THE OCCURRENCE OF HEALTH-RELATED WATER QUALITY INDICATOR BACTERIA ASSOCIATED WITH CONTAMINANT BUILD-UP IN VARIOUS TYPES OF DOMESTIC WATER STORAGE CONTAINERS

Dissertation submitted by

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DECLARATION OF INDEPENDENT WORK

I, **Nkope Jemina Ntsherwa,** Identity Number [redacted] and Student Number [redacted], do hereby declare that this research project, submitted to the Central University of Technology for the degree **MAGISTER TECHNOLOGIAE: ENVIRONMENTAL HEALTH**, is my own independent work.

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

..... **SIGNATURE OF CANDIDATE**

2004/03/31 **DATE**

Summary

This study assessed the occurrence of health-related microbiological indicator bacteria as well as of indicators of biofilm that might form inside various types of drinking-water storage containers in a domestic environment. The susceptibility of different container types to environmental contamination (dust, flies, ants etc) as well as the formation of contaminant build-up were also compared in order to identify the container type least likely to support biofilm formation. Previous studies have indicated that the way water is stored and used at home has often led to deterioration of its microbiological quality to a point where it posed a risk to consumer health. This appears to be a result of contaminant build-up (i.e. "biofilm" formation) in storage containers because of poor container hygiene and handling of containerised water by individuals. The results of this study indicated contaminant build-up formation in various types of containers, which contributed to the deterioration of water quality.

Container water quality was assessed *before* and *after* dislodging biofilm in the containers. A significant increase was found in the indicators of contaminant build-up (turbidity and heterotrophic bacteria) *after* the samples of mixed suspension were analysed. The level of turbidity and heterotrophic bacteria supported the assumption of contaminant build-up in all the types of drinking water storage containers. High counts of total coliforms, *Escherichia coli* and *Clostridium perfringens* were also observed *after* dislodging the contamination build-up. This showed a strong association between these indicators and those of contaminant build-up. It was therefore evident that biofilm did form as organic or inorganic surface deposits as well as microorganisms contributing significantly to the potential presence of pathogenic microorganisms in the container water.

The health-related quality of water did not comply with the values and limits proposed by various guidelines used for the study.

The level of contamination was found to be much higher in containers with maximum environmental contamination (uncovered / unrinsed) than in the containers with minimum environmental contamination (covered / rinsed), as had been expected. This contamination might have resulted from dusts and other environmental pollutants of the containerised water. Higher levels of microbial contamination and decreased water quality were associated with wide-mouthed storage containers (e.g. bucket-type containers) that are inadequately protected (uncovered or poorly covered). The water in the uncovered containers generally appeared to be subjected to contamination from the outside environment (such as dusts, flies etc). Floating bacteria and inorganic particulate matter that might be introduced into the water probably attached to the inner surfaces of the containers and formed the contaminant build-up, thereby

causing deterioration of water qu s of indicators of organic pollution (total coliform bacteria) were found in the plastic than in the metal containers. Polyethylene (from the plastic material) has been described in a number of studies as hydrophobic material, enhancing bacterial attachment and growth.

The container type least prone to contaminant build-up was determined by using the "*after*" data sets (worst scenario data sets). The quality of water in the screw-top containers differed significantly (lower indicator counts) from that of the water in the bucket-type plastic and metal containers. The screw-top containers were found to be the container types least prone to promoting build up of contaminants. Their smaller mouth-tops minimised contamination and therefore they appeared to be more suitable for use.

It was evident that improving household water collection and storage is one option for achieving a beneficial health effect. Household water collection and storage deserve due consideration in the prioritization and implementation of water, sanitation and hygiene measures for use at household, community and regional levels.

anorganiese partikulêre goed wat in and, het aan die binnekante van die houers vasgeheg en het die besmette opbou gevorm en sodoende die agteruitgang van die waterkwaliteit veroorsaak. Hoër vlakke van indikators van organiese besoedeling (totale kolivorme bakterieë) is in die plastiese as in die metaalhouers gevind. Poli-etileen (van die plastiese materiaal) is in verskeie studies beskryf as hidrofobiese materiaal wat bakteriële vashegging en groei verhoog.

Die houertipe wat die minste geneig is tot besmette opbou is bepaal deur die "na"-datastelle (die ergste scenario-datastelle) te gebruik. Daar is gevind dat die waterkwaliteit in die houers met skroefproppe betekenisvol (laer indikatortellings) verskil het van die van die water in die emmertipe plastiese en metaalhouers. Daar is gevind dat die skroefprophouers die houertipe is wat die minste geneig is om die opbou van besmetting te bevorder. Die houers met kleiner openinge het besmetting verminder en daarom het hul meer geskik vir gebruik voorgekom.

Dit het duidelik geblyk dat die verbetering van die opgaar en berging van huishoudelike water een opsie is om 'n voordelige gesondheidseffek te verkry. Die opgaar en berging van huishoudelike water moet deeglik oorweeg word in die prioritisering en implementering van water, sanitasie en higiëne-maatreëls vir gebruik op huishoudelike, gemeenskaps- en streeksvlakke.

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CHAPTER 2: METHODOLOGY

1 BACKGROUND

The purpose of this study was to find out whether the occurrence of health-related microbiological indicator bacteria in household water storage containers could be associated with organic and inorganic contaminants building up and adhering to interior sidewalls of various types of drinking water storage containers in a domestic environment, Motshabi Square, Free State Province, South Africa. The study also compared the susceptibility of different container types to such contaminant build-up. The container type least contaminated could then, in educational programmes, be recommended for preferred use by communities.

The rationale behind this study was that most people in informal settlements are not supplied with in-house or on-site (yard) taps and are likely to be dependent, for some time to come, on the use of containers to source, transport and store drinking water at their homes. Biofilm that forms in these containers may harbour pathogens introduced by a number of routes including the entry of dust in highly populated areas, aerosol-containing pathogens, as well as water handling with unwashed hands and utensils. Repeated use of such containers, after contamination, may lead to repeated consumption of contaminated water that in turn may result in the transmission of infectious diseases. Service providers that distribute treated water are facing a complex and challenging task of ensuring that safe drinking water supplies reach communities. The information from this study will assist public health workers to advise those people who are still relying on containers on how to optimally source, collect and store drinking water.

Previous studies in the area (Jagals, Grabow and Williams, 1997; Bokako, 2000) indicated that the manner water is stored and used at home often leads to deterioration of its microbiological quality. Studies in other areas also indicate that even though the microbiological quality of the municipal supply is good, the quality of the water worsens once sourced at communal standpipes and stored in the households (O'Connor and O'Connor, 1996; Genthe, Straus, Seager, Vundule, Maforah, and Kfir, 1997). This appears to be the result of some type of contaminant build-up (i.e. "biofilm" forming) in storage containers because of poor container hygiene and handling of containerised water by individuals in households (Jagals, Bokako and Grabow, 1999; Bokako, 2000; Nala, 2002; Jagals, Nala and Joubert, 2003).

Jagals et al. (2003; 1999; 1997) investigated whether the deterioration of water quality in storage containers could be associated with biofilm. They detected substantial numbers of

microbiological indicators, particula acteria, in contaminant layers that formed on the inside of container walls. This was reported by these investigators as probably a type of biological film similar to what was found in distribution systems by Piriou, Dukan, Levi, and Jarrige (1997) as well as Schaule and Flemming (1997).

Sidewall-adhering contaminants, usually in the form of a slimy, sticky substance, also occur in water distribution systems, and are referred to as biofilm (Kastl and Fisher, 1997). For the purposes of studying these occurrences in domestic water storage containers, the expression *biofilm* may also be used (Joubert, Jagals and Theron, 2003).

Three types of storage containers, bucket-type *plastic* and *galvanised metal* containers, as well as plastic screw-top containers were chosen for this study. It was observed that the particular communities mostly use screw-top containers, rolled or pushed with a wheelbarrow, to fetch water from communal taps. Any biofilm that might have formed on the inside of this type of containers and shaken loose with the rolling would settle at the bottom of the container once placed in the home. Bokako (2000) established that the quality of water deteriorated in such containers regardless of whether the storage containers were protected (closed) or unprotected (wide open at the top). The effect of container material type on general water quality was investigated (Bokako, 2000), with no significant differences observed in microbiological quality for plastic or galvanised metal containers. This investigation, however, did not include plastic screw-top containers and did not differentiate between factors such as wide-mouthed versus closable containers since the focus was at that stage the general microbiological quality of stored water.

The current study focused on the development of contaminant build-up, indicated by turbidity and heterotrophic bacteria, in various storage containers, and their association with the specific health-related microbiological indicator organisms total coliforms and *Escherichia coli*. The formation of contaminant build-up (and their differences) in various storage containers (their exposure to contaminant build-up or their prodivity to allow its formation) was also investigated.

One household was randomly identified in the study area and invited to participate. As an add-on to the one-roomed dwelling of the participating household, a sheet-metal shack had been erected in which the types of storage containers typically used in the area were stored and the water used from them. This simulated the real life situation experienced by the people in the area especially the factors that lead to container contamination.

Boyne (1997) stated that public drinking water provides a viable home for a variety of opportunistic pathogens, and these microorganisms create a thick, self-protective slime, better known as biofilm.

Fass, Dincher, Reasoner, Gatel and Block (1996) reported that the main sources of microorganisms in drinking water distribution systems are those present in the water after treatment (and supply) and sloughed from biofilms within the system.

Reiff (1996) reported that the formation and development of biofilm is influenced by a number of factors:

- presence of microbial nutrients in the water (organic pollution);
- characteristics of the wall such as the type and roughness of the material;
- microbial and chemical quality of the finished water; and
- water temperature and pH.

Camper, LeChevallier and Huck (2000) reported that the low nutrient environment present in drinking water distribution systems did not appear to be a hospitable environment for bacterial growth. Yet biofilms are found on almost every submerged surface in distribution systems (Flemming, Percival and Walker, 2002). Like other living creatures, bacteria require certain nutrients for growth, and therefore high-nutrient water may possibly result in excessive bacterial growth (Edstrom Industries Incorporation, 2003).

Pasmore (2001) reported that almost all conventional water distribution systems contain bacteria as well as many organic substances. Although water represents the main component of biofilms (Schaule and Flemming, 1997), it is the bacteria that begin to adhere to surfaces in aqueous environments and that excrete slimy, glue-like substances that anchor them to metals, plastic or any kind of material (Kastl and Fisher, 1997). In this process organic and inorganic contaminants are trapped within the substance, contributing to the build up of the filmy layer (Ladd and Costerton, 1990). Lindsay and Von Holy (1997) found out that in aqueous environments bacteria occur in two forms: planktonic (free floating) and those that are attached to the surfaces, with the attached state the most predominant form of microorganism survival.

The formation of biofilm associated with events of regrowth or after-growth in water distribution systems is one of the main reasons for the deterioration of the bacteriological quality of drinking water (Momba, Kfir, Venter and Cloete, 2000).

2.1 Attachment phenomena to form biofilm

Biofilm development is the result of a successful attachment and subsequent regrowth of microorganisms on a surface (Momba et al., 2000). Almost immediately after attaching itself to the walls, biofilm begins building upon itself, adding layer upon layer, forming a biological coating (Chlorine Chemistry Council, 1998).

The formation of biofilm is far from a random process and follows the following course (Lovell, 2001):

- reversible adsorption of bacteria (seconds);
- irreversible attachment of bacteria (seconds-minutes);
- growth and division of bacteria (hours-days);
- exo-polymer production and biofilm formation (hours-days); and
- attachment of other organisms to biofilm (days-months).

Lindsay and Von Holy (1997) also discovered and reported that biofilm formation occurs by a stepwise process including reversible attachment, irreversible attachment and colonisation. They also reported that bacterial cell attachment and subsequent biofilm formation on a variety of metal and non-metal surfaces can occur within contact times as short as twenty minutes.

Bacterial cells attach to surfaces conditioned with organic residues and frequently produce extra cellular polymeric substances (EPS), which allow for cell-to-cell bridges and also cement individual cells to surfaces (Bryers and Sharp, 1997; Lindsay and Von Holy, 1997; Schmitt and Flemming, 1999).

2.2 Biofilm occurrence in drinking water storage containers

In the context of this study, the expression *biofilm* was used to refer to the film or slimy layer of organic and inorganic material that forms on the inner container sidewalls or introduced by environmental factors (dust etc.) following sourcing, transportation and storage.

Biofilm in distribution systems is characterised in various ways. It is not only comprised of various organisms, but also consists of pieces of organic and inorganic matter, as well as microorganism by-products that bind the colonies together (extra cellular materials) (http: www.ci.sf.ca.us/puc/wqfs/biofilms.htm). Such studies have not yet discussed domestic drinking water storage container as part of such distribution systems. This was recently done so by Jagals et al. (2003), Nala (2002), Bokako (2000) and Momba et al. (2000). Biofilms are also reported to harbour bacteria, some of which might be hazardous to human health when ingested with the water from the distribution system (Jagals et al., 2003; Schaule and Fleming, 1997).

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Schaule and Fleming (1997) repo to confirm assumptions about whether deterioration in water quality is linked to biofilm, appropriate sampling should be done. According to the Standard Methods for the Examination of Water and Wastewater (1998), the levels of assimilable organic carbon (AOC) could be used as a gross measure for the potential of water to sustain bacterial growth, thereby indicating the potential for biofilm to develop on those surfaces that are in contact with water (Miettinen, Vartiainen and Martikainen, 1997). In this study, the characterisation of biofilm was not done due to the high costs involved. Measuring assimilable organic carbon (AOC) is a complex and costly biochemical procedure, which was beyond the scope of this study. Instead, a simple brushing technique, used by Bokako (2000) and Nala (2002), was used to determine whether the perceived film/layer contributed to the deterioration of the health-related microbiological quality of the stored water (Jagals et al., 2003). This brushing technique was adapted by Bokako (2000) from a swabbing technique originally used by Jagals et al. (1997). Nala (2002) and Jagals et al. (1997) also reported using it in their studies. In a study done to evaluate dislodging methods for the biofilm grown under laboratory conditions, Lindsay and Von Holy (1997) showed that surface scraping (scrubbing) could remove up to 97% of biofilm cells attached to stainless steel surfaces.

Turbidity was associated with the occurrence of biofilm, and other health-related indicator organisms, such as total coliforms and *E coli,* gauged the occurrence of microorganisms that might harm the health of people who used water from the containers (Jones and Bradshaw, 1996; Bokako, 2000; Nala, 2002).

As DiGiano, Zhang, Francisco and Wood (2001) found that water quality deteriorates within drinking water distribution systems, so did the previous studies in this area indicate that stored water at dwellings deteriorates to a quality not suitable for human consumption. This was reported to be a result of poor container hygiene and handling of water by individuals in households (Pinfold, 1990; Jagals et al., 1997; Bokako, 2000). Sobsey (1999) also reported that microbial contamination of collected and stored household water is caused, not by the collection and use of faecally contaminated water that was not safe to begin with, but by contamination of initially microbiologically safe water after its collection and storage.

According to the US Environmental Protection Agency (USEPA, 1996), the occurrence of excessive coliform bacteria in domestic water distribution systems is due to biofilm that grows on the material of the water distribution system, and it is also likely that biofilm exists at some level throughout drinking water distribution systems (MMWR, 1998). Jagals et al. (2003) also reported coliforms occurring in biofilms on the inside of domestic storage containers.

DiGiano et al. (2001) showed that bacterial regrowth is more likely to be seen at locations with long water residence time. Residence time is a key factor in determining the extent to which

bacteria can regrow. Residence tir portant factor in the current study since water was stored in containers and long periods of storage might have had an effect on the development of contaminant build-up such as biofilm.

Contaminated water that is biologically unstable, with a high bacterial growth rate, can support growth of bacteria on the inner walls of distribution systems, while biologically stable water (low bacterial growth rate) would not support such growth (Du, 1997).

