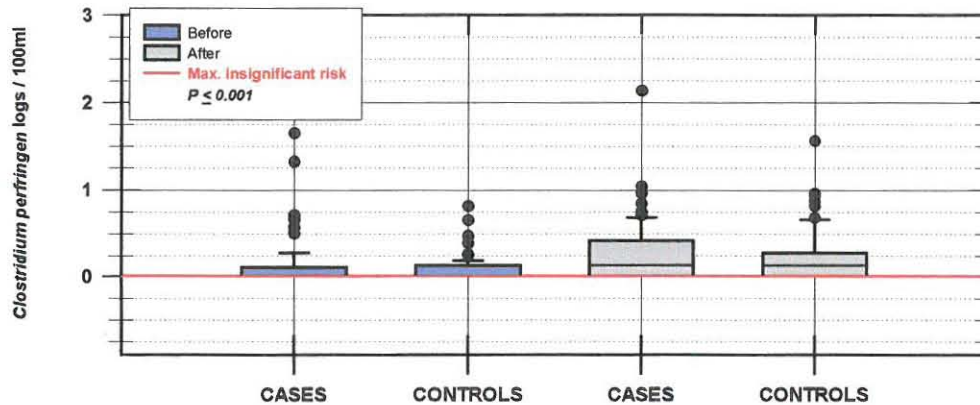


FIGURE 3.5.5: *Clostridium perfringens* in container-stored water before and after suspending biofilm

Figure 3.5.5 illustrates the log-transformed results of the *Clostridium perfringens*. The results shows that the median levels for *C. perfringens* in container water used by controls were lower than the limits for *insignificant risk* (1 organism / 100ml) in terms of *Water Quality Criteria* (Aucamp and Vivier, 1990). However, the occurrence of *Clostridium perfringens* in the water of both cases and controls after suspending biofilm was generally higher than in the water quality before suspending biofilm.

3.6 PLASTIC AND METAL CONTAINER WATER QUALITY

This section investigates whether container types had any effect on the water quality. Water quality data for plastic and metal containers was analysed.

The data in both sets from Table 3.6.1 failed the Kolmogorov-Smirnov test for normality ($P \leq 0.001$). The results indicated that the difference in the median value between the water qualities in plastic and metal container was not great enough to exclude the possibility that the difference was due to sampling variability. This implied that there was no statistically significant difference in the water quality stored in plastic and metal containers ($P \leq 0.001$).

Turbidity levels in both the plastic and metal containers were above the *significant risk* limits according to the DWAF (1996). The water in the metal containers was more turbid than in the plastic containers. The results indicated that there was a statistically significant difference in the water quality in both the container materials ($P = 0.012$). The data for plastic and metal containers were showed in Table 3.6.1.

Table 3.6.1: Levels of all indicators in plastic and metal containers.

| | Plastic | Metal | Comparing groups for supply types |
|--|---|---|--|
| Heterotrophic bacteria / 100ml | n = 210 Geometric Mean = 6.07×10^5 Min = 167 Max = 9.73×10^7 (log-transformed data) Median = 5.98 25 th Percentile limit = 4.65 75 th Percentile limit = 6.88 95 % Confidence Intervals = ± 0.18 | n = 72 Geometric Mean = 5.06×10^5 Min = 256 Max = 1×10^8 (log-transformed data) Median = 5.61 25 th Percentile limit = 4.37 75 th Percentile limit = 7.00 95 % Confidence Intervals = ± 0.33 | No significant difference P = 0.723 H ₀ accepted Normality: Failed (P ≤ 0.001) Mann-Whitney Rank Sum |
| Turbidity NTU's | n = 218 Arithmetic Mean = 19 Min = 0.11 Max = 16 (log-transformed data) Median = 1.28 25 th Percentile limit = 0.69 75 th Percentile limit = 1.94 95 % Confidence Intervals = ± 0.21 | n = 72 Arithmetic Mean = 42 Min = 0.19 Max = 30 (log-transformed data) Median = 1.63 25 th Percentile limit = 0.87 75 th Percentile limit = 3.39 95 % Confidence Intervals = ± 1.44 | Significant difference P = 0.012 H ₀ rejected Normality: Failed (P ≤ 0.001) Mann-Whitney Rank Sum |
| Total coliforms / 100ml | n = 224 Geometric Mean = 132 Min = 0 Max = 6.60×10^5 (log-transformed data) Median = 1.80 25 th Percentile limit = 0.60 75 th Percentile limit = 3.28 95 % Confidence Intervals = ± 0.22 | n = 76 Geometric Mean = 220 Min = 0 Max = 7.20×10^5 (log-transformed data) Median = 2.11 25 th Percentile limit = 1.28 75 th Percentile limit = 3.31 95 % Confidence Intervals = ± 0.36 | No significant difference P = 0.268 H ₀ accepted Normality: Failed (P ≤ 0.001) Mann-Whitney Rank Sum |
| Escherichia coli / 100ml | n = 224 Geometric Mean = 2 Min = 0 Max = 2.92×10^4 (log-transformed data) Median = 0 25 th Percentile limit = 0 75 th Percentile limit = 0 95 % Confidence Intervals = ± 0.10 | n = 76 Geometric Mean = 3 Min = 0 Max = 4.87×10^4 (log-transformed data) Median = 0 25 th Percentile limit = 0 75 th Percentile limit = 0 95 % Confidence Intervals = ± 0.23 | No significant difference P = 0.092 H ₀ accepted Normality: Failed (P ≤ 0.001) Mann-Whitney Rank Sum |
| Clostridium perfringens / 100ml | n = 224 Geometric Mean = 1 Min = 0 Max = 133 (log-transformed data) Median = 0 25 th Percentile limit = 0 75 th Percentile limit = 0.19 95 % Confidence Intervals = ± 0.04 | n = 76 Geometric Mean = 2 Min = 0 Max = 1.32×10^4 (log-transformed data) Median = 0.13 25 th Percentile limit = 0 75 th Percentile limit = 0.53 95 % Confidence Intervals = ± 0.14 | Significant difference P = 0.010 H ₀ rejected Normality: Failed (P ≤ 0.001) Mann-Whitney Rank Sum |

In terms of *C. perfringens*, the plastic and metal containers starts to show a significant difference. Even though the metal containers shows a higher contamination than plastic containers, there was a statistically significant difference in the water quality in both container materials.

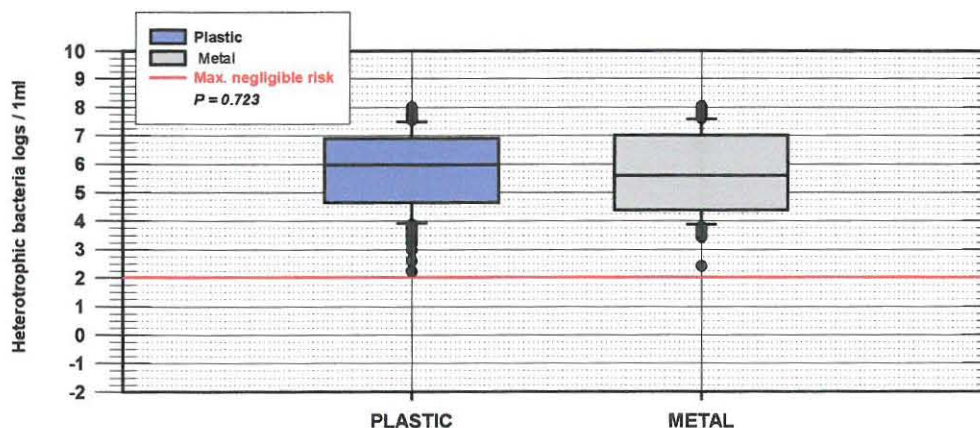


FIGURE 3.6.1 (a): Heterotrophic bacteria In plastic and metal containers

The results in Figure 3.6.1 (a) shows that the median values for heterotrophic bacteria in both groups were above the *negligible risk* (≤ 100 organisms / 100ml) limits proposed by the DWAF (1996). However, the occurrence of heterotrophic bacteria in the plastic container water was generally higher than the metal container water.

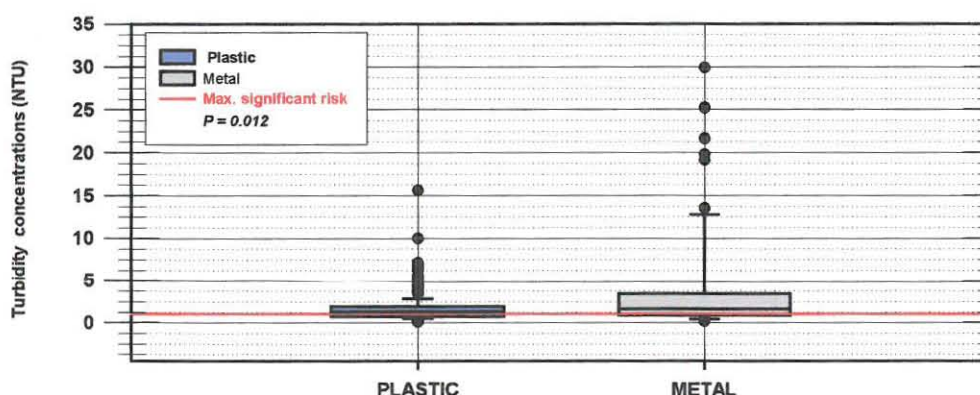


FIGURE 3.6.1 (b): Turbidity In plastic and metal containers

The results in Figure 3.6.1 (b) indicated a slightly high median value for turbidity in both the container types. Both data sets did not comply with the *significant risk* (0-1

NTU) limits proposed by the *however, turbidity levels in the metal container water were higher than in the plastic container water. There results indicated that there was a statistically significant difference in the water quality in both container materials (P = 0.012).*

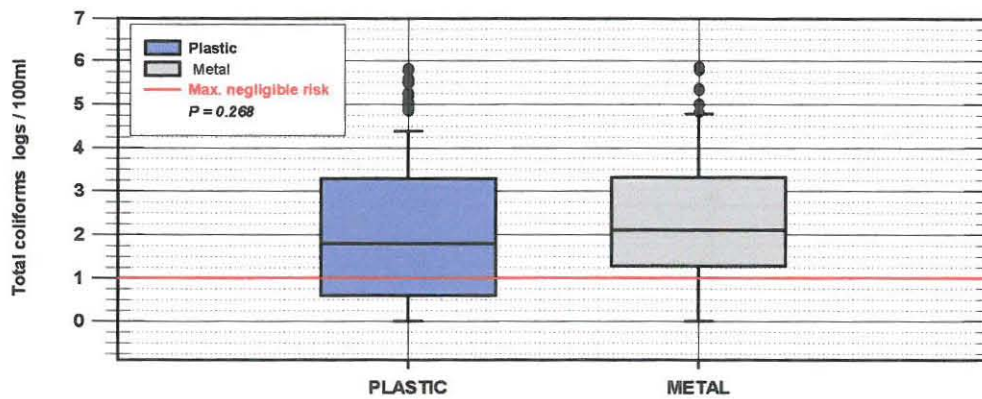


FIGURE 3.6.1 (c): Total coliform in plastic and metal container water

Figure 3.6.1 (c) indicated that both sets of data did not comply with the limits for *negligible risk* (10 organisms / 100ml) of microbial infection proposed by the DWAF (1996) as well as the Assessment Guide: *Quality of Domestic Water Supplies* (1998) (0-5 organisms / 100ml). Higher total coliform counts were observed in the metal containers than the plastic containers.

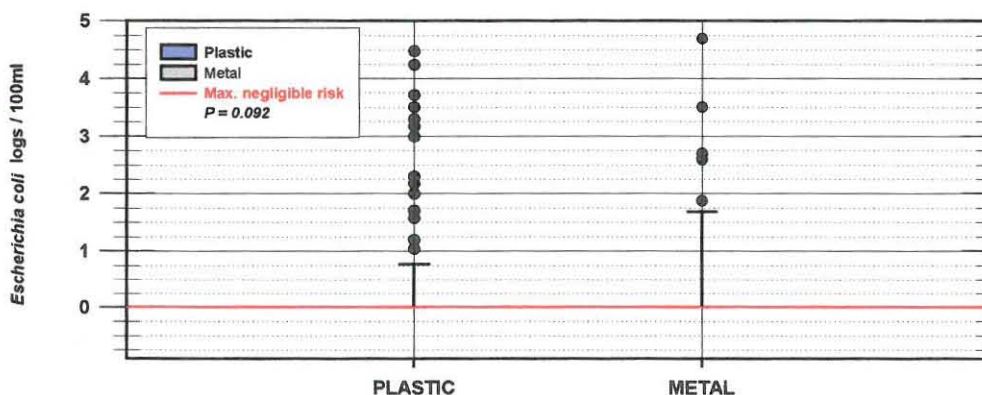


FIGURE 3.6.1 (d): *Escherichia coli* in plastic and metal container water

Figure 3.6.1 (d) illustrates the log-transformed results of the *E coli* in plastic and metal containers.

The data represented in Figure 3.6.1 (e) indicated that both sets of water did not comply with the *negligible risk* (zero organism / 100ml) limits proposed by the DWAF (1993) for drinking water quality. The results further indicated that the metal container seems to have a higher level of *Escherichia coli*.

Figure 3.6.1 (e) illustrates the log-transformed results of the *C. perfringens* in plastic and metal containers.

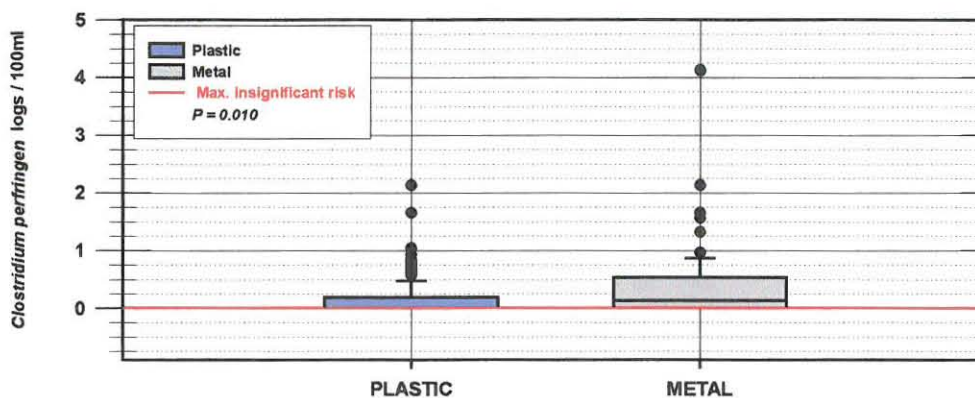


FIGURE 3.6.1 (e): *Clostridium perfringens* in plastic and metal container water

With regards to *C. perfringens* results (Figure 3.6.1 e) counts in the metal containers did not comply with the limits for *insignificant risk* (1 organism / 100ml) as proposed by the *Water Quality Criteria in South Africa* (Aucamp and Vivier, 1990). However, the results further indicated a statistically significant difference in the water quality used by both plastic and metal containers ($P = 0.001$).

CHAPTER 4

DISCUSSIONS

Chapter 4: DISCUSSIONS

This study assessed the microbiological and aesthetical quality of the water that the community consumed in order to describe any risk of microbiological infection these members might be exposed to. The risk of infection to consumers of the supply water was determined by comparing the water quality with various health-related water quality guidelines. The following guidelines were used:

- ◆ South African Water Quality Guidelines: Vol. 1: Domestic Water (Department of Water Affairs and Forestry (DWA), 1993; 1996).
- ◆ Proposed Water Quality Criteria in South Africa of the National Department of Health (Aucamp and Vivier, 1990).
- ◆ Water Research Commission: Assessment Guide: Quality of Domestic Water Supplies (WRC, 1998). Vol. 1, 2nd edition.

An observational cross-sectional study was conducted by Theron (2000) to determine the effects of various environmental health factors including water quality on human health in the same area. Nala and Jagals (1999) did a case-control follow-up study of the same households selected by Theron (2000).

In other related studies done by Jagals et al. (1997, 1999) it was found that the general microbiological quality of the municipal water supply to the community of Botshabelo area was within the acceptable limits in terms of the guidelines, but the quality of the supply water deteriorated rapidly once collected and stored in containers for domestic use. However, in this study, the supply water quality did not comply with the DWA (1996) for heterotrophic bacteria and total coliforms. However, *E. coli* and *C. perfringens* did comply with the DWA, (1993) and *Water Quality Criteria in South Africa* (Aucamp and Vivier, 1990). This water quality deteriorated to much more unacceptable levels with storage in containers.

4.1 AESTHETIC WATER QUALITY IN CONTAINERS

Seeing that the water quality deteriorated in containers, possible reasons for the deterioration were investigated. There were some indications of some type of biofilm forming on the sidewalls of the containers that could contribute to the deterioration of water. To confirm this, a simple technique was used by brushing the



inside walls of filled container

brush. This simulated the effect of the biofilm being loosened everytime an unwashed or unrinsed container was filled by a household member. The brushing technique merely accelerated this process and also maximized the contamination that could take place with filling as well as handling. By somehow loosening this biofilm from container walls and suspending the film particles in the container water and then measuring the aesthetical quality of the suspension, a conclusion could be reached whether the increased presence of suspended material in such water was due to biofilm forming in containers. The water quality in containers before and after suspending biofilm was assessed. The Wilcoxon Signed Rank test was applied to determine if there was any significant difference in the container water quality before and after suspending biofilm. It was found that there was indeed a statistically significant difference in turbidity after the suspension of biofilm in container water.

Biofilms form when bacteria adhere to surfaces in aqueous environments and begin to excrete a slimy, glue-like substance that can anchor them to all kinds of material – such as metals, plastics, soil particles, medical implant materials, and tissue (<http://www.erc.montana.edu>). A biofilm can be formed by a single bacterial species, but more often biofilms consist of many species of bacteria, as well as fungi, algae, protozoa, debris and corrosion products. Essentially, biofilms may form on any surface exposed to bacteria and some volume of water. Once anchored to a surface, biofilm micro-organisms carry out a variety of detrimental or beneficial reactions (by human standards), depending on the surrounding environmental conditions (<http://www.erc.montana.edu>). Higher microorganism counts were also observed after suspending biofilm although, the water quality in both instances were generally above the microbiological and aesthetical risk limits in terms of water quality guidelines.

Schaule and Flemming (1997) also reported that drinking water contains microorganisms that will colonise the surface of container walls with which the water is in contact after a few days and form biofilms, regardless of the material. This situation was developing in container walls used by community members in this study. When cells are eroded from biofilms or part of the biofilm matrix sloughs off, the water becomes contaminated. This leads to high colony numbers and positive results of pathogenic bacteria (Schaule and Flemming, 1997; Schaule et al., 1996,



Jesse et al., 1996). This is an indication of biofilm forming in the samples collected after loosening the biofilm.

Very little information is available on the inter-relationships between bacteria in mature biofilm, bacteria in the water and bacteria in newly formed biofilms. However, after suspending the biofilm in the water, the water became turbid. As mentioned in Section 1.7.2, turbidity was used to measure the clarity of the water and suspended matter in suspension such as loosened biofilm.

Augoustinos et al. (1992) showed that turbidity correlates with the presence of nutrients in a water system. The presence of nutrients may result in microbial growth and deterioration of water quality. Increased turbidity means an availability of a matrix for the transport of microorganisms through the system or a way of introducing the microorganisms. The turbidity in the container-stored water strengthened the assumption that there were indeed biofilm forming in container sidewalls.

4.2 MICROBIOLOGICAL WATER QUALITY IN CONTAINERS

A further possible effect of the variant container water quality on users (cases and controls) was investigated. This water quality study was done simultaneously with the study done by Nala and Jagals (1999). In general, cases and controls were found to be using water of equally poor quality.

4.2.1 HETEROTROPHIC BACTERIA

The spread plate method was used since it has been used in the previous studies by Jagals et al., (1997; 1999). Other methods are available that could have been used with better effect such as the pour plate method (Standard Methods, 1998). However, the heterotrophic bacteria still showed high counts. Although the pour plate method is reported to yield higher counts, the heterotrophic bacterial counts obtained by the method used in this study, were still above the *increased risk* of infectious disease transmission proposed by the DWAF, 1996. Heterotrophic bacteria levels in the community supply water generally exceeded the *negligible risk* limits proposed by the DWAF, 1996.

The high heterotrophic bacteria count in the municipal supply could be ascribed to the water network in the area that may have been subjected to pollution from unknown sources around the time of sampling such as pipe breaks (Jagals et al.,





1997). Increased heterotrophic bacterial levels within distribution systems are generally due to a number of factors, usually the absence of a residual disinfectant (such as chlorine) combined with either contamination from outside the distribution network or more commonly from regrowth of microorganisms (Clark and Goodrich, 1992).

During this study, residual chlorine was also routinely tested even though not reported. It was found that the residual chlorine was generally very low ($< 0.1\text{mg/l}$) at the supply point and absent in container water. Kastl and Fisher (1997) reported that the reaction of chlorine with natural organic matter in water and biofilm is usually responsible for chlorine decay during transport through the distribution system. Chlorine is not only consumed in the container water, but also by the biofilm surface. Decline in chlorine concentrations carries potential health risks due to bacterial regrowth. This may have been the cause of the increase in the heterotrophic bacterial counts in the supply water.