Sobsey (1999) reported that factors contributing to the problem of household water contamination are unsanitary and inadequately protected (open, uncovered or poorly covered) water collection and storage containers, the use of unsanitary methods to dispense water from household storage containers, including faecally contaminated hands and utensils, lack of protection against contamination introduced by vectors (flies, cockroaches, rodents, etc.) and inadequate cleaning of the container to prevent biofilm formation and accumulation of sediments and pathogens.

Jensen, Ensink, Jayasinghe, van der Hoek, Cairncross and Dalsgraad (2002) reported that the storage of water for hours or even days allows the possibility of faecal contamination of good quality water. Contamination takes place when faecally contaminated hands and utensils come into contact with the water. Jensen et al. (2002) further described this domestic pathway of contamination as domestic domain transmission corresponding to in-house contamination. This domestic pathway of contamination of the household drinking water is independent of pollution at the source.

In the context of this study potential contamination routes were assumed to be:

- contamination at source and during transportation to the household environment;
- contamination while the containers were refilled (unwashed hands coming into direct contact with water, especially after a visit to the toilet); and
- storage methods. Protection of the container contents from environmental pollution (dust etc.). One set of containers was exposed to maximum environmental contamination (net-covering) and for the other set contamination was minimised by covering with a close-fitting lid.

An earlier study done in the area, Joubert et al., (2003) indicated that in most households the containers are placed near open windows and on the ground without any form of protection from contamination by dust, young children or household pets. In the set up of this study, the

same situation was simulated. Sor rs (covered and uncovered) were placed near the window on the table and the other containers were placed on the ground.

2.3 Effects of the system material on biofilm

Schaule and Flemming (1997) stated that non-sterile water such as drinking water, contains microorganisms that may colonise the surfaces with which it is in contact, and may form biofilms, regardless of the material. Different materials of the surfaces inside distribution systems have little or no effect on the intensity of biofilm development. Stainless steel is just as susceptible to biofilm growth as plastic.

In a study by Evison and Sunna (2001), it was found that different materials of household tanks (polyethylene, fibreglass and cast iron) did not significantly influence the total bacterial count of the stored water. However, a study done by Charnock and Kjønnø (2000) determined the release potential of assimilable organic carbon (AOC) by material containing polyvinyl-chloride (PVC). Measures of AOC were based on bacterial counts and were in principle related to the phenomenon of increase in heterotrophic plate count (HPC) during distribution. Therefore the level of heterotrophic bacteria was used to measure the availability of the assimilable organic carbon. During experimental phases, AOC continued to be released and PVC material continued to contribute to bacterial after-growth and building up of biofilm over long period.

Various types of plastic material are widely used in domestic drinking water distribution systems and according to Kalmbach, Manz and Szewzyk (1997), polyethylene has been described in a number of studies as hydrophobic material enhancing bacterial attachment and growth.

Biofilm formation is usually encouraged on the surface of the material (such as materials containing PVC), if that material is able to supply the required nutrients for bacterial re-growth (Ali-Vehmas, Tsitko, Vuoriranta, Kostyal, Ahlgren, Salkinoja-Salonen, 2000; Momba et al., 2000; Hem and Aquateam, 2002). According to Schaule and Fleming (1997) biofilm not only occurs on material that releases biodegradable substances but also on inert materials. Percival, Walker and Hunter (2000) reported that our increasing tendency to use systems made of modern biofilm-encouraging materials for distributing drinking water is giving greater opportunity for biofilms to develop.

This study compared the level of contaminant build-up formation in containers made of different materials. The purpose was to draw conclusions regarding the material of the container type best supporting bacterial growth or contaminant build-up.

Apart from the presence of the heterotrophic organisms detected in biofilms, a number of pathogenic and toxigenic microbiological agents have been detected (MacKay, 1997; Schultz and Ely, 2000). Biofilms serves as a focal point where bacteria and other microorganisms interact (Momba et al., 2000). The metabolic by-products of one organism can provide nutrients for other organisms. This enables proliferation in those organisms that would be unable to grow by themselves (Water Quality and Public Health, 2002).

The main concern would be that pathogenic organisms slough away from the biofilm and be released into the water (Noguera, Okabe and Picioreanu, 1999). It is when these pathogen cells erode from biofilms or the biofilm matrix that water becomes contaminated and high colony numbers detected, together with positive results for pathogenic bacteria if they are present (Schaule and Fleming, 1997; Momba et al., 2000).

DiGiano et al. (2001) reported that some fraction of attached bacteria is susceptible to detachment both because of its physiological state and the shearing action of water moving through the distribution system. This can also occur during filling of containers at a tap. The splashing of the water may cause the detachment of the biofilm from the container side walls, thereby contaminating the water with potential pathogens.

Momba et al. (2000) reported on the occurrence of biofilms that harbour various types of microorganisms. The most alarming results were the presence and multiplication of pathogenic and opportunistic pathogens such as *Salmonella, Pseudomonas, Aeromonas* and others which occurred within the biofilms.

Little is known of the behaviour of specific pathogenic microorganisms within drinking water distribution systems. Warnecke (1996) and Havelaar (1997) reported that the drinking water environment can bring about physiological changes in organisms, leading to difficulties in their detection and changes in behaviour different from those seen in other, more laboratory-oriented environments.

For this study, it would have been too costly to isolate each potential pathogen in the container water to confirm pathogen development. Instead, total coliforms and *Escherichia coli* bacteria, as well as the presence of vegetative spores of *Clostridium perfringens*, were used to indicate pathogen bacteria present in container biofilm. According to Jones and Bradshaw (1996) biofilms can be produced by members of the Enterobacteriaceae (coliforms and faecal coliforms). It therefore made sense to use these indicators in this study. Not only do they indicate the potential presence of pathogens, but they also serve as a confirmation of biofilm activity if present in excessive numbers. The use of indicators is discussed in Section 6.1.

4.1 Availability and accessibility of water

According to the World Health Organisation (1997), supplying safe, quality water to people is an important consideration in the protection of human health and well-being. Other factors such as accessibility, reliability of the supply and the cost involved must also be considered (WHO, 1997).

Jagals et al. (1997) reported that certain urban communities had to travel substantial distances (up to 300m and sometimes more) to collect water from communal standpipes, and the enroute environmental inputs such as dust may already have contaminated the water. It was also reported (Genthe and Seager, 1996, Jagals et al., 1999) that even when standpipes were brought into closer proximity of dwellings, the long source-to-consumption sequence still posed a problem (contamination of water along the route as a result of poor hygiene and handling practices).

The individual households in the study area were not supplied with in-house taps or standpipes in their yards. These people had to use containers to source drinking water from communal standpipes, which were quite far from their homes, and to store it in their houses.

Water sourcing and storing can be seen as distribution-related activities that negatively affect the quality of the water. Jagals et al. (1997; 1999) reported that supplying a community with treated piped water does not necessarily mean that water-related health risks will be totally eliminated, because water becomes contaminated during collection, storage and handling by the consumers.

Most water supply sources are not reliable or sufficient and the communities are often compelled to use substantial numbers of containers to collect and store their water. This bulk storage of water, often already contaminated during sourcing, leads to the development of biofilm, thereby posing risk of infection to the consumers. The various types of containers normally used by the communities are of plastic and/or metal, with or without covering lids or screw caps – the latter of which the inside is difficult to clean (Bokako, 2000).

4.2 Safe water

Due to difficulties in accessing clean drinking water supplies, the residents in the study area store water in various types of containers at their homes. Rijal and Fujioka (1998) reported that this type of household water containers might contain high levels of faecal indicator bacteria, indicating a greater possibility that pathogens are present in those water containers.

Safe and clean water is vital for healthy living practices as well as for consumption and is one of the primary requisites for healthy human life (Jagals, 2000). Drinking water should be suitable

excreted in the faeces of the infecte ngested by others in the form of faecally contaminated water (Grabow, 1996).

Bacterial regrowth has been associated with the likelihood of waterborne illness (DiGiano et al., 2001). Pathogens such as *Salmonella* and *Shigella* are associated with faecal pollution, and may be found in any water source subject to such contamination.

5.1 The effects of water storage on safety for use

Bokako (2000) reported that it is especially after collection and during storage that the health-related microbiological quality of container stored water deteriorates to such an extent that such waters pose a risk of infection to the consumers.

Of the many causes of drinking water quality deterioration in distribution systems, biological phenomena (organic contaminants) are undoubtedly the subject of most studies. They are also the most closely monitored because of short-term public health risks (Piriou, Dukan, Levi, Guyon and Villon, 1996).

In most developing communities (including the study area) with severe water shortages and large populations, distribution mains (communal taps) may be located very far away or may only supply water for few hours each week; the householders must store the water in buckets in their homes (Evison and Sunna, 2001). When these intermittent supplies are stored over a period of several days, water quality may rapidly deteriorate, posing a risk of infection to the consumer (Evison and Sunna, 2001).

Water contaminated with microbiological constituents can lead to a variety of diseases and can also play a major role in the spread of such diseases (Nevondo and Cloete, 1999). Genthe and Seager (1996) also reported that communicable water-related diseases, especially diarrhoea, are the most widespread health problems related to consumption of contaminated water at the point of use.

In developing communities, it is important to educate the population about good hygiene, maintenance of water delivery systems and safe storage of water in the household (Ford, 1997). Jagals et al. (1999) reported that improving water supply in developing urban areas without educating people on how to make use of such improvements might not achieve the improvement in quality of household water that should be expected with such improvements.

In this study, the focus was mainly to determine the occurrence of health-related water quality indicator bacteria (total coliforms, *E coli* and *C. perfringens*) associated with the biofilm (indicated by turbidity and heterotrophic bacteria).

The effects of environmental c the container-stored water were also considered. To determine whether incidental environmental contamination (dust, etc.) could be associated with biofilm formation, the volunteer household were requested to keep one of the two types of buckets covered to *minimise* environmental contamination. The others were to be kept open to allow for *maximum* environmental contamination. The water from the particular study containers was not used for drinking purposes because of possible microbiological contamination.

6 BIOFILM BUILD-UP IN CONTAINERS AND ITS EFFECT ON HEALTH-RELATED WATER QUALITY

Contaminated water constitutes a serious threat to public health worldwide because of the presence of microorganisms. These microorganisms constitute a threat to the safety of drinking water because they indicate the potential of the water to cause an outbreak of waterborne disease (Muyima and Ngcakani, 1998).

Momba et al (2000) reported that regrowth of microorganisms in drinking water distribution systems is caused by the utilization of biodegradable compounds which are either present in treated water or originate from materials in contact with drinking water (contamination during filling, storage and handling). Research has indicated that coliforms in distribution systems originate from biofilms and their levels increase throughout the system (Momba, Cloete, Venter and Kfir, 1999; Camper, Jones and McFeters, 2001).

Distribution systems also contribute to the deterioration of the water quality, since many factors (environmental, behavioural, etc.) can introduce bacteria into drinking water during distribution and use (Muyima and Ngcakani, 1998). Two of the main factors that were found to increase the numbers of bacteria in distribution systems are also considered causes for increasing bacterial numbers containers. These are:

- Microorganisms introduced from external sources by a number of means such as environmental contamination (dust, etc). Open containers are especially susceptible in this regard.
- Internal regrowth (contaminant build-up) or after-growth of bacteria and the associated formation of biofilms.

Microbial safety of drinking water has primarily been determined by testing for bacterial indicators of faecal pollution, mainly *Escherichia coli* and total coliforms. These indicators are used to assess the potential public health risk of drinking water and their presence or absence

are key elements of most drinking elines and water supply operating licences (Stevens, Ashbolt and Cunliffe, 2001).

To determine the risk of infection to the communities in the study area, the occurrence of biofilm-like contaminant build-up (measured by turbidity and heterotrophic bacteria) and health-related water quality indicators were assessed in various drinking water storage containers.

6.1 INDICATORS (PHYSICAL AND MICROBIOLOGICAL)

Turbidity was used with heterotrophic bacteria to indicate the occurrence of biofilm in container-stored water. Total coliforms and *E coli* were used as indicators of hazardous microbiological pollution of stored water. Total coliform indicator bacteria were used to measure the level of environmental contamination (potential level of organic pollution) of water stored in containers, whereas *E coli* were used to indicate the worst and most dangerous form of microbiological pollution, namely faecal pollution.

The cause and effect relationship between bacterial regrowth and water quality parameters has not been well established due to the many interdependent variables involved: resident time, temperature, the amount of utilizable carbon, inactivation rate by disinfectants have been identified as important (DiGiano et al., 2001).

6.1.1 Indicators of contaminant build up

6.1.1.1 Turbidity

This physical test measures the concentration of suspended matter (particles) in water by measuring the clarity of water (WHO, 1997).

Turbidity is caused by the presence of suspended and settleable matter, which normally consists of a mixture of organic and inorganic matter and other microscopic organisms (Water Research Commission, 1998). According to Coulson (2000) organic and inorganic matter enters storage containers during collection, transportation and during unprotected water storage (in open containers) at home. Depending on the nature of origin of the suspended matter causing turbidity, associated health effects can be expected (WRC, 1998).

Turbidity is an optical property of water that interrupts light transmission through water. This causes incident light to be scattered and absorbed. Turbidity results from the presence of suspended solids in water (Bromberg, 1995). The human eye can spot signs of "turbidity" due to the water's cloudy appearance or particle-laden characteristics. Direct association between the occurrence of microorganisms and turbidity has been reported, since turbid water often

contains higher general bacterial co ...uspended matter in turbid water can be both organic and inorganic matter (World Health Organisation, 1996).

For this study, turbidity was used as a physical parameter to indicate contaminant build-up. Variations in measured turbidity were therefore used in conjunction with assessing the occurrence of heterotrophic bacteria to measure the level of biofilm formation.

6.1.1.2 Heterotrophic bacteria

Heterotrophic bacteria are used as practical indicators of general microbiological water quality (Standard Methods, 1998; Lisle, 2003). Their presence indicates post-treatment contamination or regrowth of bacterial microorganisms in distribution systems (South African Water Quality Guidelines, 1996). For this study this was also assumed applicable to storage containers. A large variety of heterotrophic bacteria have been isolated from biofilm in distribution systems and the most alarming results are the presence and multiplication of pathogens (Momba et al., 1999). These bacteria are very widespread throughout water and may be distributed anywhere in a water system. Large heterotrophic bacterial counts in water indicate a deterioration of water quality and are a warning signal that more dangerous bacteria (pathogens) may be present and contaminating the water (Bromberg, 1995).

Heterotrophic bacteria test is a simple, inexpensive test that yields results in a relatively short time. The test detects a wide variety of organisms, primarily bacteria (Renfrew Water Analysis, 2002), which give an indication of the general microbiological quality of the water (WHO, 2002; Planzinska, 1998). Heterotrophic bacteria obtain their carbon and energy from organic compounds (American Water Works Association (AWWA), 2003). Camper et al. (2000) reported that monitoring Heterotrophic Plate Counts alone does not provide much useful information on the status of biofilm in distribution systems.

However, although high heterotrophic bacterial counts do not necessarily constitute a health risk, they are the sign that a particular network (storage buckets in the case of this study) is subject to biological disorders, which can protect pathogenic species (Piriou et al., 1997).

Heterotrophic bacteria were used in this study as a gauge of the changes in the levels of contamination caused by the biological component of the biofilm (Nala, 2002).

6.1.2 Indicators of hazardous microbiological quality

6.1.2.1 Total coliforms

The total coliform indicator group comprises mainly a vaguely-defined group of facultative anaerobic, gram-negative, non-spore forming, rod-shaped bacteria, which ferment lactose and

produce acid and gas within an : 24-48 hours at 35 to 37°C (Standard Methods, 1998).

Total coliform bacteria were used to measure the level of organic pollution. These are the primary indicators of the potability and suitability for consumption of drinking water. Their presence in water may indicate faecal contamination (Standard Methods, 1998).

The occurrence of total coliforms in the stored water also provided useful information on environmental contamination of the stored water (Jagals, 2000).

Coliform organisms have long been recognized as a suitable microbial indicator of drinking-water quality, largely because they are easy to detect and enumerate in water (Cartwright, 1996; Strecker, 1998; Standard Methods, 1998).

The presence of total coliforms in drinking water is a "general warning light" that the quality of the water is undesirable. Since total coliforms are bacteria that colonise the intestines of humans and warm-blooded animals, they serve as a signal that other pathogens may exist in the water (Bromberg, 1995). Total coliforms indicate microbial growth in distribution systems or may be indicators of post-treatment contamination of drinking water (USEPA, 1996; South African Water Quality Guidelines, 1996). For this study total coliforms were used as indictors for organic pollution of container-stored water (Standard Methods, 1998; Capital Regional District Water Department, 2001).

6.1.2.2 *Escherichia coli (E coli)*

Total coliform testing is less reliable as an indicator of faecal pollution (Standard Methods, 1998). Stevens et al. (2001) reported that total coliforms have been shown to be a poor parameter for measuring the potential for faecal contamination of drinking water due to their presence as normal inhabitants of soil and water environments, and their ability to grow in drinking water distribution systems. These factors, therefore, mean that it is difficult to interpret the sanitary significance of their presence (in the absence of *E coli*) or to have confidence in water quality in their absence (Stevens et al., 2001).

E coli is a member of the faecal coliform group of microorganisms, which generally inhabits the intestines of warm-blooded animals and is regarded as the best indicator of faecal contamination of water (Grabow, 1996; Bromberg, 1995). According to the South African Water Quality Guidelines (1996), *E coli* have been found to constitute approximately 97% of faecal coliform bacteria in human faeces.

Jagals et al. (2001) reported that the presence of *E coli* in water also represents useful indication of the risk of infection to users. *Escherichia coli* is highly specific for the faeces of humans and

warm-blooded animals (Momba et lity of *E coli* to spread via drinking water was recognised earlier and this bacterium became the standard marker of faecal contamination in drinking water.

Pilot scale water distribution networks have been used to study the fate of *E coli* in drinking water systems (Parent, Fass, Dincher, Reasoner, Gatel and Block, 1996) and it was shown that low levels of these bacteria enter the distribution system and are able to adapt and grow. Since *E coli* can develop in drinking water biofilm, it is not surprising to find some *E coli* in distributed waters, even when they are below detectable concentrations in treated water (Parent et al., 1996; Sibille, Sime-Ngando, Mathieu and Block, 1998).

For this study, *E coli* were used to measure the level of potential faecal contamination of the container-stored water.

6.1.2.3 *Clostridium perfringens*

Clostridium perfringens is an anaerobic, spore-forming, gram-positive, key species of the sulphite-reducing clostridia commonly found in human and animal faeces (Water Quality and Public Health, 2002). The spores are highly resistant to a range of environmental conditions and can survive in water for a long time (Planzinska, 1998; Francis, Lockley, Sartory, Watkins, 2001). Their presence can indicate occasional or intermittent pollution of the drinking water source.

Clostridium perfringens is present in faeces in smaller numbers than the *E coli* and it is less sensitive as an indicator of faecal contamination (Water Quality and Public Health, 2002). Low numbers may occasionally occur in water supplies, but they do not represent a risk to health. These bacteria will not grow to significant numbers or produce toxins in water supplies, as conditions are usually unsuitable (Water Quality and Public Health, 2002). Nevertheless, these organisms indicate direct as well as remote faecal pollution. No bacteria of this group should be detected in drinking water (Payment, 1995).

This indicator was used to indicate the presence of resistant microorganisms such as protozoan parasites that could be harmful to human health because they are also spore-forming and can survive adverse conditions (Payment, 1995).

7 STUDY AIMS

7.1 Rationale

Biofilm that forms in household drinking water storage containers may harbour pathogens, and repeated use of such containers, after contamination, may lead to increased biofilm build-up and consumption of contaminated water that may result in the transmission of infectious diseases.

7.2 Research Problem

It was uncertain whether container type played a role in the support and even propagation of container biofilm.

7.3 Aim

The aim of the study was, therefore, to assess the occurrence of health-related indicator bacteria as well as of indicators related to biofilm that might form inside various types of drinking water storage containers in a domestic environment.

7.4 Objectives

In order to achieve the aim, the following objectives had to be met:

- To indirectly assess the occurrence of biofilm in various types of water storage containers, using *heterotrophic bacteria* and *turbidity* as indicators.
- To assess the occurrence of health-related indicator bacteria (*total coliforms*, *E. coli* and *C. perfringens*) associated with biofilm indicators in the water storage containers.
- To compare these occurrences under different domestic storage conditions and to offer an opinion on the container type least likely to support the contaminant build-up and formation of biofilm.

7.5 Scope of the study

- The mean data values as well as the values at the 95th percentile are inclusive of all seasons since the scope of this study is to form an overall impression of contaminant build-up.

Chapter 2 (Methodology) will cover the overall study set-up, equipment and procedures used for data collection and analyses. Chapter 3 (Results and Discussion) will focus on the results of indicators of biofilm (contaminant build-up) and health-related water quality from the uncovered and covered bucket-type plastic and metal containers, as well as from rinsed and unrinsed screw-top container sets, before and after dislodging the contaminant build-up.

1 STUDY AREA

The study was done in one of the lesser-developed urban areas of the Mangaung Local Municipality in the Free State Province of South Africa, i.e. Motshabi Square in the city of Bloemfontein. This is a rapidly expanding high-density informal settlement with low socio-economic development and limited sanitary facilities and drinking water provision. None of the households had individual in-house taps or yard standpipes. The people used water storage containers to collect drinking water from communal standpipes, which in some cases can be some distance away, and store it in their houses.

2 POPULATION (HOUSEHOLD) SAMPLE SELECTION

To increase the focus of the study, one household was selected from a random group of households and invited to participate on a voluntary basis. The study needed a reliable and cooperative family living in the area. A Technikon Free State Community Development Officer identified such a household, which was subsequently invited to participate.

3 EXPERIMENTAL ENVIRONMENT

A sheet-metal shack was erected in the yard as an add-on to the one-roomed dwelling of the volunteer household. The shack was meant to simulate the real life situation experienced by the people in the area. Various types of drinking water storage containers were provided and the handling required for the containers, which included storage in the shack, was explained to the household members.

3.1 Storage container set-up

3.1.1 Storage container types

The study used three types of storage containers, with a minimum capacity of at least 20 litres (to ensure prolonged water retention time between fillings).

- Two wide mouthed bucket-type *plastic* containers – of which one had a close-fitting lid.
- Two bucket-type *galvanised metal* containers – of which one had a close-fitting lid.
- Two plastic screw-top (small-orificed) containers.

3.1.2 Stored container-water ap

It was made clear to the participating household that the water was not to be used for direct drinking purposes because of possible microbiological contamination during the experimental phase. They continued using containers of their choice for fetching and storing their drinking water.

The containers were all filled routinely as water was used for other domestic purposes such as washing clothes and dishes, as well as for body washing.

For reasons discussed later in the text, *rinsing* the containers instead of *washing* (e.g. with detergents) was preferred. Furthermore, this fitted in with the general situation where the community did not have the resources to wash their containers constantly with detergents and therefore those that díd have a sense of container hygiene would tend merely to rinse the container at the filling point.

3.1.2.1 Setting up the bucket-type containers

The wide-mouthed, bucket-type plastic and galvanised metal containers used to store water are shown in Appendix E, Figures 1, 2 and 5. The volunteer household diligently kept one bucket of each container type (one plastic and one metal) covered with its close-fitting lid whenever water was not being taken from the bucket. These containers were also rinsed with every filling. This was to *minimise* environmental contamination. For reporting purposes, the expected pollution profile is therefore described in the context of *minimum contamination.*

The other two buckets (one plastic and one metal) were left open at all times (and never rinsed during filling) to allow for *maximum* environmental contamination as well as to encourage biofilm to form *(maximum contamination).*

The above-mentioned set-up aimed at determining whether incidental environmental contamination (dust etc) could be associated with biofilm formation.

3.1.2.2 Setting up the screw-top containers

Two screw-top containers (Figure 3, Appendix E) were used to store water. The two plastic screw-top containers whether their small orifice were being capped or not, almost inadvertently lent themselves to minimum environmental contamination. The upshot was that these are difficult to even rinse effectively. One was never rinsed while the other one was rinsed as best as the household members could before each filling.

This determined, with incidental environmental contamination already minimised because of the small opening, whether biofilm formed quicker on the inner sidewalls of this type of container (Chapter 1; 1.2.2).

3.1.2.3 Setting up the control co

A bucket-type plastic container, comparable to those used in the experimental environment, was kept in the laboratory on a bench with the lid near-permanently kept on (Figure 4, Appendix E). It was only opened when samples were taken and during refill. The container was never rinsed but was slowly and completely emptied and refilled with fresh water on a weekly basis.

The rationale for doing this was to determine whether the expected contamination of the six experimental containers was indeed introduced from the study area environment. The approach was to compare the results obtained from this container to those of the other six storage containers as the water in the control container was assumed to be less exposed to environmental conditions (dust, etc.) as well as negative hygiene and handling practices.

4 HEALTH-RELATED WATER QUALITY ANALYSES

4.1 Water sampling

Twenty-six sampling sessions were conducted for a period of one year (covering all four seasons). Water samples were collected on a weekly basis in sterile Whirlpacks® and immediately transported, at temperatures less than 10°C, to the water-quality laboratory of the Water and Health Research Unit of the Technikon Free State, Bloemfontein. Analyses were completed within six hours of collection (Standard Methods, 1998).

4.2 Municipal water

Samples were also taken from the municipal water supply (communal tap) in the area every time the container water was sampled. This was to assess the microbiological and aesthetic quality of water supplied at the communal taps installed by the municipality before any conclusions could be drawn about container water contamination and container biofilm. Where possible, the tap water samples were collected at the same time that at least one filling of the containers took place. This was generally achieved.

To achieve external disinfection, water was run for 5 minutes and the mouth of the tap flamed prior to sampling. The samples for microbiological analysis were treated with sodium thiosulphate to stop continuing the bactericidal action of any free chlorine still present in the water.

4.3 Sampling container water

To establish whether indicator organisms were resident in the biofilm, or whether they were incidental to the internal water volume in the containers, samples were collected from the same container *before* (sampling undisturbed container water) and *after* loosening the contaminant

build-up from the inner sidewalls (i sample). The following summarises the procedure:

- The water sample was firstly taken from the container without disturbing the contents to any great extent (the *before* sample).
- Using a sterile long-handled brush, the inner walls of each of the abovementioned containers were scrubbed to dislodge whatever contaminant build-up (including biofilm-like substances) that might have formed on the side walls. During brushing any organic or inorganic contaminant that might have built up on the container inner sidewalls was loosened into the container contents.
- Introduction of any substance from the outside environment was carefully avoided. For instance, the analysts avoided touching the water or creating excessive floating dust in the dwelling. Sampling was not done on windy days.
- By swirling the container, any dislodged contaminant build-up (biofilm and other particulates) that might have been introduced into the containers was suspended in the water. A sample of the mixed suspension was then taken (the *after* sample).

4.4 Indicators of water quality

Indicators of health-related water quality were used to determine the level/extent of contaminant build-up in various types of drinking water storage containers.

A single comprehensive guideline for all four of the contaminant indicators did not exist. Several different health-related water quality guidelines had to be used to evaluate the results of the water quality indicators in the various water samples. The following guidelines were used:

- For heterotrophic bacteria counts: the South African Water Quality guidelines: Vol. 1: Domestic Water (Department of Water Affairs and Forestry (DWAF, 1996).
- For turbidity: the Water Research Commission: Assessment Guide: Quality of Domestic Water Supplies (WRC, 1998). Vol.1, 2nd edition.
- For *Escherichia coli*: the World Health Organisation: Guidelines for drinking water quality (WHO, 1996) (2nd ed) Vol2: Health criteria and other supporting information. Geneva, Switzerland.
- For *Clostridium perfringens*: the Proposed Water Quality Criteria in South African (Aucamp and Vivier, 1990).

4.4.1 Indicators of contaminant build-up in storage containers

4.4.1.1 Turbidity

Changes in the concentration of suspended matter were assumed to indicate changes in the levels of particles suspended in containers (i.e. indicator of contaminant build-up as discussed in Chapter 1, Section 6.1.1). The level of turbidity was determined by comparing the *before* and *after* samples. This was done to indicate whether the brushing/scraping had any effect on the increased or decreased level of the contaminant build-up and associated microbiological indicator bacteria. An increase in turbidity, after brushing, was assumed to be a function of the dislodged build-up of contaminants on the inside walls of the storage containers.

A HACH 2100 turbidity meter was used to measure turbidity levels. The measurements were recorded as Nephelometric Turbidity Units (NTUs).

Two risk-limits were used as guidelines according to the Assessment Guide Volume 1: Quality of Domestic Water Supplies (Water Research Commission, 1998). The lower limit was for *insignificant potential health effects* (≤0.1 NTU) and the upper limit for *slight potential health effects* (at 1 NTU).

4.4.1.2 Heterotrophic Plate Counts (HPC)

Heterotrophic bacteria counts were used to indicate changes in the general microbiological quality of the stored water *before* and *after* contaminant build-up suspension. This was used as a gauge of the changes in the levels of contamination caused by the biological component of the contaminant build-up i.e. biofilm (Nala, 2002).

Heterotrophic bacteria were assessed by a pour plate method (Appendix A), using Heterotrophic Plate Count (HPC) media (Standard Methods, 1998).

The criteria in the South African Water Quality Guidelines (Department of Water Affairs and Forestry, 1996) were used. The limits ranged from *negligible risk of microbial infection limit* (0 - 100 counts/1-mℓ), *slight risk* of microbial infection (100 - 1000 counts/1-mℓ), to *increased risk* of infectious disease transmission (>1000/1-mℓ).

4.4.2 Indicators of hazardous microbiological pollution of water in storage containers

4.4.2.1 Total coliforms (TC)

Total coliforms were used to indicate hazardous microbiological pollution of the stored water associated with contaminant build-up.

TC and *Escherichia coli* were simultaneously detected by the membrane filtration (MF) technique (Appendix A) using Chromocult® Coliformen agar (Merck, 1996).

Guidelines on infection risk limits v he Assessment Guide Volume 1: Quality of Domestic Water Supplies (WRC, 1998). The risk limits were *insignificant chance of infection limit* (0 - 10/100 mℓ) as well as the upper limit above which *clinical infections* may occur (100 / 100 mℓ) in sensitive groups. In the final counting of the TC colonies, the *E. coli* counts were included in the total numbers of TC since these form part of the total coliform bacteria group.

4.4.2.2 *Escherichia coli (E. coli)*

E. coli were used to measure the extent of faecal pollution as well as their association with the contaminant build-up in the stored water.

The *E. coli* results were interpreted according to the Guidelines for Drinking Water Quality (World Health Organisation, 1996) because the South African Water Quality Guideline sets do not provide for *E. coli* criteria. Safe water in terms of the WHO (1996) guidelines is regarded as water with zero (0) counts of *E. coli* per 100 mℓ. According to this guideline, any *E. coli* in drinking water is an indication that the water should not be ingested.

4.4.2.3 *Clostridium perfringens* (CP)

This indicator was used to indicate the presence of resistant microorganisms such as protozoan parasites that could be harmful to human health (Payment, 1993). CPs were detected by membrane filtration (Appendix A), using Perfringens agar (Oxoid Manual, 1990).

The results were interpreted according to the Water Quality Criteria in South Africa by Aucamp and Vivier (1990). An *insignificant risk* upper limit of 1 organism / 100 mℓ, as proposed by the guideline, was used.

4.5 Colony Verification

Colony verifications for TC and *E. coli* were done with the multi-test identification system (Analytical Profile Index, API® 20E Multi-test Galleries of bioMerieux®). Rapid ID® 32 A Multi-test Galleries (bioMérieux®) were used for the colony confirmation of *Clostridium perfringens*.