Higher heterotrophic bacterial counts were found after loosening and suspending the biofilm in container water.

Excessive heterotrophic bacterial counts can be caused by factors such as bacteria, which migrate from a biofilm layer to a water body and vice-versa (Kastl and Fisher, 1997). Film activity destroys residual chlorine, allowing greater survival of bacteria on the surface of the film. This can also explain the zero levels of residual chlorine in the container. Chlorine also evaporates in open containers and on exposure to sunlight.

Biofilms can develop even under oligotrophic conditions, such as those prevailing in drinking water distribution systems. Such biofilms not only represent a potential contamination source for heterotrophic plate count bacteria, but they also provide protective habitats for pathogenic organisms.

Apart from possibly being accommodated by the biofilm, high heterotrophic bacteria levels in stored water used by both cases and controls may also be attributed to poor hygienic handling of water from containers within households. Jagals et al. (1999) reported that water was generally scooped with a mug placed next to the container. Such mugs were exposed to unhygienic conditions such as flies, dust and unwashed hands of consumers. Theron (2000) also found that unhygienic mugs played a role in families that reported excessive diarrhoea.



There is no clear-cut evidence that heterotrophic bacteria as a whole pose a public health risk. Most heterotrophic bacteria in drinking water are not human pathogens (Rusin et al., 1997). However, some of the genera are opportunistic pathogens. Rusin et al. (1997) defined opportunistic pathogens as those that usually cause disease when the host immune system is weakened. *Pseudomonas* and *Aeromonas species* can be such opportunistic pathogens and may be included as part of heterotrophic bacteria. In this study, these species were detected amongst the total coliforms through confirmation tests (API Test) as non-coliform genera (false positive).

Heterotrophic bacteria counts posed an *increased risk* of microbiological infection to consumers in terms of the DWAF (1996) because this levels generally exceeded more than 1000 organisms / 1 ml in all the waters.

4.2.2 TOTAL COLIFORMS

Statistically significant higher total coliform levels were observed in the stored water compared to the supply water ($P \leq 0.001$). The log median values for total coliforms in the municipal supply were on the limits for *negligible risk*. Total coliform levels in stored container water were within the risk of infectious disease transmission with continuous exposure and a *slight risk* with occasional exposure in terms of the DWAF (1996), as well as the *Assessment Guide: Quality of Domestic Water Supplies* (1998). This in general indicated a presence of organic pollution in the container-stored water.

Total coliforms levels exceeded the limits for *increased risk* of infectious disease transmission in consumers in the container water quality after suspending biofilm because the levels generally exceeded more than 100 organisms / 100ml. Total coliforms levels were statistically higher after the release of biofilm in the container water than before the release of biofilm.

Although total coliforms levels in water used by the cases and controls before and after suspending the biofilm, showed no statistically significant difference in the water quality, the water used by controls contained more total coliforms than the water used by cases. The number of total coliforms used by cases before suspending biofilm indicated that the water quality was within the risk limits of infectious disease transmission (DWAF, 1996) with continuous exposure and slight



risk with occasional exposure to consumers. *Escherichia coli* levels were slightly higher after suspending the biofilm.

Jones and Bradshaw (1996) reported that regrowth of coliform bacteria in distribution systems could cause problems for water quality. Total and other species of the faecal coliform group, other than *Escherichia coli* may readily grow in water (Sjogren and Gibson, 1981 cited in Jagals et al., 1997).

In general, the consumers were exposed to continuous *slight risk* of infectious disease transmission when consuming water especially after suspending biofilm.

4.2.3 *Escherichia coli*

Although the median values were always zero, the geometric mean values showed that *Escherichia coli* was intermittently found in all waters. This was an indication of occasional faecal pollution in the water. This posed a *slight risk* of microbial infectious disease with occasional exposure to the consumers in terms of the DWAF (1993). The reason for the presence in the stored water might have been because of poor personal hygiene practices as well as unhygienic domestic environments.

Escherichia coli levels in the supply water were generally lower than in the stored water, but both showed an occasional exposure to consumers, with more occasions due to the container water quality.

The results indicated that *Escherichia coli* levels before and after suspending biofilm and also in both cases and controls had similar geometric mean values, which was within the limits for *slight risk* of microbial infection with continuous exposure to consumers in terms of the DWAF (1993).

Daly et al. (1996) reported that in water distribution networks, potentially hazardous situations could arise where small numbers of pathogenic organisms adhere to and reproduce within the biofilm despite the unfavourable conditions in the water phase. It was apparent that *Escherichia coli* must be in some way protected as a result of its association with the biofilm found in this study. As *Escherichia coli* is a faecal pollution indicator, these results are important in the context of potential problems caused by biofilm development in containers.

The presence of *Escherichia coli* at similar levels before and after suspending the biofilm, can be attributed to poor water hygiene and handling practices of stored water. During the interviews by Theron (2000), the information obtained showed



generally poor domestic environment conditions such as presence of flies in the kitchen and toilet in both the case and control households.

Henry et al., (1990) reported that some studies which attempted to identify potential sources of *Escherichia coli* within the home, have shown that mothers' and children's hands were important vehicles of risk for the transmission of diarrhoea disease.

Keeping domestic and other farming-related livestock within household limits is customary in developing regions. These usually substantial numbers of animals also contribute to faecal pollution of the environment (Jagals and Grabow 1996; Moe et al., 1991), which could have landed in container water.

Moe et al. (1991) found that when a water supply was contaminated with *E. coli*, the rate of diarrhoeal disease was significantly higher, and it appeared that in this situation water becomes a major source of exposure to faecal contamination and diarrhoea pathogens. No direct association between diarrhoea and water supply contaminated with *E. coli* was investigated during this study.

4.2.4 *Clostridium perfringens*

Clostridium perfringens were detected in water sampled at public standpipes as well as in container stored water. Both the geometric mean values for standpipe water and container water were within the limits for minimum *slight risk* of microbial infection disease to consumers according to the *Water Quality Criteria* (Aucamp and Vivier, 1990). Even though the median values were zero, there was a statistically significant difference between the water quality at the supply point and in the container water. Both the supply water and container water had similar geometric mean values. However, the levels of pollution were low. This indicated a minimal remote faecal pollution or resistant spores in the drinking water supply at the standpipes as well as in the container water. *Clostridium perfringens* spores are quite ubiquitous in the environment and that might have landed in containers during filling and handling (Payment and Franco, 1993).

Clostridium perfringens levels before the biofilms were suspended slightly higher than after suspending biofilm. Similar geometric mean counts were also observed in the cases and controls. There was a statistically significant difference in the water quality used by both the cases and controls. Both types of waters posed a minimal

indication of faecal pollution in the container water according to the *Water Quality Criteria* (Aucamp and Vivier, 1990).

Although the median values indicated a zero presence before suspension of biofilms in containers, the geometric mean value showed that *Clostridium perfringens* were intermittent found.

Clostridium perfringens levels in general were within the maximum *insignificant risk* limits, which posed an indication of faecal pollution in the container water especially after suspending biofilms.

4.2.5 BACTERIOPHAGES

No indications of bacteriophages were found in the supplied municipal water at public standpipes or in the container-water. This was also no indication of enteric viruses being present in the drinking water supply. There were no phages present in container water before and after suspending biofilm and also in container water. This indicated that the technique for detecting phages in the drinking water samples was not sensitive enough or that there were no phages present.

4.3 EFFECTS OF DISTANCES

To further investigate possible reasons for the significant differences between the water quality before and after suspending biofilm in containers, the effect of distance in the microbiological water quality in containers was investigated.

In general, no statistically significant differences were observed in the container water quality whether fetched from yard taps or from communal taps at various distances. However, the Mann-Whitney Rank Sum Test did indicate that there were some statistical significant differences in the total coliform levels in container water.

Further investigation was done to determine exactly the effect of the distances. A Kruskal-Wallis One Way ANOVA (based on rank transformation) was applied. The results indicated that the differences were found in container water used by cases carried over distance more than 10m but less than 100m. The same differences were also found even when using the Turkey multiple comparison method.

This finding implied those households within a maximum 10m distance from the taps rinse their containers more often because taps are nearer the homes. They also

tend to replenish water quite c observed that small containers were used to collect water in these households.

Households where taps had more than 100m away tend to use more screw-top containers. These screw-top containers were usually rolled or pushed with a wheelbarrow on the way back home. It could be that any biofilm that might have formed on the inside of these type of containers as shaken loose with the rolling action, and would therefore settle at the bottom of the container once placed in the home. This possibility was not investigated during this study. Also less contamination was experienced since the container had small opening and was kept closed during transport.

Households where the taps were between 10m and 100m away used big open containers to fetch water. Jagals et al. (1997) indicated that water was exposed to the environment or surrounding conditions, especially on the way home. The supplied water might become contaminated by en-route environmental inputs such as dust. This water could also be subjected to careless handling. At home water containers were generally stored either on a table at a window or on the floor underneath the table. Open containers generally left uncovered or covered with a cloth or board, which was not closely fitted. It was also easier to dip a scoop or mug straight into the water container. The implication of these differences for the case group (no differences observed for the controls) needs to be further investigated.

4.4 EFFECTS OF CONTAINER MATERIALS

Further investigation was done to determine the effect of container material types on general water quality. In general no significant difference was observed in the microbiological water quality either in plastic or metal containers. The only difference observed was in the turbidity of the water and the *Clostridium* spores, which were found in both the plastic and metal container. The turbidity was higher in the metal containers than the plastic containers.

4.5 REASONS FOR POOR WATER QUALITY

Theron (2000) reported that an increasing risk of diarrhoea was associated with poor knowledge regarding the causes and prevention of diarrhoea and poor household water hygiene. At home in the study area, the water containers were



generally stored either on a table or on the floor underneath the table. Furthermore, Theron (2000) also found that the containers were stored open and were subjected to environmental contaminants such as dust and flies, which could give rise to deterioration of the water quality in the storage container.

Nala and Jagals (1999) observed that the methods of extracting water from a container also indicated possible contamination of such water. In most instances, water was scooped with a mug kept uncovered. Such mugs were generally exposed to unhygienic conditions such as flies, dust and unwashed hands of consumers. In most cases, the containers after standing empty during the night were rinsed only with water at the taps before filling. The containers were generally not cleaned and disinfected thoroughly (Jagals et al., 1997).

The community was generally at risk of microbial infection posed by poor handling and storage of container water.

CHAPTER 5

CONCLUSIONS

Chapter 5: CONCLUSIONS

5.1 SUMMARY

- The microbiological quality of water supplied to the community of Botshabelo, Section K did not comply with the microbiological limits in terms of the *South African Water Quality Guidelines* (1996) for heterotrophic bacteria and total coliforms. However the *Escherichia coli* and *Clostridium perfringens* levels did comply with the *South African Water Quality Guidelines* (DWAF, 1993) and *Water Quality Criteria in South Africa* (Aucamp and Vivier, 1990). This water quality deteriorated to much more unacceptable levels with storage in containers.
- The quality of supplied water deteriorated (up to 9.60×10^7 orders of magnitude) in containers due to the circumstances surrounding storage and handling of such water from the supply point up to the point of by consumption.
- Biofilm formed on the insides of container walls, which apparently affected the quality of the water in containers.
- The quality of water deteriorated even further after biofilm was mechanically released to the water.
- Levels of all the indicator organisms except somatic coliphages were markedly higher after suspending biofilm to container water than before mechanical release.
- Case and control groups were found to have equally poor microbiological quality after collection and storage.
- It was found that differences occurred in the water quality of container water, which had to be carried over a distance of between 10m and 100m. Households used big open containers to fetch water that were then exposed to the environment or surrounding conditions especially on the way home. Water became even more contaminated by en-route environmental inputs such as dust.

- Container-stored water was handled with careless handling. At home water containers were generally stored either on a table at a window or on the floor underneath the table. Containers were generally left uncovered or covered with a loosely fitted cloth or board.
- The methods of extracting water from a container also indicated possible contamination of such water. In most instances, water was scooped with a mug kept uncovered. Such mugs were generally exposed to unhygienic conditions such as flies, dust and unwashed hands of consumers. In most cases, the containers after standing empty during the night were rinsed only with water at the taps before filling. The containers were generally not cleaned and disinfected thoroughly.
- No difference was observed in the microbiological water quality in plastic or metal containers.

5.2 RECOMMENDATIONS:

- Only a few surveys related to water quality and associated problems have been conducted in South Africa, since the benefits of this type of research are often underestimated. The majority of those studies approach the problem on a macro-scale, which all too often excludes most rural communities, and thus the risk of population exposure to water-related diseases is often underestimated. The findings of this study, therefore, seem to be of great importance. This is also due to the fact that the information highlights the particular problems in Botshabelo settlement, which is probably representative of many such areas in South Africa. This information should therefore serve the purpose of highlighting the necessity to deal with water quality issues as a matter of urgency.
- Immediate remedial action needs to be taken before the situation worsens.
- Appropriate education can meet the immediate needs to prevent the transmission of water-related diseases in rural communities. Interventions to improve water quality excreta disposal, which are associated with better hygiene practices, produce greater impacts than improvements in water quality. This is particularly so in highly contaminated environments where diarrhoea rates are high. Because the use of more water is not automatic following the installation of

water supplies, hygiene e
et al., 1991).

necessary part of the intervention (Esrey

- In addition, the people should also be advised to maintain water free of contamination in the household. These might ultimately result in improvements in the health standard of our population.

The following specific recommendations are made:

5.2.1 ENGINEERING INTERVENTION

- Access to the water supply should be as close to the home as possible. This will shorten the tap-glass sequence, in order to foster the use of larger amounts of water for personal as well as environmental hygiene practices. Priority should be given to those communities whose existing water sources are furthest from their houses.

5.2.2 EDUCATION INTERVENTION

- Community of Section K Botshabelo is at risk of microbial infection posed by poor handling of container water. Appropriate hygiene education programmes can cause behavioural changes, which can reduce the transmission of enteric pathogens and thereby reduce diarrhoea morbidity or mortality rates.
- Personal and domestic hygiene practices are essential for controlling water and sanitation-related diseases. Thus, communities with good sanitation facilities are also frequently better educated, have higher levels of personal and domestic hygiene, and may have better access to safe drinking water.
- It is essential for prior or concurrent improvements in water supply (quality, availability as well as quantity), as well as a vigorous and sustained community education programme. Thus a combined approach that includes the provision of safe and potable drinking water, hygienic disposal of excreta, and health education about water-use practices and domestic hygiene is needed.

5.2.2.1 Behaviour to be recommended in hygiene education:

Water collection:

- Drinking water should be collected in clean containers without coming into contact with hands and other materials.

- Water should be transported in a covered container.

Water storage:

- Water should be stored in containers that are covered and regularly cleaned.
- Drinking water should be stored in a separate container from other domestic water wherever possible.

Water used for drinking:

- Drinking water should be taken from the storage container in such a way that hands, mugs, or other objects couldn't contaminate the water.

Excreta disposal:

- Adults and children should use latrines.
- The stool of infants and young children should be safely disposed of.
- Hand-washing facilities and soap should be available and hands should always be washed after defecation.

5.2.3 CONTAINER HYGIENE

Poor hygienic handling of water from containers used by the consumers was identified within their households. Water was generally scooped with a mug placed next to the container. Such mugs were exposed to unhygienic conditions such as flies, dust and unwashed hands of consumers.

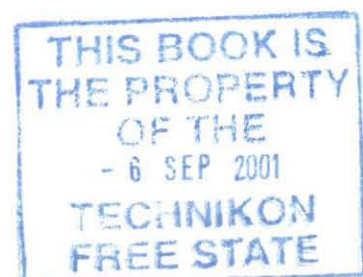
5.3 FUTURE RESEARCH

Because of the risk for infection indicated by the levels of the indicators in the water, further research needs to be done to establish the possible mechanisms of introduction and survival of the organisms in the film. This aspect could be an indication of the level of environmental health impacts released from the surrounding domestic environment and introduced into the storage containers.

Water is scarce in this country (South Africa) and further deterioration of the quality of the already limited sources should not be allowed to happen.

CHAPTER 6

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Chapter 6: REFERENCES

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APPENDICES

BACTERIOLOGICAL ANALYSIS

Equipment and preparation

MEMBRANE FILTRATION

Equipment and procedures for bacteriological analysis by membrane filtration were based on generally accepted guidelines (SABS, 1984 & 1987; Millipore corporation, 1992; Standard Methods, 1998).

1) FILTER & VACUUM ASSEMBLY

3 x Millipore[®] 3-place PVC manifolds. 9 x glass 47-mm diameter Millipore[®] filter holder sub assembly comprising:

Glass funnels ± 250-mL capacity.

Fritted glass base support for filter membrane.

Clamp to secure funnel on base after loading filter membrane.

2 x EDWARDS[®] 1.5 Two-stage 220/240 V 50/60 Hz vacuum/pressure pump. Two sets of 1-litre vacuum filter glass flasks for trapping moisture before vacuum pump.

The assembly is connected by means of silicone rubber tubing.

2) STERILISATION

Steam sterilisation of equipment was done in an autoclave at 121°C / 15 psi for 20 minutes after each completed filtration session of all samples.

Dry sterilisation of equipment was done in an oven at 180°C for 10 min. Dry sterilisation was done between each sample filtration session.

The filtration sub-assemblies are immersed in boiling water for 10 min to decontaminate between indicator-group filtration within each sample filtration session. Forceps were immersed in alcohol and flamed before every filter handling between batches.

3) PHOSPHATE BUFFER

Stock phosphate buffer solution and stock magnesium chloride solution were prepared according to Standard Methods (1998). Sterile working solutions of buffer were made up by adding 1.25 ml of phosphate (34 g KH_2PO_4 / L distilled water) buffer and 5 ml of magnesium chloride solution (81.1 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ / L distilled water) to 1 litre of reagent grade water and autoclaving.

4) MEMBRANE FILTERS

Sterile Millipore[®] HA-type 0.45 μm pore size membranes were used. The membranes were 47 mm in diameter, white and grid-marked.

5) INCUBATION

- 5.1 Incubators with circulating air (fan induced) were used. Temperatures varied within 0.5°C accuracy - especially within stacks of incubated plates.
- 5.2 25-litre water baths with uniformly distributed heating elements in the steel inner jacket to ensure constant temperature distribution was used. The baths were equipped with gabled covers to aid temperature maintenance within 0.2°C of setting.

6) PIPETTES

Pipetting for 1 ml and smaller volumes were done with Gilson[®] adjustable pipettes with sterile disposable tips. Errors in calibration were checked not to exceed 2.5%. Larger volumes were dispensed with standard graduated glass pipette.

7) DILUTIONS

The following dilution procedure was followed to achieve the ideal colony range of between 20 and 60 (Standard Methods, 1998):

| Types of water samples | Chromocult [®] Coliform | <i>Clostridium perfringens</i> | Heterotrophic plate counts |
|------------------------|----------------------------------|--------------------------------|----------------------------|
| Container water | 10, 0, -1, and -2 ml | 100 and 10 ml | -2, -3, -4, and -5 ml |
| Municipal supply | 10, -1, and -2 ml | 100, 50, and 10 ml | -2, -3, and -5 ml |

8) COUNTING

Colonies hosted by membrane filters were counted under a ZEISS® stereo microscope.

BACTERIOLOGICAL INDICATOR ANALYSIS

Methods and Techniques for Organism recovery and enumeration

SAMPLING

Samples were taken in 900-ml sterile Whirlpacks[®] from the public standpipes as well as in containers stored water in selected households and placed in cooler bags (7°C–10°C) for transportation to the laboratory. The samples were analysed within 6 hour of collection. Samples for the detection of coliphages were transported to a laboratory in another city and analysed within 24-30 hours from collection.

MEMBRANE FILTRATION

1) THE FILTERING TECHNIQUE

3 sets of Millipore[®] 3-place vacuum manifold, complete with filter holder sub-assemblies (Appendix A) were used. Vacuum was created by the electric vacuum pumps evacuating through a dual moisture trap system comprising 1-litre capacity vacuum flasks.

Each glass assembly was separately wrapped in tin foil and sterilised before each session of filter plating (Appendix A). Constant sterilisation and decontamination of the glass sub-assemblies (Appendix A) was done during filtration sessions between samples to avoid cross contamination. Filter plating of the same sample was done in decreasing dilution order to avoid contamination.

A sterile phosphate buffer was used (Appendix A) for diluting samples and rinsing funnels after filtration (Millipore Corporation, 1992). Pre-sterilised membrane filters (Appendix A) were used. Membranes were loaded grid side up, onto the fritted glass support base of the funnel holder with a sterile forceps (Appendix A) and the funnel clamped onto the filter base.

The sample was re-mixed by vigorously shaking the bottle for several seconds. 20 - 30 ml of sterile buffer were poured into the funnel and a volume of sample was pipetted into the buffer.



Volumes of between 10-ml a le were pipetted for apparently clear water. All sample portions suspended in dilution were filtered within 30 min to avoid inactivation or multiplication of organisms in the dilution.

Vacuum was applied while slightly swirling the manifold unit to ensure uniform suspension of the sample in the volume of buffer during filtering. The funnel walls were rinsed repeatedly (3 times) with approximately 30 ml of sterile buffer. Buffer was drawn into a syringe and ejected through a sterile Sterivex[®] (Millipore[®]) filter to avoid contamination.