This was done to calculate more reliably the detected indicator numbers by excluding the false positives. False positives are non-indicator organisms that manage to grow on the selective medium within in the same colour range that is prescribed and used for colony identification (Standard Methods, 1998).

5 STATISTICAL DATA A

5.1 Data Management

Data were captured in Microsoft Excel (XP) spreadsheets. To facilitate analyses, the microbiological data were log transformed (log_{10} values) to remove excessive variance and get the data more symmetrical (Helsel and Hirsch, 1995). Since it was expected that the variation in turbidity data would not be as severe as that of the microbiological data, turbidity values were not log-transformed. The data were statistically described according to the arithmetic mean of the logs and the median as central values, sample size, range, 25th and 75th percentiles, and 95 % confidence intervals.

The 95th percentile was used to measure compliance. This work dealt with Drinking Water. The South African Water Quality Guidelines (DWAF, 1996) hints towards the use of the 95th percentile for measuring compliance of drinking water quality.

5.2 Analysis of variance (ANOVA) in data sets

The statistical bases of this study were differences between data groups (e.g. data *before* and *after* brushing). This required ANOVA (Appendix D). Since data in studies of this nature are seldom normally distributed around the mean (Helsel and Hirsch, 1995), this study used non-parametric statistical tests throughout. Non-parametric tests do not assume normal distribution of the data.

The statistical computer programme Sigma Stat® Version 2.0 (1997) was used to calculate and test for sample sizes and statistical significant differences (ANOVA) between data sets.

Tests used in this study were (Helsel and Hirsch, 1995):

- The Wilcoxon Signed Rank test was used to test for statistically significant differences in the paired *before* and *after* data (per container). The Signed Rank test is used for paired sets of non-normal data.
- The Mann Whitney Rank Sum Test was used to test for significant differences between two unpaired and unequal data sets.
- The Kruskal-Wallis ANOVA on Ranks was used to test for changes between more than two data sets. For this study, this test was used to test for changes in different types of containers, i.e. the bucket-type plastic and metal containers, and plastic screw-top containers.

5.3 Minimum sample size

The minimum sample size was determined before the experimental sessions commenced. This was done to ensure that ANOVA and association testing was done at a statistically acceptable level. An initial minimum sample size of 15 samples for each microorganism group used for each container category was applied, based on the minimum number of samples prescribed by Standard Methods (1998) for proficiency in a laboratory programme. For this study, the approaches used to determine sample sizes are discussed in Appendix D. Up to 26 samples per indicator per container were eventually taken.

6 STATISTICAL HYPOTHESES FOR THIS STUDY

Statistical hypotheses were formulated for each of the sections in Chapter 3: Results and Discussion. For this study, data were statistically analysed to address a particular hypothesis within each section in Chapter 3, based on whether the hypotheses were accepted or rejected. The approaches that follow are summarised in Tables 1, 2 and 3 at the end of Section 7.

6.1 Chapter 3: Sections 3.1 and 3.2: Comparing water quality data from covered and uncovered bucket-type containers as well as *before* and *after* data

The occurrence of water quality indicators associated with contaminant build-up was determined by analysis of water samples from the covered/rinsed (minimum contamination expected) and uncovered/unrinsed (maximum contamination expected) buckets, *before* and *after* dislodging and suspension of the container contents.

The following hypotheses were developed:

6.1.1 Paired container data (Table 1) – all the *before* versus *after* data sets

The Wilcoxon Signed Rank test was used because each of the data sets was paired.

Null hypothesis (H_0)

There would be no significant difference in the levels of indicators of contaminant build up or hazardous microbiological pollution in the *before dislodging* and the *after dislodging* results of the containers for either maximum or minimum exposure to environmental contamination.

Expected outcomes

- There would be a significant increase in indicators *after* brushing (inner side walls of the containers) because of the dislodging of loosened biofilm into the water content.
- It was expected that the increase in the levels of contamination in the containers with maximum environmental contamination (*before* results < *after* results) would be much higher

than the increase in the contamin containers with minimum environmental contamination (*before* < *after* results).

Interpreting the findings

Rejection of the H_0 would imply that there was a significant increase (or decrease) between the *before* and *after* results. This would indicate some event that brought about the significant changes, e.g. the brushing and container swirling had released biofilm and environmentally-introduced particulates into the container water content. This effect is then discussed.

6.1.2 Unpaired container data (Table 1) – all the *maximally-contaminated* versus *minimally-contaminated* data sets

The data were not paired in the sets. The Mann Whitney Rank Sum Test was therefore used.

Null hypothesis (H_0)

There would be no significant difference in the contamination levels of water in the covered/washed containers and uncovered/unwashed containers for all the container types.

Expected outcomes

- It was expected that there would be no significant increase of indicators in the *before* results for the *maximally contaminated* versus the *minimally contaminated* containers because the samples were taken from the water content only – before dislodging and suspending the contaminant build-up.
- A significant increase was expected in the *after* results (*maximally contaminated* versus the *minimally contaminated*) because a greater contaminant build-up was likely in the maximally exposed containers than in the minimally exposed ones.

Problems experienced

The collection of the *after* sample might have had some effect on the results. Since the screw-top containers have smaller openings, it was very difficult to brush the inner sidewalls effectively but the effort was made to shake the container vigorously so that the mixed suspension could be sampled.

Interpreting the findings

Acceptance of the H_0 would simply imply no contamination increase. However, rejection of the H_0 would imply that there had been a significant change in the numbers of the indicators. This

would indicate that there had been as the covering and/or washing of the containers, that had brought about the significant changes. This effect is then discussed.

6.2 Chapter 3: Section 3.3: Comparing container types (Table 2)

The Mann Whitney Rank Sum as well as Kruskal-Wallis Tests were used to determine the container type least prone to contaminant build-up under the test circumstances. For this only the *after* data sets (worst scenario data sets) were used. The rationale for using these data was that if all three container types were prone to promoting build-up of contaminants, at least one would be more likely to be so than the others.

The following two **Null hypotheses (H_0)** were developed:

- There would be no significant differences in the water quality of samples obtained from the plastic and metal bucket-type containers (both with minimum [covered] and maximum [uncovered] environmental contamination). These two types were dealt with first because it was expected that these containers, because of their wide open mouths, would be more prone to environmental contamination.
- There would be no significant differences in the water quality of samples obtained from the bucket type containers and the screw-top-type containers (both with minimum [covered for the buckets and regularly rinsed for the screw-tops] and maximum [uncovered for the buckets and never rinsed for the screw-tops] environmental contamination). Multiple comparison tests enabled assessment of all three container types.

Expected outcomes

- There were no expectations in respect of the open-mouthed containers. It was not possible to predict which container-type would be most likely to foster contaminant build-up.
- It was expected that there would be a significant difference in the results of the three container-types.

Interpreting the findings

Rejection of the H_0 would imply that there had been a difference in the water quality sampled from the three container types. The most contaminated type would be assumed the most likely to have harboured contaminant build-up.

6.3 Data analyses approach

The approach followed with the analyses of data and formulation of the hypotheses (as illustrated in Tables 1 and 2 will be used in the discussion of the results in Chapter 3. This will be done in sections.

Table 1: ANOVA for data of water quality in various covered and [illegible]re and *after* suspending the contamination build-up

Contaminant build-up potential	Plastic container (bucket-type)		Paired data Wilcoxon Signed Rank Test	Metal container (bucket-type)		Paired data Wilcoxon Signed Rank Test	Plastic containers (screw top)			Paired data Wilcoxon Signed Rank Test
	Before (B) scrubbing	**After (A) scrubbing**		**Before (B) scrubbing**	**After (A) scrubbing**		**Container contamination potential**	**Before (B) scrubbing**	**After (A) scrubbing**	
Maximum Uncovered buckets	H_0 B =A	H_0 B =A	Significant increase in indicators expected Reject H_0 if MaxB ≠ MaxA	H_0 B = A	H_0 B = A	Significant increase in indicators expected Reject H_0 if MaxB ≠ MaxA	**Maximum** Unrinsed container	H_0 B = A	H_0 B = A	Significant increase in indicators expected Reject H_0 if MaxB ≠ MaxA
Minimum Covered buckets	H_0 B=A	H_0 B=A	Significant increase in indicators expected Reject H_0 if MinB ≠ MinA	H_0 B = A	H_0 B = A	Significant increase in indicators expected Reject H_0 if MinB ≠ MinA	**Minimum** Rinsed container	H_0 B = A	H_0 B = A	Significant increase in indicators expected Reject H_0 if MinB ≠ MinA
Mann-Whitney Rank Sum Test	No increase of indicators expected Accept H_0 if Min=Max	Significant increase of indicators expected Reject H_0 if Min≠Max	**What if the increase of** MaxB ≠ MaxA ≥ MinB ≠ MinA	No increase of indicators expected Accept H_0 if Min=Max	Significant increase of indicators expected Reject H_0 if Min≠Max	**What if the increase of** MaxB ≠ MaxA ≥ MinB ≠ MinA	Mann-Whitney Rank Sum Test	No increase of indicators expected Accept H_0 if Min=Max	Significant increase of indicators expected Reject H_0 if Min≠Max	**What if the increase of** MaxB ≠ MaxA ≥ MinB ≠ MinA
	Explain differences			**Explain differences**				**Explain differences**		

Table 2: ANOVA to determine the container type least prone to contaminant build-up under the test circumstances

Contaminant build-up	Plastic and metal buckets-type containers Mann-Whitney Rank Sum Comparison
Max	*After* Data - H_0 Plastic = Metal
Min	*After* Data - H_0 Plastic = Metal
	If differences are significant then reject H_0 and explain differences

Plastic screw top containers	Plastic bucket-type containers	Metal bucket-type containers	ANOVA on Ranks Kruskal-Wallis test
After Data	*After* Data	*After* Data	H_0 Screw-top = Plastic = Metal
After Data	*After* Data	*After* Data	H_0 Screw-top = Plastic = Metal
If differences are significant then:reject H_0 and explain differences			

This chapter is divided into sections in accordance to the data analyses approach (Tables 1 and 2 in Chapter 1). In the sections that follow, indicator results as found in various types of storage containers used in the study, will be illustrated graphically and discussed. The first section (A - first phase) will deal separately with the water quality results from the bucket-type plastic and galvanised metal containers, plastic screw-top containers, as well as the plastic container (control). Section B covers the second phase of the study, in which the bucket-type (wide-mouthed) plastic and metal containers are compared. In Section C, the plastic, metal wide-mouth containers and the plastic screw-top container are compared under maximum-exposed circumstances.

SECTION A: RESULTS FROM ALL THE CONTAINER TYPES

3.1 Bucket-type plastic containers (covered and uncovered)

This section describes the health-related water quality results for both the covered and uncovered bucket-type plastic containers *before* and *after* dislodging the contaminant build-up. "Maximum contaminant build-up potential" refers to the uncovered containers and "minimum contaminant build-up potential" refers to the covered containers. "*Before*" refers to undisturbed samples (Chapter 2; Section 4.3) and "*after*" refers to the sample of the mixed suspension.

The guidelines used in this study were the South African Water Quality Guidelines (DWAF, 1996); Assessment Guide for Quality of Domestic Supplies (WRC, 1998); Drinking Water Quality Guidelines of the World Health Organisation (WHO, 1996); and the Water Quality Criteria in South Africa (Aucamp and Vivier, 1990).

3.1.1 Turbidity indicating contaminant build-up in plastic bucket-type containers

Table 3.1.1 (Appendix F) summarises the results. Figure 3.1.1 shows that turbidity levels increased significantly ($P \leq 0.001$ and $P = 0.004$) *after* scrubbing of the inner container sidewalls of both the uncovered and covered containers. This increase indicated that the brushing and swirling of the containers dislodged and released biofilm particulates into the container water content. Bokako (2000) and Nala (2002) also reported that after the containers' inner sidewalls were brushed to release potential biofilm, the sample would contain increased particulate matter that would influence turbidity.

According to the Assessment Guide for Quality of Domestic Water Use (WRC, 1998) NTU levels above 0.1 indicate a slight risk of potential health effects should the water be used for

drinking and food preparation. Alt itself does not have direct health effects, it is one of the indicators of microbiological water quality, and depending on the nature of origin of the suspended matter there may be associated health effects (WRC, 1998). The water did not comply since both the mean values (the red line in each box) as well as the values at the 95th percentile (the black dot above the horizontal caps at the end of the upper whiskers) were above the 0.1 NTU limit (blue line).

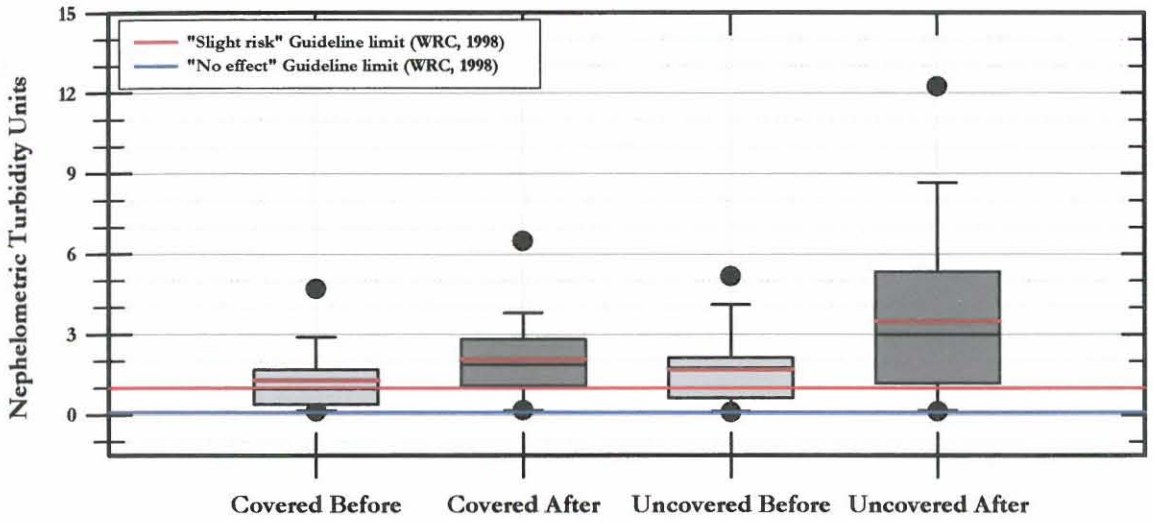

Figure 3.1.1: Comparing turbidity levels of water *before* and *after* dislodging contaminant build-up in covered and uncovered bucket-type plastic containers

Turbidity levels of water in both the uncovered and covered bucket-type plastic containers, *before* dislodging the contaminant build-up, were compared. There was no significant increase (P=0.126), as expected, and the hypothesis was not rejected. A significant increase was expected in uncovered (compared to covered) containers, *after* loosening the biofilm because a greater contaminant build-up was likely in the maximally-exposed containers than in the minimally-exposed ones. There was, however, no significant increase (P=0.198). This implied that there was no difference in the potential of either the uncovered (maximally-exposed) or the covered (minimally-exposed) containers to support contaminant build-up. In a study done by Nala et al. (2003), lower levels of turbidity (indicating less biofilm) were measured after an education intervention into unhygienic water use by Dywili and Jagals (1999) as well as Nala (2002), which focused on container cleaning practices.

Although not significant, the greater increase (Figure 3.1.1) in turbidity levels in the containers with maximum contamination (uncovered) as compared to the one with minimum contamination (covered) implied that incidental environmental contamination (dust, etc.) might have played a role in the formation of contaminant build-up. Turbidity levels were above the

slight risk limit (1 NTU) even *before* ilm into the containers, which indicated continuous contamination of the water through environmental factors such as dust.