Vacuum was broken and the membrane lifted with a sterile forceps, and put grid side up, onto a selective medium in petri dishes, ensuring no trapped air under the membrane. The dishes were marked and inverted to be incubated (Millipore Corporation, 1992; Standard Methods, 1998).

The incubation temperatures and times for each indicator organisms group are described in Appendix C.

2) SPREAD PLATING TECHNIQUE

Petri dishes (90-mm diameter) with appropriate media were placed into an incubator at 37^oC for 30 minutes to slightly dry the media.

0.3 ml of sample was pipetted into the petri dish, the volume was spread over the surface of the medium using a 3 mm diameter glass rod (resembling an ice hockey stick) inside a laminated flow cabinet to minimise cross contamination. Plates were left for sample moisture to settle onto the medium surface and then inverted to be incubated (Standard Methods, 1998).

3) DILUTIONS

All samples were filtered in triplicate (3 filters) per dilution. Dilutions were made up to ideally achieve counts of between 20 to 60 colonies per plate (Standard Methods; 1998). Tillet (1993) described various factors that could lead to inaccuracies or unacceptable variation in counts of the same sample at the point of sampling and in the laboratory. Even vigorous mixing of a sample in the laboratory before extraction could not prevent variation in counts due to natural random distribution of organisms in such a sample.



Dilution procedures in the laboratory should ideally be adapted to minimise variations while diluting from the sample (Appendix A). Undiluted sample applications varied between 1 ml and 100 ml. These applications were single extractions by pipette or decanted into sterile 100 ml measuring cylinders from the raw sample after the sample had been vigorously shaken.

4) COUNTING

After incubation for appropriate periods of time, colonies were counted according to the prescriptions for each group of organisms. To achieve reliable statistical quantification of the final count per 100 ml and also per 1 ml per sample was calculated as follows (Standard Methods, 1998):

$$\frac{[(\text{Plate 1} + \text{plate 2} + \text{plate 3}) / 3] \times 100}{\text{Sample size (Volume)}} \\ \text{Sample dilute}$$

A formula was programmed in a Microsoft Excel[®] spreadsheet (Appendix G). The analyst enters ① the counts from each of the 3 plates (membranes) ② sample volume (maximum 1-ml for diluted samples) as well as ③ the dilutions expressed as 0.1; 0.01; etc. (minimum 1 ml for undiluted samples).

Counts are expressed as number of organisms per 100 ml and for heterotrophic bacteria per 1 ml.

BACTERIOLOGICAL INDICATOR ANALYSIS

Media, Reagents and Procedures

HETEROTROPHIC BACTERIA

Culture media Agar (Merck, 1996)

The following ingredients were used:

| | |
|---------------------------------|--------|
| Peptone | 3.0 g |
| Soluble casein | 0.5 g |
| K ₂ HPO ₄ | 0.2 g |
| MgSO ₄ | 0.05 g |
| FeCl ₃ | 15 g |

- ◆ All the ingredients were added to 500 ml-distilled water (2 bottles).
- ◆ pH was adjusted to 7.2 before autoclaving.
- ◆ Then the mixture was boiled gently to dissolve the powder.
- ◆ The mixture was then autoclaved at 121^oC for 15 min.
- ◆ After autoclaving, the liquid was poured into 90-mm petri dishes, 5 mm in depth.
- ◆ Fresh plates were stored in the dark inside sealed plastic bags (for moisture retention) at < 8^oC. Unused plates were discarded after 6 months.
- ◆ Heterotrophic bacteria were enumerated from the samples by spreading 0.3-ml sample dilutions onto a non-selective medium.

Incubation: The prepared plates were inverted and incubated aerobically in an incubator at 37^oC for 48 hours.

Identification: Heterotrophic bacteria appeared as all the colonies on the plates.

THE COLIFORMS

1) TOTAL COLIFORMS

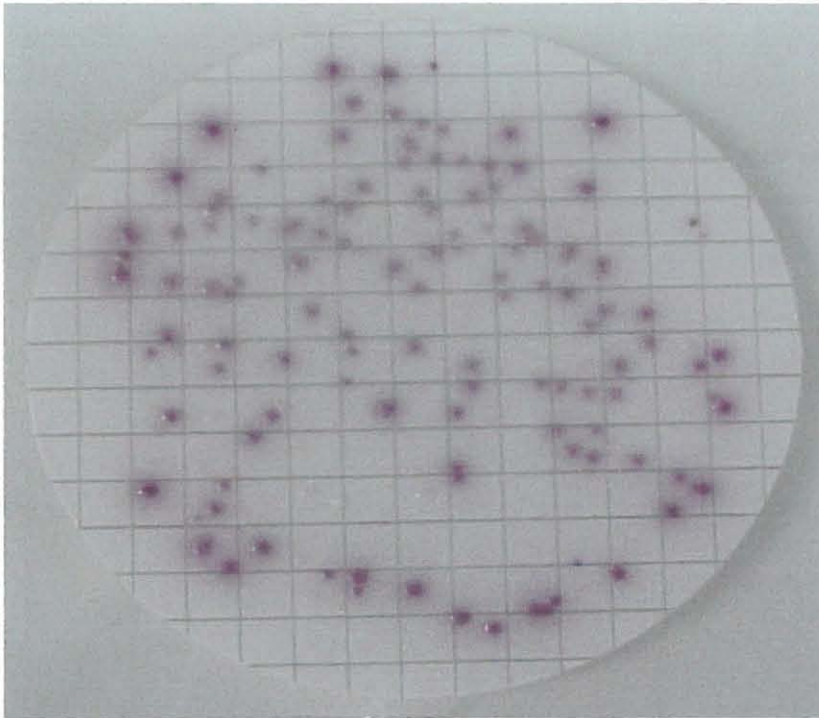
Chromocult[®] Coliform Agar (Merck, 1996):

- ◆ 26.5 g of the powder was suspended in 1 litre of distilled water.
- ◆ The mixture was heated in a flowing water bath while gently being stirred until the powder was totally dissolved.
- ◆ The medium was cooled to 40-50°C and the Cefsulodin solution (10-mg Cefsulodin in 2 ml of distilled water) was added to the 1 litre of medium by gently shaking to homogenise. Merck (1996) reported that Cefsulodin solution can be added if high accompanying flora is expected, especially *Pseudomonas spp.* and *Aeromonas spp.* The solution deactivates the occurrence of *Pseudomonas spp.* and *Aeromonas spp.*
- ◆ The liquid was poured into 90-mm petri dishes, 5 mm in depth. This medium does not require autoclaving.
- ◆ Fresh plates were stored in the dark inside sealed plastic bags (for moisture retention) at < 8°C.

Incubation: The plates were inverted and incubated at 35°C - 37°C for 24 hours.

Identification: Total coliform colonies appeared in various shades of salmon to red (Merck, 1996).

Confirmation: API[®] 20E (bioMérieux[®]) (Appendix D)



TOTAL COLIFORM COLONY MORPHOLOGY

2) *ESCHERICHIA COLI*

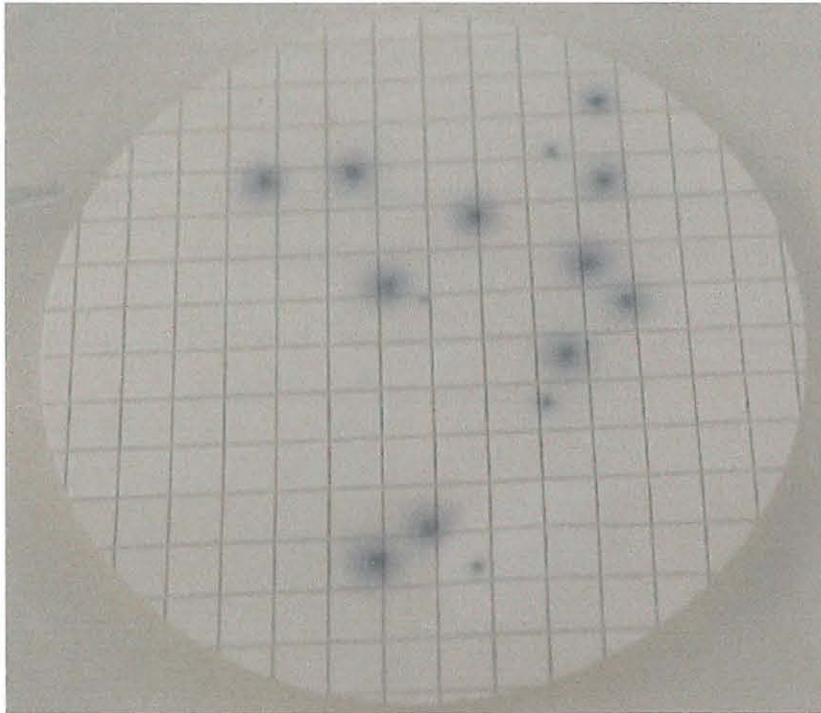
Chromocult[®] Coliform Agar (Merck, 1996):

- ◆ The same procedure as with total coliforms were used.

Incubation: The plates were inverted and incubated at 35°C - 37°C for 24 hours.

Identification: *E. coli* colonies appeared in various shades of dark blue-to-violet (Merck, 1996).

Confirmation: API[®] 20E (bioMérieux[®]) (Appendix D)



***ESCHERICHIA COLI* COLONY MORPHOLOGY**

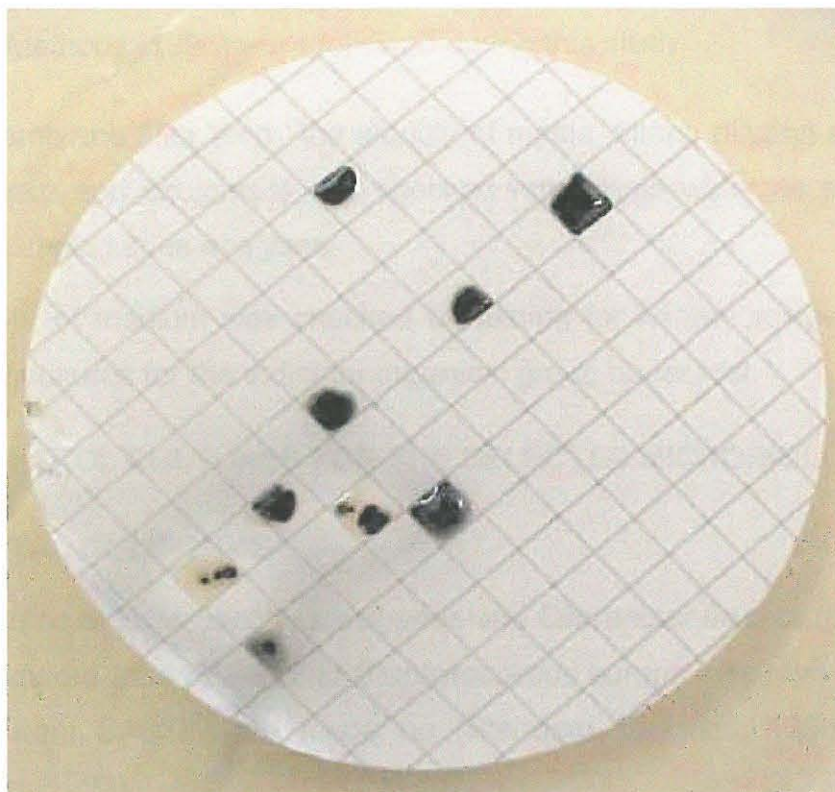
3) *CLOSTRIDIUM PERFRINGENS*

Perfringens (OPSP) agar (Oxoid, 1990):

- ◆ 22.8 g of the powder was added to 500 ml-distilled water, the mixture was boiled gently to dissolve the powder.
- ◆ The mixture was then autoclaved at 121°C for 15 min.
- ◆ After cooling to 50°C, rehydrated supplements A (SR76) and B (SR77) were added. The mixture was mixed well and then poured into 90-mm diameter petri dishes, 5 mm in depth.
- ◆ After cooling, the plates were stored in darkness in plastic bags (to maintain moisture content) at < 8°C. Unused plates were discarded after 2 weeks.

Pasteurisation: Samples (presumably containing *C. perfringens* spores) were pasteurised in a water bath at 80°C for 10 minutes (Oxoid, 1990; Ashbolt et al., 1993; Ferguson et al., 1996).

- Incubation:** The plates and incubated anaerobically in an incubator at 37°C for 48 hours. Oxoid gas generating kits producing atmospheres of 95% hydrogen and 5% carbon dioxide were used.
- Identification:** *Clostridium perfringens* colonies appeared as partially or fully discoloured dark brown to black colonies.
- Confirmation:** Cultured isolates confirmed on Rapid ID[®] 32A galleries (bioMérieux[®]) (Appendix D).



***CLOSTRIDIUM PERFRINGENS* COLONY MORPHOLOGY**

ANALYTICAL QUALITY CONTROL

1 GENERAL QUALITY CONTROL PROCEDURES

Accuracy of results obtained from the analyses done for this project was of paramount importance. An effective quality assurance programme was established. According to Standard Methods (1998), it is especially important that laboratories performing only a limited number of microbiological testing exercise strict quality control. The guidelines for minimal quality control programmes recommended by Standard Methods (1998) were followed during this study.

- ◆ For membrane filter tests, the sterility of media, filters, dilution and rinse water, glassware and equipment was checked with sterile water as a sample during each sample series analyses.
- ◆ Each lot of medium was checked by testing for known positive and negative control cultures for the indicator organism group under test.

1.1 CONTROL CULTURES FOR THE SELECTED MICROBIOLOGICAL TESTS

1.1.1 TOTAL COLIFORMS

Stock cultures of *Enterobacter aerogenes* and *Citrobacter freundii* (positive control - culture acquired from SABS) and *Staphylococcus aureus* (negative control - culture acquired from SABS) were made up (Standard Methods, 1998; Merck, 1996; bioMérieux, 1996).

1.1.2 *ESCHERICHIA COLI*

Stock cultures of *Escherichia coli* (positive control - culture acquired from SABS) and *Enterobacter aerogenes* and *Citrobacter freundii* (negative control - culture acquired from SABS) were made up (Standard Methods, 1998; Merck, 1996; bioMérieux, 1996).

1.1.3 *CLOSTRIDIUM PERFRINGENS*

Stock cultures of *C. perfringens* (positive control - culture acquired from SABS) and *C. bifermentans* (negative control - culture acquired from SABS) were made up

(Oxoid, 1990).

1.2 PROCEDURES FOR MEDIUM CHECK

Volume units of 1-ml of the solution were filtered through membranes and the membranes placed on petri dishes containing the various selective growth media. Parallel analyses were done at least once a month for the duration of the project.

The specific colony colour identification and distinction was standardised by the analyst group (making sure everyone see and understand the same colour – including the various nuances / shades) and used to identify the various indicators tested for on the various media.

2 METHOD PRECISION

Precision was calculated with duplicate for each different water type. The test laboratory is continually involved in surface and drinking water quality testing and has a set precision criterion based on $3.27\bar{R}$ (Standard Methods; 1998). This criterion was updated every 3 months, using the 15 most recently set of duplicate results.

Duplicate testing of at least 10% of all samples is a monthly routine and includes duplicates for each analyst involved. Results are transformed and the range calculated.

Results from a series that show excessive variability was not accepted. The analytical problem will be identified and resolved.

3 COLONY VERIFICATION

The actual selectivity / specificity of the various selective growth media has been found in many reports to be inconsistent (Dionisio and Borrego, 1995; Figueras et al., 1996). Various reasons are given for this. Probably one of the most common reasons is the vast array of species and sub-species often to be found in a single indicator organism group or species as well as in the multitude of non-indicator groups. Amongst these variants one will inevitably find non-indicator organisms that find the selectivity of a specific medium accommodating and may even manifest in the colours prescribed to the analyst for identification.

To establish the accuracy of or levels as well as the selectivity of the various media for detecting the selected indicators, a verification programme was designed and followed according to Standard Methods (1998). Representative selections of colonies were made of various bacterial pollution-indicator organisms detected in water samples from the target catchment.

Standard Methods (1998) recommends at least 10 colonies picked randomly per month from known positive samples and verified. Because this study critically examined the specificity of the various media selective for the various selected indicator groups, this number of verifications was increased.

3.1 PREPARATION FOR COLONIES FOR VERIFICATION

Selections were made only from colonies that could be counted as the actual indicator on the various selective growth media. These counts would be based on various colour-related identifications (counting the specific coloured colonies) as prescribed by the relevant authoritative manual such as Standard Methods (1998) and guidelines from manufacturers.

Between 12% and 40 % of all the colonies cultured on the various media were randomly selected. Before verification began with multi-test identification system galleries such as the API[®] and RAPID ID[®] by bioMérieux[®], the coloured selected colonies was first stripped of the coloration that facilitated the selectivity of the growth medium. This was to eliminate all possible interference with the functions of the Identification System Galleries.

3.1.1 THE COLIFORMS

The Coliform colonies were picked up from the membranes with inoculum needles and streaked out on the same selective medium and incubated at the prescribed temperature. This was to obtain pure single colonies (but without the membrane) with the same colour that had originally been used to identify the specific colony as being from the relevant coliform group. In fact this could be seen as further affirmation of the original selection of the colony as alien particles trapped on the membrane could sometimes lead to coloration of the membrane, making the colour identification of the colony difficult.

Membrane-grown colonies to detect *E. coli* on the Chromocult Coliform[®] medium were only partially picked up because the remaining colony was used for



intermediate *E. coli* verif:

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CS' indole reagent according to the user

manual (Merck, 1996). It was feared that the indole reaction might influence further refinement of the selected colony. The partially recovered material generally proved enough to produce strong single colonies during the next round of streaking-out.

In order to confirm *E. coli* detection, the dark blue to violet coloured colonies were coated with a drop of KOVACS' indole reagent. A cherry-red colouring after some seconds confirmed a positive indole formation and consequently the presence of *E. coli*.

This method proved to be very useful for positive identification of *E. coli* when plates inoculated with heavily polluted waters were used. However, KOVACS' indole reagent on plates containing weaker colonies of *E. coli* actually lessened the efficiency of the reagent because a drop of the reagent tended to colour the whole membrane cherry-red, making the positive identification of especially small *E. coli* colonies very difficult.

Single colonies on the selective media were then streaked out and grown on Plate Count Agar (Standard Methods, 1998) to strip the colonies of their colour. This part of the process was the last step in which the colonies were touched with the metal-eye of an inoculum needle. Picking the isolated colony from the Plate Count Agar to be used for identification on the API strip was done with sterile swabs to exclude possible interference from the metal eye of an inoculum needle with the oxidase test.

3.2 THE PRINCIPLE OF IDENTIFICATION SYSTEM GALLERIES

Selections were made from the plates where the particular dilution yielded growth of between 20 and 80 colonies. Of this, a constant percentage exceeding 10% of the identified coloured colonies were selected and processed (Section 3.1 above) for transfer to the various types of confirmation galleries.

The various identification systems (confirmation galleries) consist of strips with a characteristic number of micro-tubes containing dehydrated substrates. These substrates support specific enzymatic activity or fermentation of sugars. Each micro-tube is inoculated with a dense bacterial suspension made up of the original selected colony, which at the same time reconstitutes the substrates. Metabolic end-products are produced during incubation which produces spontaneous colour changes or revealed colours afterwards by the addition of reagents.

The various reactions are read into a Reading Table. The identification is obtained from an Identification Table or a computerised Analytical Profile Index.

4 THE COLIFORMS

4.1 CONFIRMATION OF THE VARIOUS PRESUMPTIVE COLIFORMS:

4.1.1 CHROMOCULT[®] COLIFORM AGAR:

Salmon to red (Total coliform on Chromocult[®] Coliform Agar) and deep blue-to-violet (*E. coli* on Chromocult[®] Coliform Agar) were selected. The colony morphology was carefully noted and included colour, size, shape, composition, and edge appearance. These would be colonies that the analyst will count as the coloured coliform colonies on a given specific growth media. A note was also made of the number of colonies counted from every particular plate (membrane) as well as the number taken for verification by the API[®] 20E-identification system. The colonies were then purified as described in Section 3 above.

4.1.2 API[®] 20E MULTI-TEST GALLERIES (bioMérieux[®])

API[®] 20E are standardised identification systems for *Enterobacteriaceae* and other non-fastidious Gram-negative rods. The systems use 12 and 20 miniaturised biochemical tests (respectively) in strips, and a related database. These systems can be used to identify a substantial number of species that included the most important species used in this study.

4.1.3 PREPARATION OF THE INOCULUM

Homogeneous bacterial suspensions, of the selected (and purified) colonies were made according to the prescriptions contained in the manual provided with the commercial identification kit (bioMérieux[®]).

4.1.4 INOCULATION OF THE STRIPS

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

4.1.5 READING THE STRIPS

After the incubation time, the spontaneous colour reactions from each strip were

recorded. Reagents were he prescribed tubes and the colour reaction recorded. All these recording were done on result sheets provided with the kit.

4.1.6 IDENTIFICATION

The pattern of each of the reactions obtained was hand-coded, on the result sheets, into a numerical profile. These numerical profiles are then read into the ANALYTICAL PROFILE INDEX as a number. The Index then provides the name of the species that matches the code.