3.1.2 Heterotrophic bacteria indicating contaminant build-up in plastic bucket-type containers

There was a significant increase ($P \leq 0.001$), as expected, in the level of heterotrophic bacteria *after* scrubbing the inner sidewalls of both groups of containers. The results are shown in Table 3.1.2, Appendix F. This increase showed that the contaminant build-up that had formed on the inner sidewalls of the containers contained microbiological elements, the basis for biofilm. Although the heterotrophic bacteria group does not include all the bacteria in water, it does represent the potential total number of bacteria in water, and the higher this level of bacteria the greater the chance of all kinds of bacteria not related to the coliform group that may be ingested (Department of Water Affairs and Forestry, 1996). Figure 3.1.2 shows the results.

The organism numbers at the 95th percentile exceeded those stipulated in the guidelines (DWAF, 1996). This meant that the water did not comply with the guideline limits (slight risk and negligible risk limits, indicated by the red and blue lines in the graph).

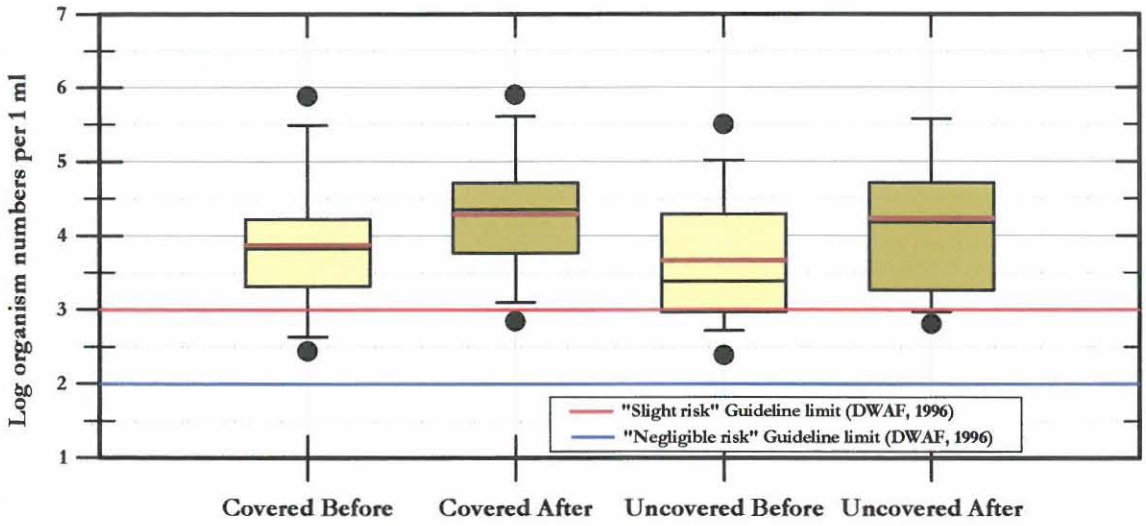

Figure 3.1.2: Comparing heterotrophic bacteria numbers of water *before* and *after* suspending contaminant build-up in covered and uncovered bucket-type plastic containers

According to the South African Water Quality Guidelines (DWAF, 1996), heterotrophic bacteria levels above 1000 organisms per 1mℓ pose an *increased risk* of infectious disease transmission. Their presence in drinking water poses a difficult problem because it is not clearly known whether they are really innocuous or whether they are harmful. They could be unimportant whatever their number or they could be opportunistic pathogens if allowed to multiply in large numbers (Payment, 1995). Heterotrophic bacteria levels for both uncovered

and covered plastic bucket-type cont the guideline limit, indicating a slight risk of infection to the users.

There was no significant increase (P = 0.413 and P = 0.489) when the vertical data sets were compared (Appendix F, Table 3.1.2). This implied that both maximally and minimally-exposed containers supported contaminant build-up. There was no difference in the level of contamination, *after* dislodging, for the uncovered and covered bucket-type plastic containers. However, as with turbidity, the results showed a greater increase in heterotrophic bacteria in the containers with maximum contamination (uncovered) as compared to the one with minimum contamination (covered). This supports the supposition that incidental environmental contamination (dust, etc.) might have played a role in the formation of contaminant build-up. Theron (2000) and Bokako (2000) reported that water in uncovered containers is generally subjected to contamination from the outside environment (such as dusts, flies etc.). They furthermore explained that floating bacteria and inorganic particulate matter that might be introduced into the water can attach to the surface of the containers and form part of biofilm, thereby contributing to deteriorating water quality (Theron, 2000; Bokako, 2000 and Mutevu, 2002). Nala (2002) also emphasized that excessive levels of heterotrophic bacteria can be introduced into relatively clean-looking water supplies by negligent water handling practices.

3.1.3 Total coliforms indicating hazardous microbiological pollution in plastic bucket-type containers

There was a statistically significant increase ($P \leq 0.001$) in the total coliform levels *before* and *after* the suspension of the contaminant build up (Table 3.1.3, Appendix F).

This indicated that total coliform levels increased due to some form of organic contamination that built up on the inner sidewalls of the containers and was released into the water following scrubbing and swirling. The presence of total coliforms can be an indicator of faecal contamination (Commercial Environmental Monitoring, 2003). LeChevallier (1999) described biofilm as a collection of organic and inorganic, living and dead material collected on a surface. Jones and Bradshaw (1996) reported that reduction in biofilm could lead to lower levels of total coliform numbers in water distribution systems.

There was no significant increase (P = 0.242 and P = 0.790) in the vertical data sets (Table 3.1.3, Appendix F). This indicated that there was no difference in the susceptibility of both the uncovered and covered containers to encourage and promote build-up of contaminants by organic matter. Both maximally and minimally-exposed containers allowed contamination of the water by organic pollutants that contributed to the formation of contaminant build-up.

Figure 3.1.3 shows the numbers of te ia detected in water from the uncovered and covered bucket-type plastic containers *before* and *after* dislodging the contaminant build-up. According to the Assessment Guide: Quality of Domestic Water Supplies (WRC, 1998), an insignificant chance of infection exists if the level of total coliforms exceeds 10 organisms per 100 mℓ (red line on the graph). The organism numbers at the 95th percentile exceeded this limit, posing the risk of clinical infections and serious health effects should the water be used for drinking and food preparation.

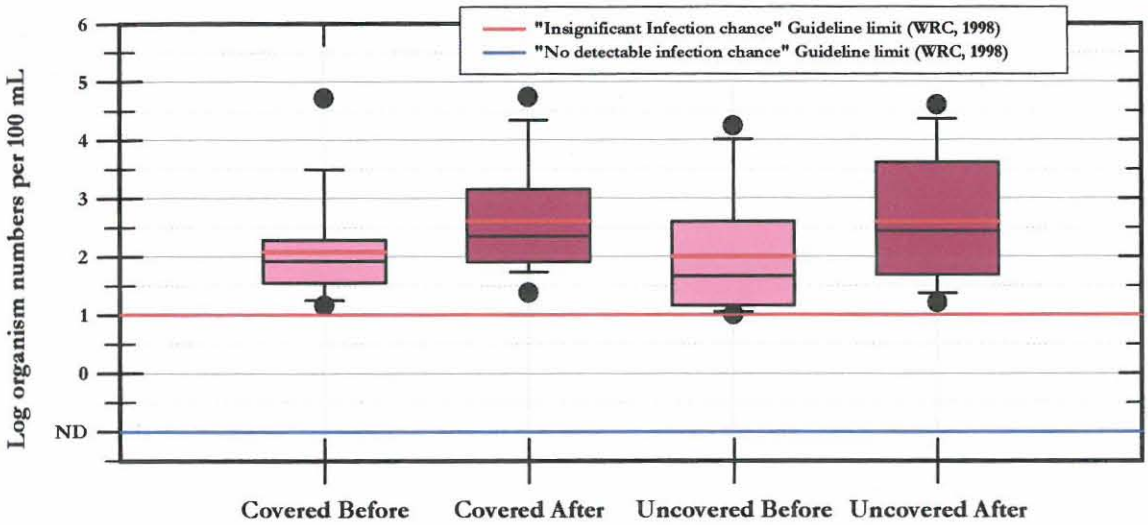

Figure 3.1.3: Comparing total coliform numbers of water *before* and *after* dislodging contaminant build-up in covered and uncovered bucket-type plastic containers

3.1.4 *E coli* indicating faecal contamination in plastic bucket-type containers

The levels of *E coli* were assessed in both the undisturbed and the mixed suspension samples. Table 3.1.4 (Appendix F) shows the *E coli* numbers in water from the covered and uncovered bucket-type plastic containers *before* and *after* dislodging the contaminant build-up.

As with the previous indicator groups, the level of *E coli* increased significantly *after* scrubbing of the inner sidewalls of the containers. There was a statistical significant increase ($P \leq 0.001$) in the levels of *E coli* after dislodging the contaminant build-up in the container water content. The results (Figure 3.1.4) suggested that the water was being faecally contaminated and that *E. coli* was being harboured in the container biofilm. Parent et al. (1996) showed that *E coli* have the ability to survive or grow in biofilm.

The organism numbers for both sets of containers, at the 95th percentile, exceeded those stipulated in the guidelines for *negligible risk* limit (0 organism detectable (ND) in 100 mℓ water, indicated by the red line in the graph) as proposed by the World Health Organisation for drinking water (WHO, 1996).

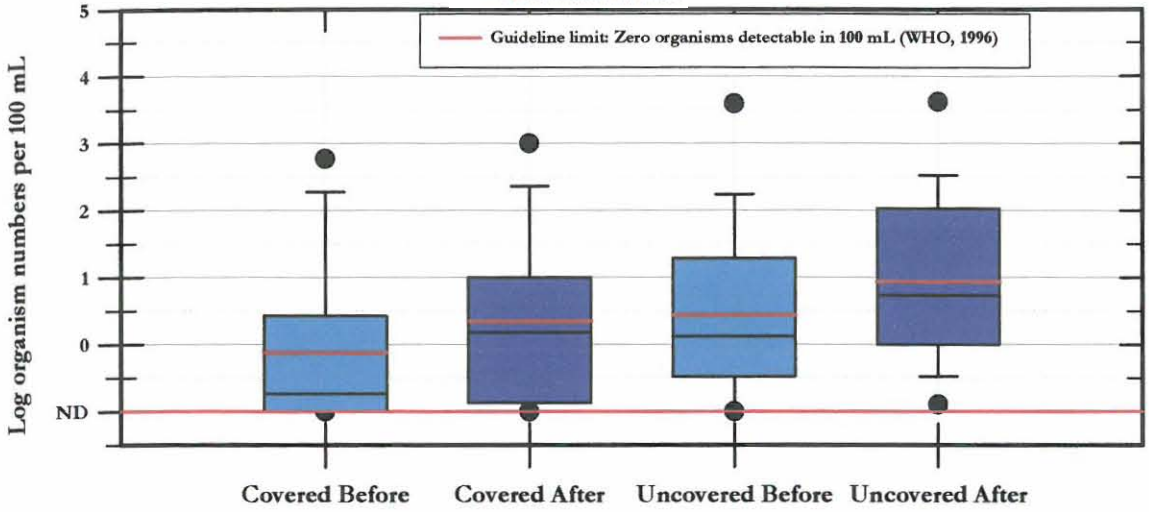

Figure 3.1.4: Comparing *E coli* numbers of water *before* and *after* dislodging the contaminant build-up in covered and uncovered bucket-type plastic containers

Potential contamination routes such as container hygiene between fillings, and environmental contamination during storage, might have introduced these faecal materials into the water. Jagals et al. (1999; 1997), Bokako (2000) and Nala (2002) also found *E coli* in the container water and the occurrence of these bacteria were attributed to poor personal hygiene practices, especially after toilet use by household members. However, these intermittent occurrences of *E coli* were reduced significantly during and immediately after a hygiene education intervention by Nala (2002).

There was no significant increase (P = 0.083 and P = 0.142) when the vertical data sets were compared (Table 3.1.4, Appendix F). These results showed that both the uncovered and covered containers allowed for faecal contamination of the water, indicated by the level of *E coli*. The *E coli* in the contaminant build-up may have found their way into the containerised water and were able to adapt and grow (Parent et al. (1996).

3.1.5 *C perfringens* indicating the presence of resistant microorganisms in plastic bucket-type containers

Clostridium perfringens were detected in both the *before* and *after* water samples from the bucket-type plastic containers with a significant increase in *C perfringens* numbers *after* scrubbing the inner sidewalls of the containers, indicating that resistant/spore-forming microorganisms were present in the biofilm and could end up in the stored drinking water. Neither data sets (*before* and *after*) complied with the guideline limits (indicated by the blue and red lines in Figure 3.1.5 proposed by the Water Quality Criteria in South Africa (Aucamp and Vivier, 1990). The organism numbers at the 95th percentile exceeded those stipulated in the guidelines. This meant that there was a risk of infection to the users should the water be consumed.

The results in Table 3.1.5 (Appenc istical significant increase (P = 0.181 and P = 0.241) between vertical data sets and the hypothesis was not rejected. This indicated that water from both the uncovered and covered containers encouraged the build-up of contaminants, therefore giving the spore-forming bacteria a suitable environment in which to grow.

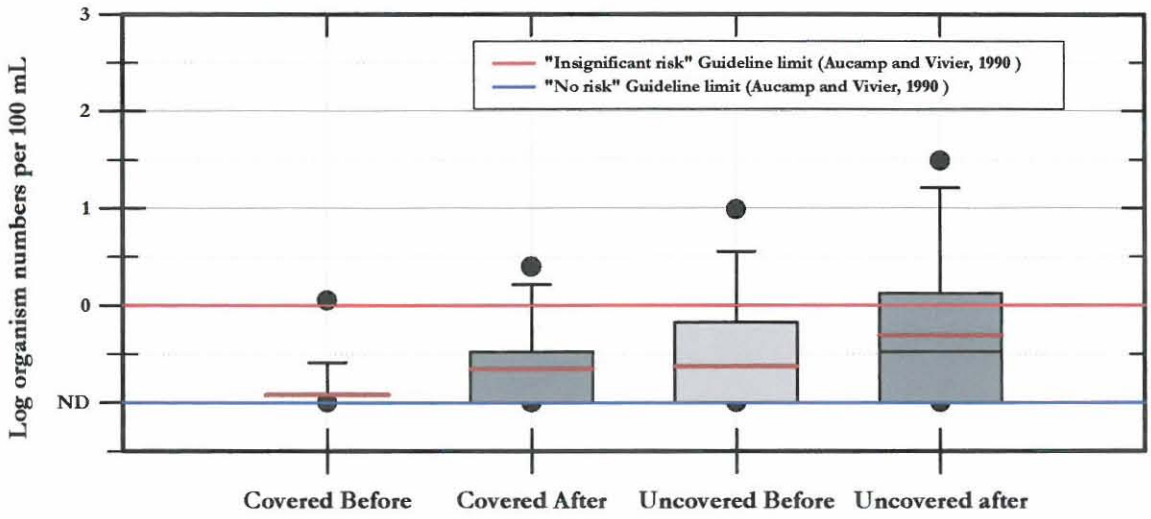

Figure 3.1.5: Comparing *C perfringens* numbers of water *before* and *after* suspending contaminant build-up in covered and uncovered bucket-type plastic containers

Figure 3.1.5 shows the results of *C perfringens* as found from the covered and uncovered bucket-type plastic containers *before* and *after* dislodging the contaminant build-up. These spore-forming bacteria were intermittently found in storage container water. Since *C perfringens* are commonly found in human or animal faeces (Water Quality and Public Health, 2002), their presence in the stored water might have been due to faecal contamination that took place during filling or introduced by environmental factors such as dust etc. (Refer to Chapter 1, Section 2.2, for potential contamination routes in the context of this study).

3.2. Bucket-type metal containers (covered and uncovered)

In this section, results of indicators found in water from the bucket-type galvanised metal containers are shown and discussed. As with the previous section, "maximum contaminant build-up potential" refers to the uncovered container and "minimum contaminant build-up potential" refers to the covered container. The *before* and *after* theory, undisturbed sample and the sample of the mixed suspension, still applies as in the previous section.