4.1.7 QUALITY CONTROL

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

5 THE SPECIAL INDICATORS

5.1 CONFIRMATION OF *CLOSTRIDIUM PERFRINGENS*:

5.1.1 *PERFRINGENS* (OPSP) AGAR

Colonies exhibiting dark brown to black (*Clostridium perfringens* on Perfringens (OPSP from Oxoid® Agar), were selected. The colony morphology was carefully noted and included colour, size, shape, composition, and edge appearance. These would be colonies that the analyst will count as the various coloured *Clostridium perfringens* colonies on the various growth media. A note was also made of the number of colonies counted from every particular plate as well as the numbers taken for verification by the Rapid ID® 32A-identification system.

The colonies were then purified as described in Section 3 above. After this process, the colonies were emulsified according to prescription and the emulsion flooded onto Columbia sheep blood agar (Oxoid®) plates. The plates were incubated at anaerobically at 37°C for 24-48 hours.

5.1.2 RAPID ID® 32A MULTI-TEST GALLERIES (bioMérieux®)

Rapid ID® 32A is a standardised identification system combining 29 biochemical tests that offers a multitude of capabilities for identifying anaerobes.

5.1.3 PREPARATION OF

Homogeneous bacterial suspensions of harvested colonies from the blood plates were made according to the prescriptions contained in the manual provided with the commercial identification kit (bioMérieux®).

5.1.4 INOCULATION OF THE STRIPS

The microtubes on the prepared strips were filled according to prescription and incubated aerobically for 4 hours at 37°C.

5.1.5 READING THE STRIPS

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

5.1.6 IDENTIFICATION

The patterns of each of the reactions obtained were hand-coded, on the result sheets, into a complex numerical profile. These numerical profiles are then read into the ANALYTICAL PROFILE INDEX as a number. The Index then provides the name of the species that matches the code.

5.1.6 QUALITY CONTROL

Several QC tests were done on the various batches of Strips acquired. The stock cultures used were obtained from local medical commercial pathological laboratories. The reference organism used was *Clostridium histoliticum*.

PHAGE ANALYSIS

Media, Reagents and Procedures

SOMATIC COLIPHAGES

Somatic coliphages were enumerated, using the Plaque Assay method for Somatic Coliphages using Small Petri Dishes with Double Agar Layer (SP-DL) (ISO, 1995; Grabow et al., 1997). The method is based on conventional plaque assay for somatic coliphages (Grabow et al., 1993) in small volumes of water (generally 1.0 ml) using small petri dishes (90-mm diameter).

GROWTH MEDIUM FOR THE HOST CULTURE (Nutrient broth)

Prepare ordinary nutrient broth (Difco[®] or equivalent) according to the manufacturer's instructions, heat to dissolve, dispense in convenient containers - i.e., 100 ml quantities in 200-mL medical flats, autoclave, and store at about 4°C for not longer than 30 days.

PHAGE BOTTOM AGAR

| | |
|-----------------|---------|
| Bacto agar | 14.0 g |
| Tryptone | 13.0 g |
| NaCl | 8.0 g |
| Glucose | 1.5 g |
| Distilled water | 1000 ml |

Heat to dissolve agar, and autoclave. Pour about 20 ml in 90-mm diameter petri dishes. Store at 4°C for a maximum 10 days.

PHAGE TOP AGAR

| | |
|--|--------|
| Bacto agar | 8.0 g |
| Tryptone | 10.0 g |
| NaCl | 8.0 g |
| Glucose | 3.0 g |
| Na ₂ CO ₃ solution | 5.0 ml |
| MgCl ₂ solution | 1.0 ml |

Distilled water

1000 ml

Autoclave and cool to 55-60°C. Add naladixic acid solution if considered necessary (1.0 mL/100 ml). Distribute 2.5-mL aliquots into test tubes with caps. Store at 4°C for a maximum 30 days.

HOST CULTURE

Escherichia coli strain C (ATCC 13706) = WG4

Naladixic acid resistant mutant of WG4 = WG5

TEST SAMPLE

Water (e.g. drinking water) or liquid suspension (e.g. suspension of faecal material). Make tenfold dilution in peptone saline solution as necessary.

NALADIXIC ACID SOLUTION

Dissolve 0.5 g of naladixic acid in 4 ml of 1 M NaOH. Add 16 ml of sterile water and mix well. Decontaminate by membrane filtration, e.g. syringe filter, 0.22 µm membrane. Store at 4°C for a maximum 4 weeks.

MgCl₂ SOLUTION

Prepare 4 M stock solution by dissolving 820 g of MgCl₂.6H₂O crystals in 1000 ml of water; sterilise by autoclaving; store at room temp in the dark.

CaCl₂ SOLUTION

Prepare 1 M stock solution by dissolving 147 g of CaCl₂.2H₂O in 1000 ml water by gentle heating. Decontaminate by membrane filtration, e.g. syringe filter, 0.22 µm membrane. Store at 4°C for a maximum 6 months.

PEPTONE SALINE SOLUTION

Dissolve 1-g peptone and 8.5 g sodium chloride in 950-ml water by boiling. Adjust pH to 7.0±0.1 using 1 M NaOH or HCl. Make up to 1000 ml with water, and dispense in convenient volumes. Autoclave. Store at 4°C for a maximum 6 months.

TEST PROCEDURE

- ◆ Steam the required number of test tubes with top agar to liquefy agar and adjust to 48°C in a heating block.



- ◆ Add 0.5 ml of the host culture (overnight in stored volume of growth medium at 35-37°C) to the top agar.
- ◆ Add 1 ml of the test sample, or an appropriate dilution of the test sample, to the top agar in each test tube.
- ◆ Mix gently and pour the top agar mixture with minimum delay onto the bottom agar layer in a 90-mm phage agar plate.
- ◆ Repeat the above in tenfold to obtain counts per 10 ml. If tenfold dilutions are required, three plates should preferably be used for each dilution to obtain meaningful results.
- ◆ Incubate inverted plates overnight at 35-37°C and count plaques of somatic coliphages.

Notes

1. Tests were carried out according to basic principles outlined in:
 - 1.1. Grabow W O K, Holtzhauzen C S and de Villiers C J (1993) Research on Bacteriophages as Indicators of Water Quality. WRC Report No 321/1/93. Water Research Commission, Pretoria. pp 147.
 - 1.2. ISO/CD 10705-2:1995. Water Quality - Detection and Enumeration of Bacteriophages. Part 2: Enumeration of somatic coliphages. International Organisation for Standardisation, Geneva. Pp 15.
2. A heating block should be used for tubes with top agar instead of water bath if possible in order to avoid contamination by phages in water bath water.

STATISTICAL APPROACHES

A Techniques

B Data presentation

A TECHNIQUES

1) CHARACTERISTICS OF WATER RESOURCES DATA

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

- 1.1 A lower bound of zero – no negative values are possible.
- 1.2 Presence of outliers, observations considerably higher or lower than the most of the data. This occurs infrequently but regularly. Outliers on the high side are more common in water resources.
- 1.3 Positive skewness, due to items 1 and 2. Skewness can be expected when outlying values occur only in one direction.
- 1.4 Non-normal distribution of data due to items 1 – 3 above. Many statistical tests assume that data follow a normal distribution while water resources data often do not.
- 1.5 Data reported only below or above some threshold (censored data).
- 1.6 Seasonal patterns.
- 1.7 Autocorrelation. Consecutive observations under similar circumstances tend to be strongly correlated to each other. The most common kind of autocorrelation in water resources, high values will tend to follow high values in circumstances such as intermittent high volumes of intensive rainfall.
- 1.8 Dependence on other uncontrolled variables. Values strongly covary with discharges, rainfall or some other variable.



2) CENTRAL TENDENCY (Methods of location)

Microbiological data about populations of indicator organisms formed the backbone of this study. Since the true values of indicator organisms e.g. faecal coliforms in a volume of water is not known, all the water in that volume should be analysed to assess the value (concentration) of the faecal coliform content. This is simply neither physically nor financially possible, especially in large water bodies. To overcome this, smaller samples of the water volume are taken. The samples are then measured in such a way that conclusions about the sample may be extended to the entire target population (Helsel and Hirsch, 1995). These conclusions are estimates of the true population values.

The most popular estimate that can generally be made of the true population values is the central value or central tendency.

2.1 Estimates on indicator population in water samples

Central tendency estimates applied for this study were the following:

- ① The mean (or average) is the sum of all the data in a set divided by the sample size. However, the mean is sensitive to outlying values in data sets. Since microbiological data may vary greatly (outliers) in the same sample, the mean is generally strongly influenced and it is said that the estimate of the target population value may not be realistic. However, outliers were kept in the sets for this study (Discussed in 3 below) as they presented real events in the sampling and analysis routines such as higher activity pollution in the particular water type at the particular time.

To calculate a more realistic estimate based on the mean in a data set that contains outliers, it is best to remove as much of the variance as possible. To do this, the data units are transformed to their logarithms (Discussed in 4 below) to create data that are more “normally” distributed although the data will generally not be symmetrical. This is referred to as lognormal data. The mean of these logarithms of data in a set is calculated and then transformed back to its original units. The resultant mean is referred to as the geometric mean. The preferred best estimate of central tendencies of untransformed microbiological data such as obtained in this study, was the (Standard

Methods, 1998). The mean was used in results and discussion areas where skewed data sets were used untransformed.

- ② The median was used in discussion areas where transformed data were used. The median is only minimally affected by the magnitude of a single observation such as an outlier (Helsel and Hirsch, 1995) and will therefore be resistant to the effect of outliers, which had been kept in the data sets throughout this study.

2.2 Calculations for enumeration methods

Mean values of indicator organism concentrations in samples were also established through measures of central tendency.

Colony counts on each membrane per sample set measuring up to number of organisms were calculated to arithmetic mean values because of the predominantly symmetrical distributions of the colonies per triplicate set (the formula is discussed in Appendix B).

3 OUTLIERS

Outliers are observations whose values are quite different than others in the data set (Helsel and Hirsch, 1995; Glantz, 1997). While it is often found that analysts would discard outliers, this procedure was not followed in this study. Outliers were kept in the sets and investigated further.

Outliers generally have one of 3 causes:

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

When outliers occurred during this study, the following were investigated:

- ◆ recording errors such as erroneous entering into calculation programmes
- ◆ copying, decimal points or other obvious errors
- ◆ comparing the outlying tendency with the other indicators enumerated from the same sample to see if a similar event occurred
- ◆ re-running the sample and analysis.

Where no errors were detected, samples were kept in the sets as they presented real events in the sampling and analysis routines such as higher activity pollution in the particular water type at the particular time or treatment that may have occurred on the same samples e.g. brushing technique (Section 3.2).