3.2.1 Turbidity indicating contaminant build-up

A significant increase in turbidity levels was observed *after* dislodging the contaminant build-up in both the container with minimum contaminant build-up potential as well as the one with

maximum contaminant build-up pot ıesis was therefore rejected. The process of dislodging the contaminant build-up resulted in the suspension of particulate matter (organic or inorganic in origin) that had accumulated or grown in the containers, leading to the interruption of light transmission through water, and increased turbidity. The results verified the assumption that the contaminant build-up that formed in the containers would be dislodged and would become suspended in the container contents following the effects of the sterile brush as Nala (2002) and Bokako (2000) had found.

According to the results in Table 3.2.1 (Appendix F) and Figure 3.2.1, turbidity levels posed a risk of potential health effects in terms of the Assessment Guide: Quality of Domestic Water Supplies (WRC, 1998). Both data sets of the covered and uncovered containers, *before* and *after* dislodging, were within the limits of probable secondary health effects, should the water be used for drinking (WRC, 1998).

At the 95th percentile, turbidity levels exceeded those stipulated in the guidelines (blue and red lines in the graph) and therefore the water did not comply. The increase in turbidity, *after* scrubbing the uncovered container, was higher than the increase in turbidity of the water *after* scrubbing the sidewalls of the covered container. Again, as with the bucket-type plastic containers, the assumption that incidental environmental contamination (dust, etc.) might have played a role in the formation of contaminant build-up was verified. There was no significant increase ($P = 0.407$ and $P = 0.145$) when the vertical data sets were compared, and the hypotheses were not rejected (Table 3.2.1, Appendix F). This showed that both the covered and uncovered metal containers supported the development of contaminant build-up. There was no difference in their potential to promote the build-up of contaminants.

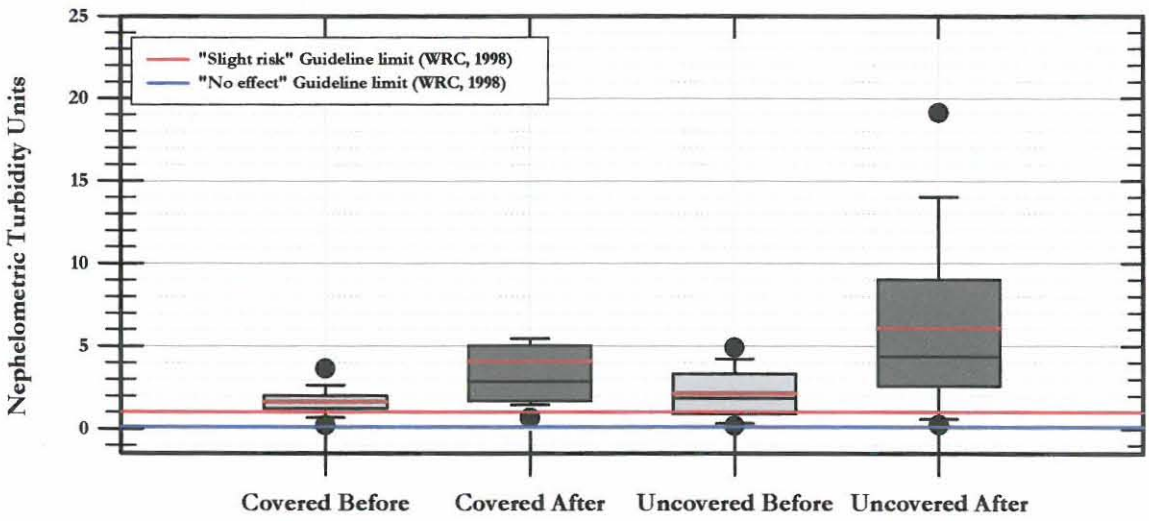

Figure 3.2.1: Comparing turbidity levels of water *before* and *after* dislodging contaminant build-up in covered and uncovered bucket-type metal container

3.2.2 Heterotrophic bacteria i inant build-up

The results in Table 3.2.2 (Appendix F) indicated a significant increase in heterotrophic bacteria *after* scrubbing the inside walls of both the covered and uncovered containers. The hypothesis was rejected. The effect of the brush and swirling of the containers released biofilm and other environmentally introduced particulates into the container water contents.

Lund and Ormerod (1995) reported that a large variety of heterotrophic bacteria, from potentially pathogenic bacteria to coliform bacteria, have been isolated from biofilms in water distribution systems. The greater increase from the uncovered container was attributed to dust and other environmental contaminants.

Vertical data sets (Table 3.2.2, Appendix F) showed no significant increase (P = 0.281 and P = 0.687), and the hypotheses were not rejected. The "no increased effect" indicated the water from both covered and uncovered containers to be subjected to contamination, which led to deposition and build-up of contaminants.

According to the South African Water Quality Guidelines (1996), heterotrophic bacteria level within 0 – 100 counts/ 1 mℓ range pose a negligible risk of microbial infection and the level above 1000 counts/ 1 mℓ indicate post treatment contamination or after-growth in the water distribution system. The organism numbers at the 95th percentile exceeded those stipulated in the guidelines and therefore the water did not comply with the guideline limits (blue and red lines in the graph). Figure 3.2.2 shows the results.

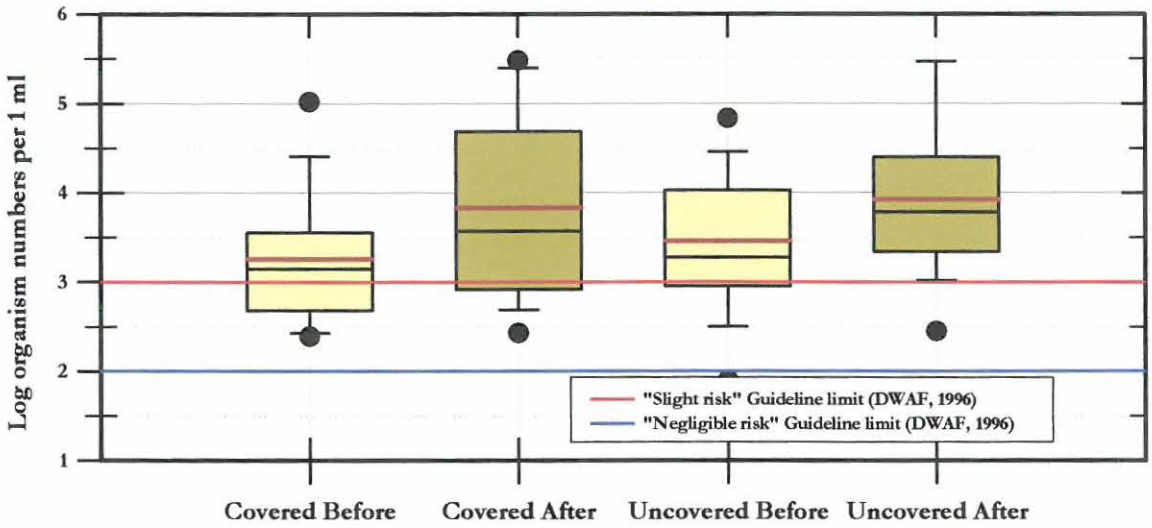

Figure 3.2.2: Comparing heterotrophic bacteria numbers of water *before* and *after* dislodging contaminant build-up in covered and uncovered bucket-type metal containers

3.2.3 Total coliforms indicating biological pollution

Table 3.2.3 (Appendix F) shows a significant increase ($P \leq 0.001$), as expected, in the total coliform level *after* scrubbing of both the uncovered and covered containers. The null hypothesis was therefore rejected. This indicated that the contaminant build-up that formed or adhered to the inside walls of the containers was also organic in origin supporting reports that the steps in biofilm development incorporates adhesion by organic molecules and free floating bacteria to the surface (Edstrom Industries Incorporation, 2003; Utah Department of health, 2002). However, the increase was much higher in the uncovered than in the covered containers, suggesting the effect of environmental contamination.

There was a significant increase ($P = 0.021$) in total coliform level *before* suspension of the contaminant build-up in both the uncovered and covered containers (Table 3.2.3, Appendix F). This indicated that the uncovered metal container might have been exposed to increased pollution (either faecal or organic) when compared to the covered container. The hypothesis was rejected. Conversely, there was no significant increase ($P = 0.072$) *after* suspension of the contaminant build-up in the uncovered and covered containers. The hypothesis was therefore not rejected. It is evident that even though the environmental contamination was minimised, contaminant build-up continued to develop in the bucket-type metal containers. It was evident that even low-nutrient conditions can support the growth and development of biofilm (Edstrom Industries Incorporation, 2003).

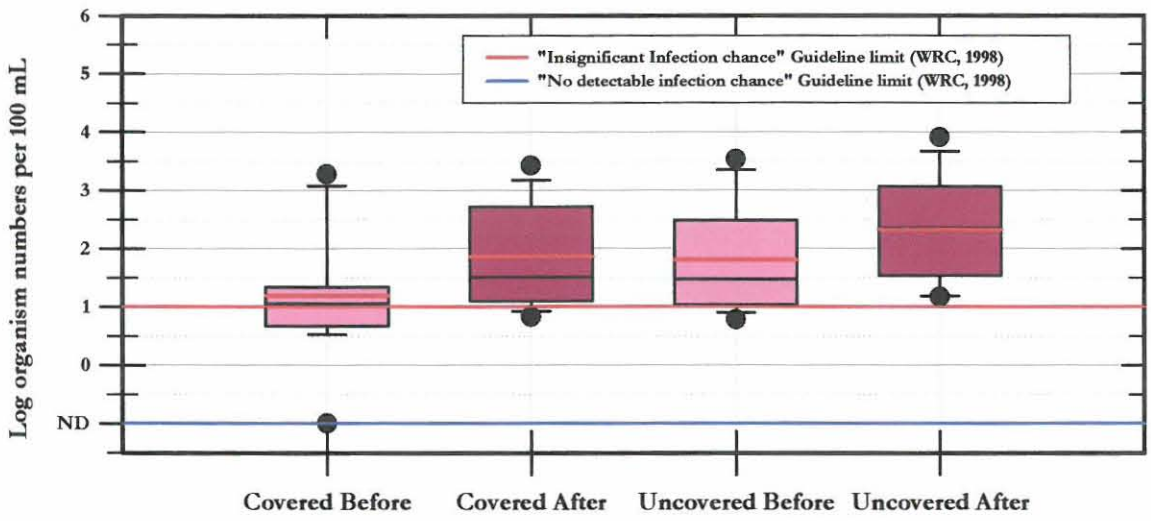

Figure 3.2.3: Comparing total coliform numbers of water *before* and *after* dislodging contaminant build-up in covered and uncovered bucket-type metal containers

According to the Assessment Guide: Quality of Domestic Water Supplies (WRC, 1998), total coliform level within the range of 0-100 counts/ 100 mℓ may pose an insignificant chance of infection and the level above 1000 counts/ 100 mℓ may pose serious health risks in all the users

when the water is used for drinking : ɔility of infection when the water is used for other domestic purposes. Organism numbers at 95th percentile were above the limit as proposed in the assessment guide and therefore the water did not comply with the required limits, indicated by the red and blue lines in Figure 3.2.3 (WRC, 1998).

3.2.4 *E coli* indicating faecal contamination

E coli presence in water indicated faecal contamination and a possible presence of pathogenic organisms (Grabow, 1996; Jagals, 2000). Water that contains any number of *E coli* is not safe for human consumption (WHO, 1996).

The results in Table 3.2.4 (Appendix F) show a significant increase ($P \leq 0.001$) in *E coli* levels *after* scrubbing the inner sidewalls of the containers. This implied that *Escherichia coli* were intermittently found in the stored water. Bokako (2000) reported that the presence of *E coli* at similar levels *before* and *after* dislodging biofilm can be attributed to poor water hygiene and handling practices of stored water. An increased level from the uncovered container was attributed to environmental contamination since the containers were never covered. Bokako (2000) further reported that water in open containers is subjected to contamination from the outside environment and floating bacteria that might be introduced into the water can attach to the surface and form part of biofilm. In addition, Nala (2002) and Jagals et al. (1997) reported that the use of open-ended bucket-type containers, left uncovered, exposed the water to unhygienic conditions that pose a greater contamination potential than when the container is covered.

A significant increase ($P = 0.049$ and $P = 0.007$) when the vertical data sets were compared indicated that the level of *E coli* of the undisturbed water from the uncovered container was high when compared to the level of *E coli* of the undisturbed sample (*before*) from the covered container (Table 3.2.4, Appendix F). The same phenomenon was also observed in the disturbed sample (*after*). This indicated that the uncovered bucket-type metal container was more susceptible to contamination. However, both the covered and uncovered metal containers allowed for contamination and development of contaminant build-up.

Guidelines for Drinking Water Quality (WHO, 1996) stipulate that no *Escherichia coli* should be present in water intended for drinking purposes. Considering the fact the organism numbers at the 95th percentile exceeded those stipulated, water from the covered and uncovered metal containers did not comply with the required limits (red line in Figure 3.2.4), and thus posed a risk of infection.

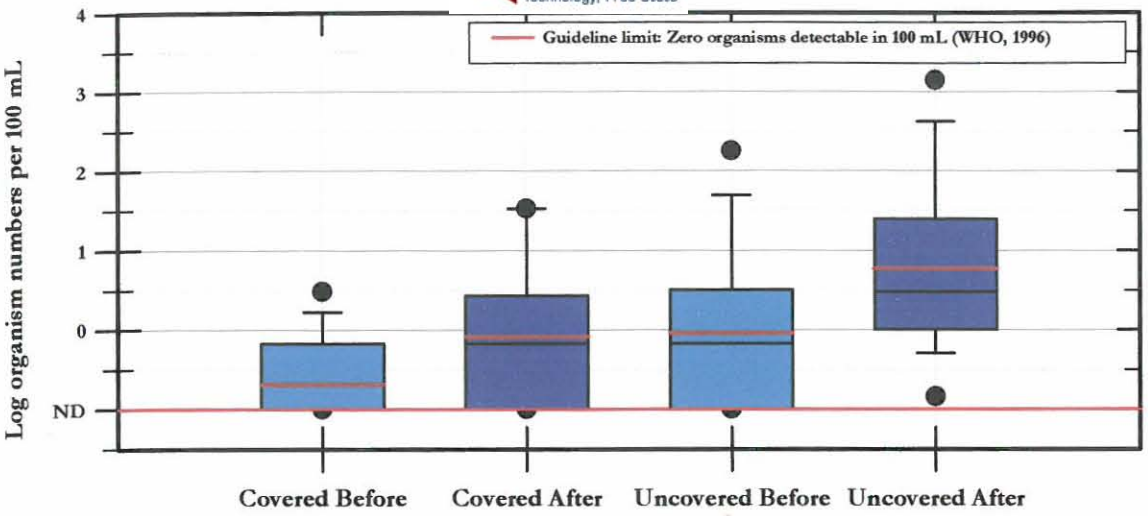

Figure 3.2.4: Comparing *E coli* numbers of water *before* and *after* dislodging contaminant build-up in covered and uncovered bucket-type metal containers

3.2.5 *C perfringens* indicating the presence of resistant microorganisms

Table 3.2.5 (Appendix F) shows a significant increase in the *C perfringens* level following dislodging of the contaminant build-up in the uncovered bucket-type container. The results indicated that *C perfringens* were occasionally found in water. According to the Water Quality Criteria in South Africa (Aucamp and Vivier, 1990) water with 1 *C perfringens* organism / 100mℓ or above poses an insignificant risk of infection. Therefore the *C perfringens* results in the bucket-type metal containers did not comply with the required limits and posed a risk of infection to the users.

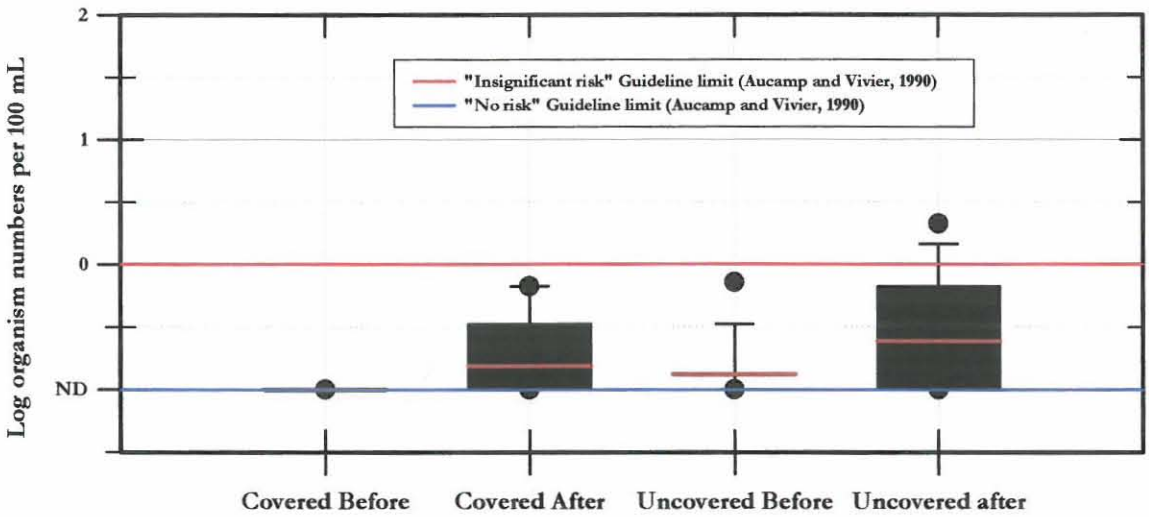

Figure 3.2.5: Comparing *C perfringens* numbers of water *before* and *after* dislodging contaminant build-up in covered and uncovered bucket-type metal containers

There was, conversely, no significant increase in *C perfringens* level *before* and *after* dislodging the contaminant build-up in the covered container. This might have been to the fact that

contamination was minimised in th :r. No significant increase is seen in the vertical data sets (Table 3.2.5, Appendix F), indicating that both maximally and minimally-exposed containers were exposed to contamination by spore-forming organisms.

3.3 Plastic screw-top containers (Unrinsed and rinsed)

3.3.1 Turbidity indicating contaminant build-up

There was a significant increase ($P \leq 0.001$) (Table 3.3.1, Appendix F) in the level of turbidity *after* dislodging contaminant build up in both rinsed and unrinsed screw-top containers. Figure 3.3.1 shows the results. Theron et al. (2000) reported that the sanitary condition of the containers could be a risk factor even with improved water supply. The hygiene education programme done by Nala (2002) in a previous study, focused on educating household members on basic container cleanliness (washing and rinsing) to reduce accumulation of biofilm. Reduced turbidity levels were found after the intervention, indicating that the containers had less biofilm.

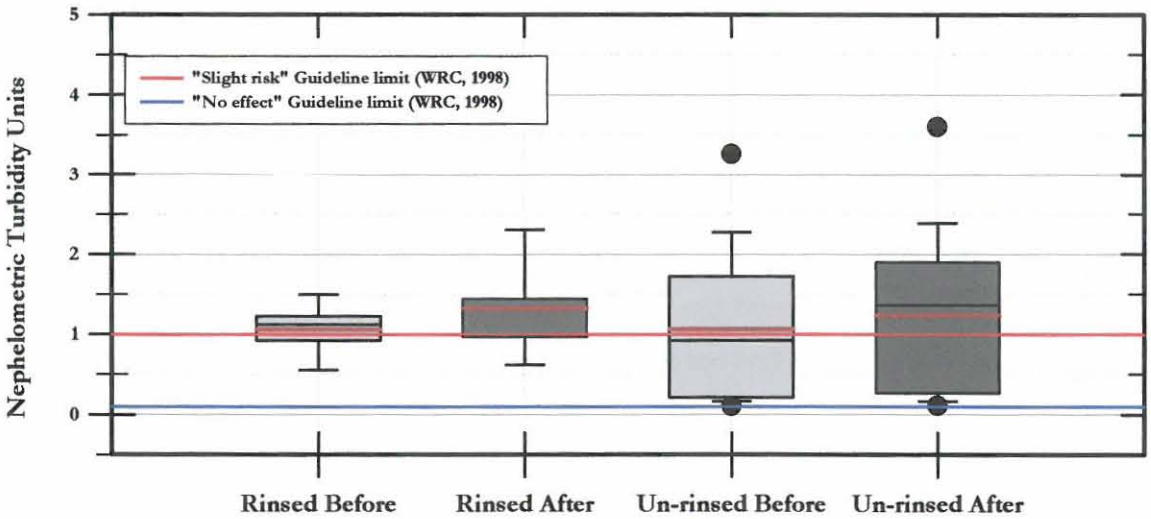

Figure 3.3.1: Comparing turbidity levels of water *before* and *after* dislodging contaminant build-up in rinsed and unrinsed screw-top containers

The increase was much higher in water from the unrinsed container compared to the rinsed container. This led to the belief that unrinsed screw-top container allowed for the build-up of contaminants to a greater extent since it was never rinsed. However, both the rinsed and unrinsed containers hosted contamination build-up.

Turbidity results, *before* and *after* dislodging, were above the limit (<0.1 NTU) proposed by the Assessment Guide Quality of Domestic Water Supplies (WRC, 1998). The water did not comply with the required limits (indicated by the blue and red lines in figure 3.3.1) since the

turbidity levels exceeded those stipu ıes and there was a slight risk of potential health effects should the water be used for drinking.

A study conducted by Nala (2002) indicated that contamination related to water handling practices during storage and use at home occurred despite improvement of container washing and rinsing. Since both rinsed and unrinsed containers allowed for contamination to build up, contamination of the rinsed screw-top container might have occurred between fillings (unwashed hands coming into direct contact with water).

3.3.2 Heterotrophic bacteria indicating contaminant build-up

There was a significant increase ($P \leq 0.001$) in heterotrophic bacteria level *after* dislodging contaminant build-up. The results are shown in Table 3.3.2 (Appendix F). Reducing biofilm in water distribution systems had been reported to reduce the occurrence of heterotrophic bacteria (Schaule and Flemming, 1997). In a study done by Nala (2002), reduced biofilm formation, indicated by lower turbidity levels, did not result in reduced heterotrophic bacteria. This was attributed to certain other factors, other than bacterial regrowth. Excessive numbers of heterotrophic bacteria were still introduced in the containers by poor handling of water in terms of hygiene (Nala, 2002).

There was no significant increase ($P = 0.130$ and $P = 0.386$) when the vertical data sets were compared and this indicated that both the rinsed and un-rinsed screw-top containers were susceptible to contamination (Table 3.3.2, Appendix F). The organism numbers at the 95th percentile exceeded those stipulated in the guidelines and therefore posed a risk to the users. (Slight risk and negligible risk limits are indicated by the red and blue lines in Figure 3.3.2).

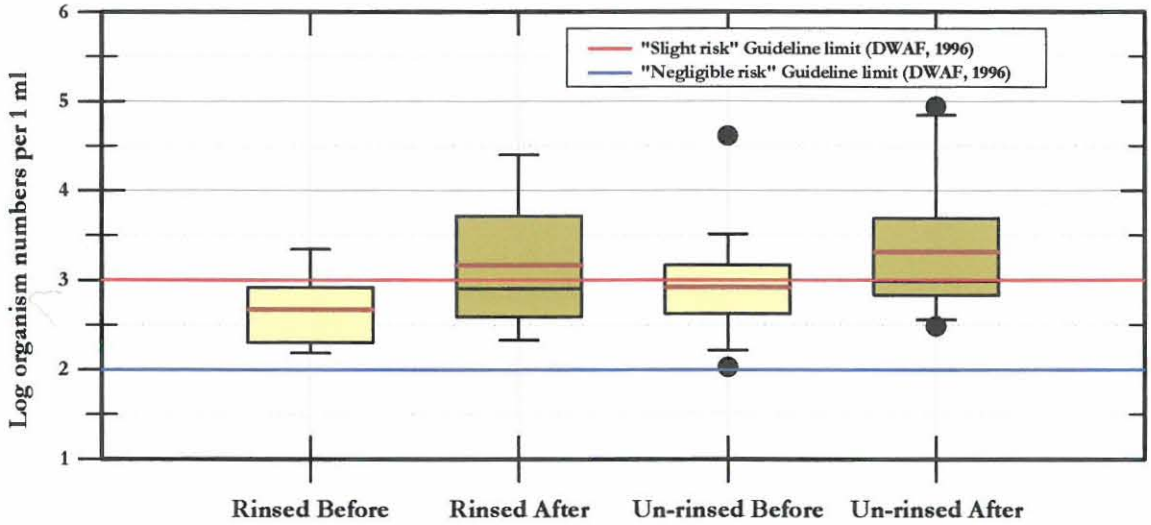

Figure 3.3.2: Comparing heterotrophic bacteria numbers of water *before* and *after* dislodging contaminant build-up in rinsed and un-rinsed screw-top containers

3.3.3 Total coliforms indicating biological pollution

Table 3.3.3 (Appendix F) shows a significant increase in the level of total coliforms *after* dislodging the contaminant build-up. Again, as with the other container-types, this increase indicated that a form of contaminant build-up (organic and inorganic) had developed on the container inner sidewalls and loosened by the process of dislodging. Jagals (2000) reported that the extent to which total coliforms are present in drinking water could indicate the likelihood of water being contaminated by organic matter from the environment. This increase was much higher in the un-rinsed than in the rinsed screw-top container.

There was no significant increase (P=345) in the level of total coliforms *before* dislodging the contaminant build-up in the rinsed and un-rinsed screw-top containers. The no increase effect, *after* scrubbing, indicated that the water from both rinsed and unrinsed containers was subjected to contamination, which led to deposition and build-up of contaminants.

According to the Assessment Guide: Quality of Domestic Water Supplies (WRC, 1998), total coliform level within the range of 0-100 counts/ 100 mℓ may pose an insignificant likelihood of infection and the level above 1000 counts/ 100 mℓ may pose serious health effects. The organism numbers at the 95th percentile exceeded those stipulated in the guidelines and therefore the water did not comply with the guideline limits.

Figure 3.3.3 illustrates the results. Guideline limits are indicated by the red and blue lines in the graph.

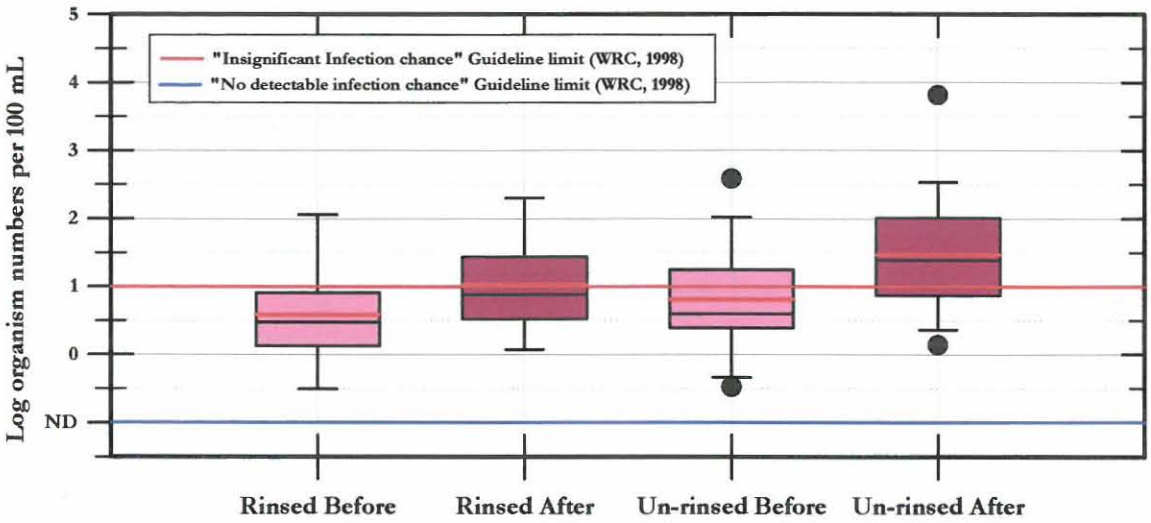

Figure 3.3.3: Comparing total coliform numbers of water *before* and *after* suspending contaminant build-up in rinsed and un-rinsed screw-top containers

3.3.4 *E coli* indicating faecal contamination

E coli results that were found subsequent to analysis of the rinsed and unrinsed screw-top container water are shown in Table 3.3.4 (Appendix F) and illustrated in Figure 3.3.4. There was no significant increase (P=0.063) in the level of *E coli after* dislodging the contaminant build-up in the rinsed screw-top container. Nala (2002) reported reduced *E coli* numbers during and immediately after the hygiene education intervention, which focused on container cleaning practices. However, less frequent intermittent occurrences indicated periodic faecal contamination related to poor personal hygiene as well as unhygienic domestic environments (Nala, 2002). In this study, it is evident that the rinsing of the container did not allow for the build-up of contaminants. There was a significant increase (P=0.002) in the level of *E coli after* dislodging the contaminant build-up in the unrinsed screw-top container. This increase showed that the unrinsed screw-top container was more subjected to faecal contamination. Parent et al. (1996) studied the fate of *E coli* in distribution systems and discovered that these organisms were able to adapt and grow in biofilm within few a hours.

Figure 3.3.4 illustrates the *E coli* results. The organism numbers at the 95th percentile exceeded those stipulated in the guidelines and water was rendered unsafe for consumption.

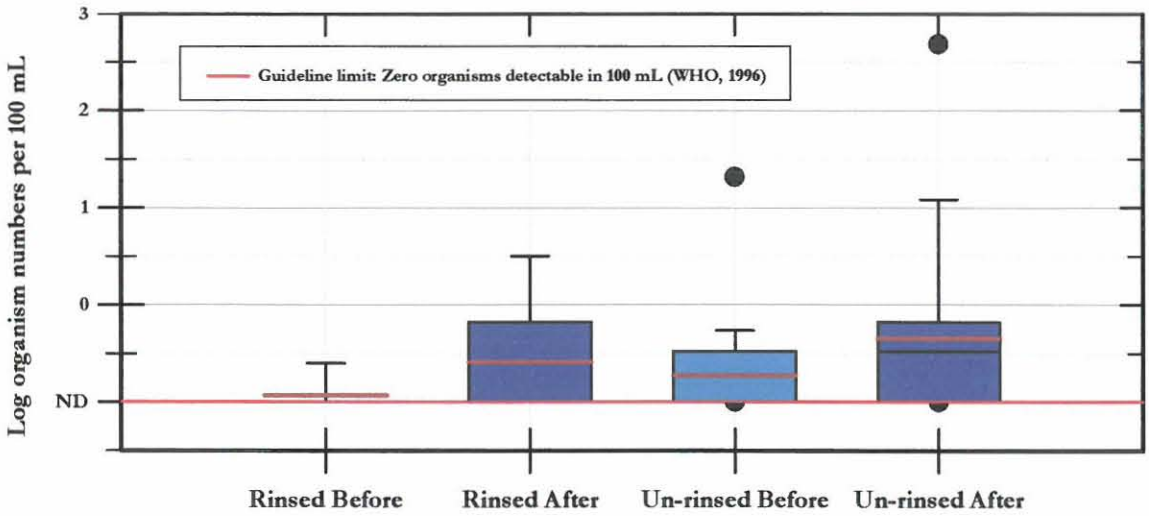

Figure 3.3.4: Comparing *E coli* numbers of water *before* and *after* dislodging contaminant build-up in rinsed and un-rinsed screw-top containers

There was no significant increase (P = 0.231) and (P = 0.412) in *E coli* levels *before* and *after* scrubbing (vertical data) the inner sidewalls of both the rinsed and unrinsed screw-top containers (Table 3.3.4, Appendix F). Although the unrinsed container allowed for more contamination there was no difference in the susceptibility of either containers to faecal contamination and build-up of contaminants.