4 HYPOTHESIS

Scientists often have prior ideas of how the systems they investigate might behave. These are called hypotheses (Helsel and Hirsch, 1995). Statistical tests are the most quantitative ways to determine whether hypothesis can be substantiated or whether they must be modified or rejected outright. Hypotheses for the various Sections contained in the Chapters for this study were formulated within each Section according to the objectives set for each Section. The significance level (α -value) is the probability of incorrectly rejecting the null hypothesis. The α -value for this study is set at default 5%. This 0.05 for the α -value is a statistical tradition but could be changed for various reasons (Helsel and Hirsch, 1995). The Sections in which this default is changed are clearly indicated in the relevant Chapter.

To compare a new technique with an established one, it will be necessary to see whether the data (adapted or otherwise) generated by these agree sufficiently for the new to replace the old (Bland and Altman, 1986). For this comparison, certain *hypotheses* had to be set prior to the stage of collecting the comparative data (Helsel & Hirsch; 1995) for the various selected methods.

The null hypothesis H_0

The H_0 is what is assumed to be true about the system under study prior to data collection, until indicated otherwise (Helsel and Hirsch, 1995).

- ◆ For this study, it was assumed that there would be no statistical significant differences between the quality of the municipal supply at standpipes and container-stored water kept in selected individual households.
- ◆ It is therefore assumed that there would be no statistical significant differences in the water quality before and after suspending biofilm.
- ◆ It was also assumed that there would be no statistical significant differences in the different types of container materials used to collect and store drinking water.

5 MINIMUM SAMPLE

The minimum samples sizes for statistical significance were determined before each series of experiments commenced at the various levels and approaches of this study.

The data in the sets used for this study were both according to normal-theory (parametric) and non-parametric estimates. In water resources measurements, these estimates generally “consider the important and frequently observed effects of seasonally or trend and so may never provide estimates sufficiently accurate to be anything more than a crude guide” (Helsel and Hirsch, 1995). Another important factor that requires careful consideration of the sample size is the availability of resources.

Nevertheless, one should determine approximately how big the sample size has to be – crude or not - in order to detect an effect or difference at a specified level of statistical difference or power (SigmaStat, 1997; Helsel and Hirsch, 1995).

The statistical programme SigmaStat Version 2.0 (1997) was used to calculate the sample size needed for statistical significance.

5.1 Sample size for ANOVA and DIFFERENCES testing

For this study, crude initial estimates of 15 samples for each microorganism group used for each water category were made based on the minimum number of samples prescribed by Standard Methods (1998) for an intra-laboratory proficiency programme.

After assessing the 1st 15 samples, the mean differences of each (n = 15) data set was used to estimate the final minimum sample size and to confirm whether the initial sample sizes were big enough.

ANOVA testing procedures (parametric or non-parametric) depend on whether the comparative data is normally distributed with equal variance. However, to determine the minimum sample size, the normality of data is generally ignored and the size determined according to the following parameters (SigmaStat, 1997, Helsel and Hirsch, 1995):

- ① The size of the minimum expected differences in the group means is entered. Based on typical null-hypotheses or data reliability theory, no differences



should be encountered means of data groups. However, standard statistical packages used to calculate the estimated sample sizes do not accept a zero entry as this is seen as statistically unrealistic (SigmaStat, 1997). Literature is also not very clear on how to approach the selection of minimum expected differences in the group means. The size of the minimum differences in the group means for the log data was therefore calculated for each comparison group individually based on the mean differences encountered after using data from the initial 15 samples.

- ② The size of the standard deviation of the data is entered. The size of the standard deviation could be the size expected (an estimate) or can be derived from previous experiments. Again, literature was unclear about what could be expected. It was decided to use studies by Jagals et al. (1997; 1999) as well as data from this study to calculate an overall mean standard deviation for each microorganism group used for each water and other category during this study. This would then be entered as the “expected” standard deviation and the calculated sample size suggested by the programme would then be used as a minimum sample size.
- ③ Desired power (sensitivity) of the test. Power is the probability that the correlation coefficient quantifies an actual association. The closer the power is to 1, the more sensitive the test. According to Helsel and Hirsch (1995), sensitivities in water resources testing is traditionally set to achieve a power of 0.80, which means that there is an 80% chance of detecting a difference / an association / a central value estimate with $1-\alpha$ confidence (i.e. 95% confidence when $\alpha = 0.05$).
- ④ Alpha (α) used to determine the sample size. The desired alpha (α) level is the acceptable probability of incorrectly concluding that there is an association. This indicates that a 1 in 20 chance of being wrong is acceptable (willing to conclude that there is a difference / an association / a central value estimate when $P = 0.05$).

6 NORMALITY OF DATA

Application of statistical techniques in the field of water resource management generally requires the assumption that data sets have symmetrical distributions

such as the normal curve. Problems can occur when statistical procedures are summarily employed assuming symmetry or linearity (Helsel and Hirsch, 1995).

In most water-quality related chemical analyses, the distribution of analytical results follows the Gaussian (normal) curve, which has symmetrical distribution of values about the mean. However, microbiological distributions are often not symmetrical (Standard Methods, 1998).

Bacterial counts often have a skewed distribution because of more low counts than high counts in a given monitoring set (Standard Methods, 1998).

6.1 Transformations

To produce data that would display normal distribution characteristics, transformations of data could therefore be used (Standard Methods; 1998). Transformations are used for three purposes (Helsel and Hirsch, 1995):

- To make data more symmetric.
- To make data more linear.
- To make data more consistent in variance.

For this study, skewed (asymmetrical) data set counts were transformed to their logarithms (ladders of power), which generally produced more symmetrical data. The rare instances where data were not log-transformed are clearly indicated in the relevant Sections.

6.2 Classification based on data distribution

Normally distributed around the mean because a lot of the variations between the subjects have been removed (Bland and Altman, 1986). These log-transformed data sets and their discussions are clearly indicated in the relevant text.

However, log-transformed data in this study often did not achieve normality despite of the general removal of variations. Even in log format, data remained slightly skewed although much closer to normal than before transformation.

Log-transformed data sets were always tested for normality before any other testing was employed.

Before every comparative test series were done, the data sets were tested for



normality (normality test). packages used for this study were SigmaStat 2.0 (1997) and SigmaPlot 5.0 (1998). These programmes use the Kolmogorov-Smirnov (K-S) normality test. The normality test-results display whether the data passed or failed the test of the assumption that the source population is normally distributed around the median. Failure of the normality test can indicate the presence of outlying influential points.

7 ANOVA

Equal Variance test results display whether or not the data passed or failed the test of the assumption that the samples were drawn from populations with the same variance (SigmaStat, 1997).

The classic technique for this comparison of data is analyses of variance (ANOVA) (Helsel and Hirsch, 1995). ANOVA is a parametric test done under the assumption that the data concerned are normally distributed around the mean with similar variance.

In instances where data sets did not pass normality testing, non-parametric testing were employed. Non-parametric testing should ideally be used wherever the distribution of data is not following the Gaussian curve of normality around the mean. These testing methods are also the ideal in instances where the sample size is between 10 and 30 – a criterion suggested by Helsel and Hirsch (1995) to distinguish between small (generally seen as 15) and big sample sizes.

7.1 Non-parametric tests

Where parametric testing loose considerable power to detect differences in non-normal data, non-parametric testing display considerable power in non-normal as well as normal data testing and display (Helsel and Hirsch, 1995). For this study therefore, non-parametric testing were used.

The following **ANOVA** tests were used:

- ◆ Rank-sum tests (Helsel and Hirsch, 1995). A rank-sum test is a non-parametric test for whether data in one group tends to differ from data in another group by being larger, smaller or larger and /or smaller. To test the hypotheses, the non-parametric Mann-Whitney Rank-sum test was used. This test was selected because:

- No assumptions were made about the normality or variance (shape) of the data.
 - It can determine whether data from each of the two groups come from the same population.
- ◆ Signed Rank tests (Helsel and Hirsch, 1995). A Signed Rank test is a non-parametric test whether the treatment differences (before and after) from the smallest to largest without regard to sign, then attaches the sign of each difference to the ranks. To test the hypotheses, the non-parametric Wilcoxon Signed Rank Test was used. This test was selected because:
- The procedure uses the size of the treatment effects and the sign.
 - If there were no treatment effect, the positive ranks should be similar to the negative ranks. If the ranks tend to have the same sign, it can be concluded that there was a treatment effect (there is a statistically significant difference before and after the treatment).
- (Helsel and Hirsch, 1995; SigmaStat[®], 1997)
- ◆ Kruskal-Wallis ANOVA on Ranks (based on rank transformation). This test was selected because:
- No assumptions were made about the normality or variance (shape) of the data distribution
 - It compares results from several different experimental groups (the three distances) that may be affected by a single factor.
- (SigmaStat[®], 1997; Helsel and Hirsch, 1995)

7.2 Rank results

- ◆ Rank transformation of data implies that the original data are replaced by ranks, which omits substantial variance and error from the multiple comparison procedure (Helsel and Hirsch, 1995).
- The P value is the probability of being wrong in concluding that there is a true difference in the groups. This implies falsely rejecting the null hypothesis. The smaller the P value ($P < 0.05$), the greater the probability that the results from the samples in the selected data sets are significantly different.

7.3 Multiple comparison t

When more than two methods are compared the interest is not only whether the three methods differed, but also which method differed from the others. Therefore, multiple comparison tests (MCT's) were applied where significant differences were encountered and the H_0 had been rejected (Helsel and Hirsch, 1995).

MCT's compare all possible pairs of group medians involved in the comparison.

The Tukey multiple comparison test because it is the more conservative of several available MCT's (SigmaStat[®], 1997) and therefore less likely to determine that a given difference is significantly different.

B DATA PRESENTATION

1 BOXPLOTS FOR SUMMARISING DATA

Boxplots are used because of their design ability to graph data representing certain statistical values (SigmaPlot[®], 1998). According to Helsel and Hirsch (1995), boxplots provide the clearest visual summaries of the following:

- ◆ The centre of the data (the median is the black centre line in the box). The median is the preferred measure of central tendency for the log-transformed data in the various Results Sections (Chapter 2). Because of log transformation, a lot of the variance had been removed although the outliers had been retained. The median is more resistant to the effects of the outliers, and would therefore tend to indicate the more realistic central point in the data. This is particularly useful when comparing data sets from various methods
- ◆ The variation or spread (interquartile range (IQR) – the box height indicates the spread of data between the 25th to the 75th percentile). The closer the data are clustered to the median within the IQR, the less variation (more stable) the data have.
- ◆ The skewness. This is also referred to as the quartile skew and is represented by the relative size of the box halves. The smaller the upper quartile skews, the more positive the data are skewed – a characteristic of water resources data (Helsel and Hirsch, 1995).
- ◆ The presence (or absence) of unusual values. The whiskers on the lines



protruding above and below the whiskers indicate the 90th and the 10th percentiles. The dot symbols beyond the last percentiles indicate outliers beyond the 90th and 10th percentiles.