3.3.5 *C perfringens* indicating re nisms

Table 3.3.5 (Appendix F) shows that there was no significant increase (P=1.00) in the level of *C perfringens after* scrubbing the inner sidewalls of the containers. The results are illustrated in Figure 3.3.5.

There was no significant increase (P = 0.988 and P = 0.828) in the *C perfringens* level *before* and *after* dislodging the contaminant build-up in the rinsed and unrinsed screw-top containers (Table 3.3.5, Appendix F). The results indicated that *C perfringens* organisms were occasionally found in the containers, posing a risk of infection.

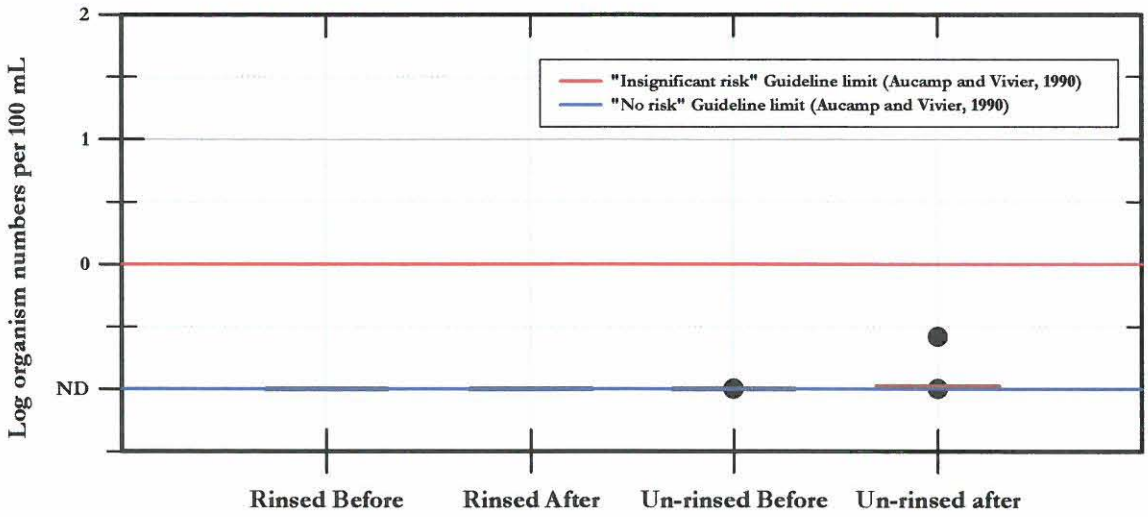

Figure 3.3.5: Comparing *C perfringens* numbers of water *before* and *after* dislodging contaminant build-up in rinsed and un-rinsed screw-top containers

3.4 Plastic bucket-type container (control)

3.4.1 Turbidity indicating contaminant build-up

The results in Table 3.4.1 (Appendix F) show that there was a significant increase (P≤0.001) in turbidity *after* the inner sidewalls of the container were scrubbed. This increase indicated contaminant build-up formation following the storage of water in the control container.

However, even *before* dislodging, turbidity levels were already above the recommended limit of <0.1 as stated in the Assessment Guide Quality for Domestic Water Supplies (WRC, 1998) indicating contamination of the water supply in the complex. Water did not comply with the required limits since turbidity levels exceeded those stipulated in the guidelines. Figure 3.4.1 illustrates the findings.

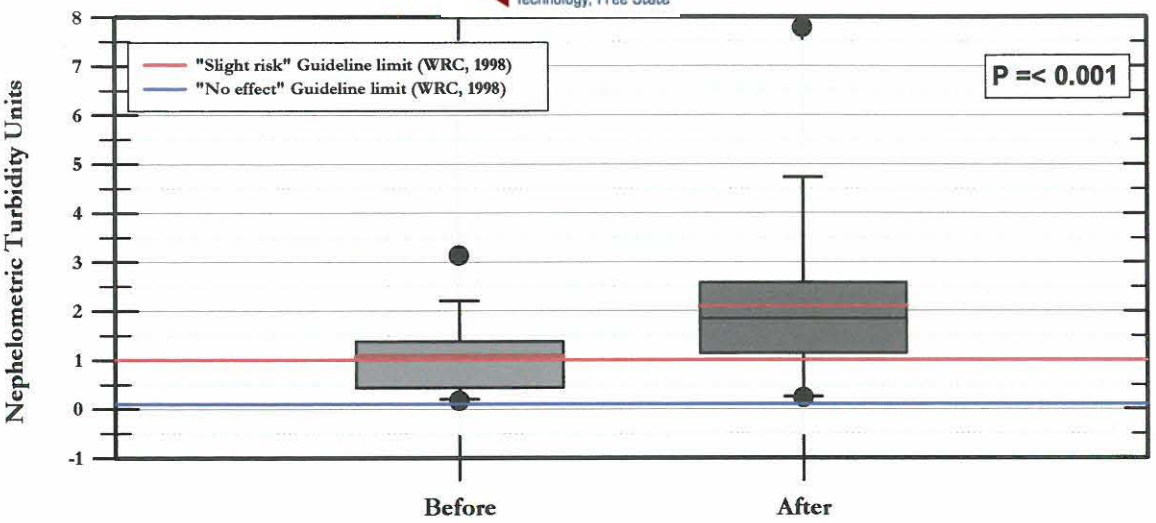

Figure 3.4.1: Comparing turbidity levels of water *before* and *after* dislodging contaminant build-up in control bucket-type plastic container

3.4.2 Heterotrophic bacteria indicating contaminant build-up

A significant increase ($P \leq 0.001$) in heterotrophic bacteria *after* scrubbing the inner sidewalls of the container led to rejection of the hypothesis (Table 3.4.2, Appendix F). The results exceeded the slight risk limit, indicated by the red line in the graph, as proposed by the South African Water Quality Guidelines (DWAF, 1996). These bacteria are not of faecal origin and are not indicators of faecal pollution (Payment, 1995).

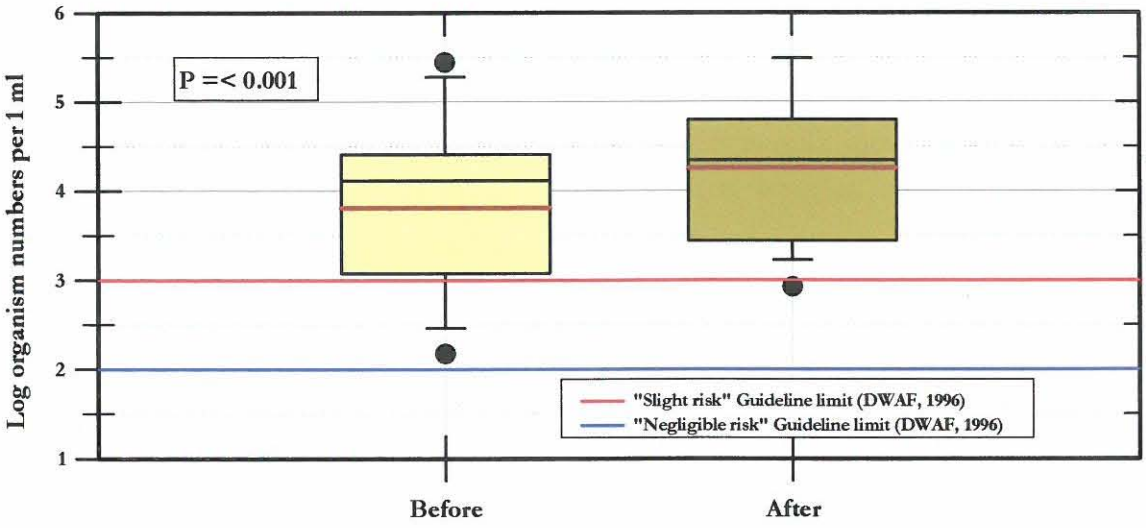

Figure 3.4.2: Comparing heterotrophic bacteria numbers of water *before* and *after* dislodging contaminant build-up in control bucket-type plastic container

Figure 3.4.2 illustrates the heterotrophic bacteria results that were found in water from the control container *before* and *after* dislodging the contaminant build-up.

3.4.3 Total coliforms indicatin obiological pollution

The results in Table 3.4.3 (Appendix F) show a significant increase in total coliform level *after* scrubbing the inner sidewalls of the container. The water did not comply with the guideline limit of not more than 10 organisms / 100 mℓ. Since their presence in water indicates organic pollution, it was evident that the contaminant build-up also constituted organic matter. Figure 3.4.3 shows the results.

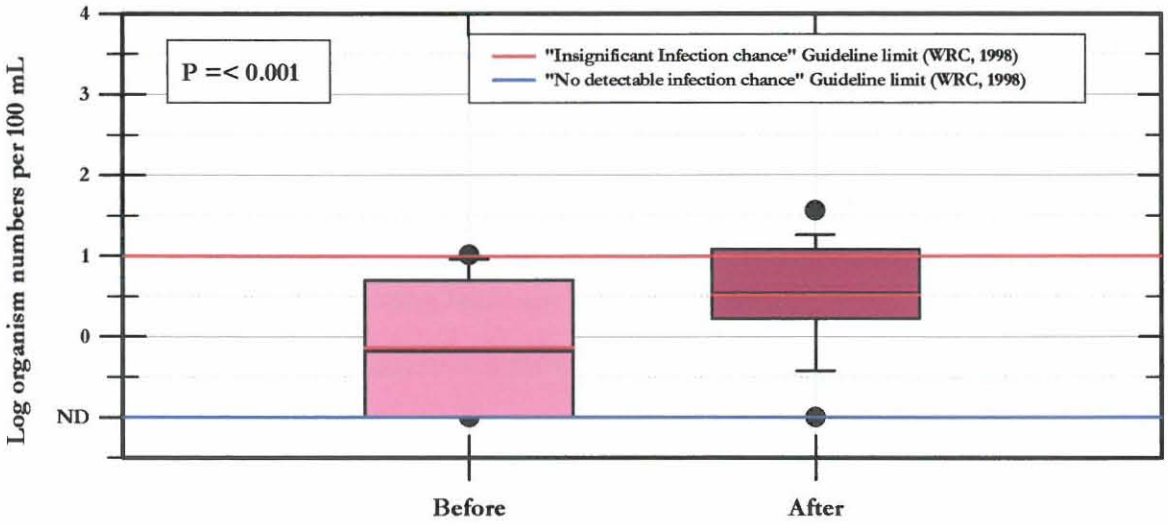

Figure 3.4.3: Comparing total coliform numbers of water *before* and *after* suspending contaminant build-up in control bucket-type plastic container

3.4.4 *E coli* indicating faecal contamination

There was a significant increase (P=0.014) in the level of *E coli after* dislodging the contaminant build-up and the hypothesis was rejected. *E coli* is a highly specific indicator of faecal pollution that originates from human and warm-blooded animals (Grabow, 1996). Their presence indicated some form of faecal contamination to the stored water. *E coli* organism numbers at the 95th percentile exceeded those stipulated in the guidelines and therefore the water did not comply with the guideline limits.

Figure 3.4.3 illustrates the *E coli* results that were found in water from the control container *before* and *after* dislodging the contaminant build-up.

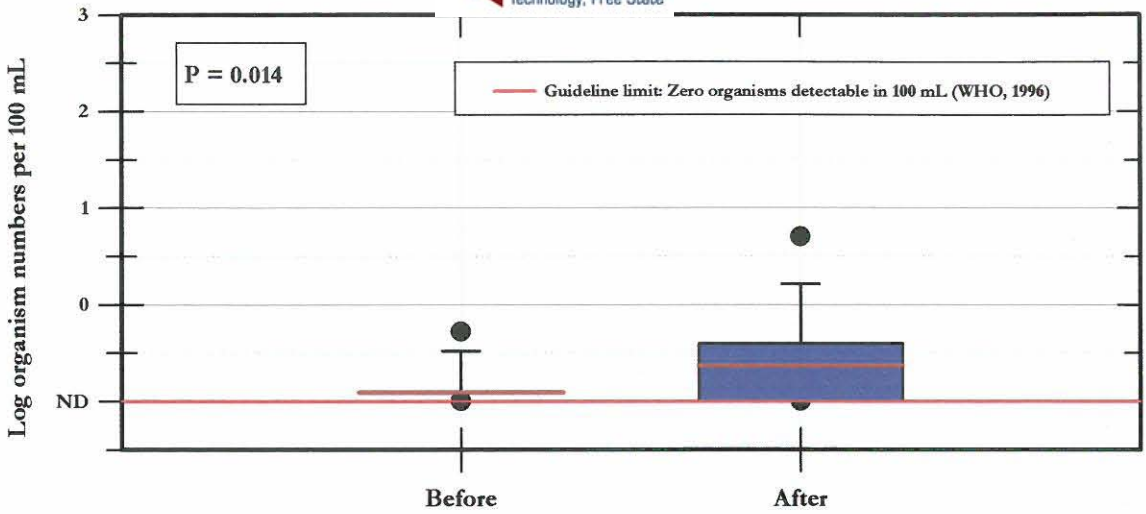

Figure 3.4.4: Comparing *E coli* numbers of water *before* and *after* dislodging contaminant build-up in control bucket-type plastic container

3.4.5 *C perfringens* indicating resistant microorganisms

There was no significant increase (P = 0.500) in the level of *C perfringens after* scrubbing the inner sidewalls of the container. The results showed that *C perfringens* were occasionally detected in the control container. The level of pollution was low, indicating minimal faecal pollution or resistant spores in the container. The results are illustrated in the figure below.

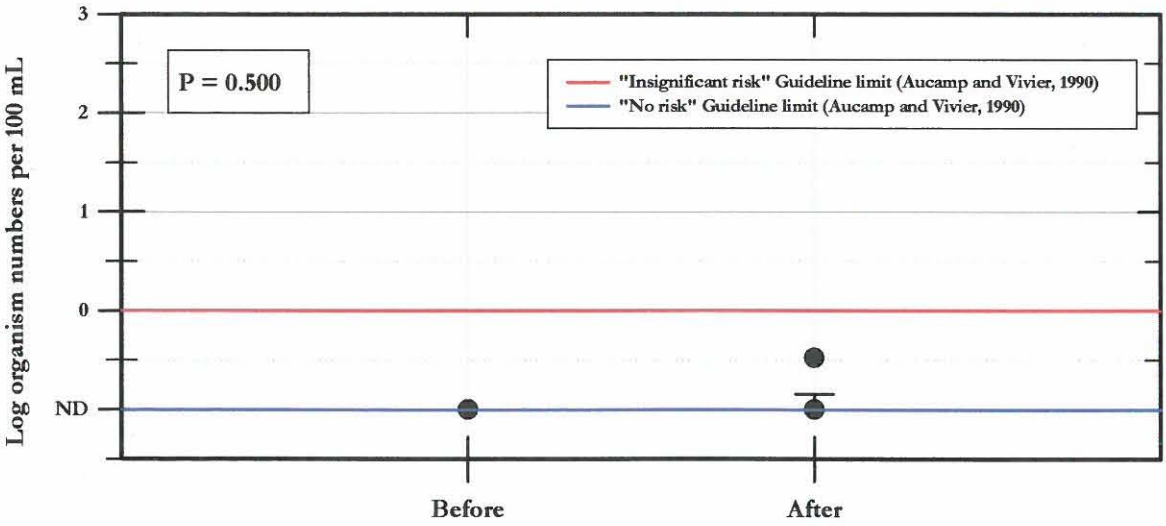

Figure 3.4.5: Comparing *C perfringens* numbers of water *before* and *after* suspending contaminant build-up in control containers

3.5 Bucket-type plastic and metal containers

In the previous sections, contaminant build-up formation in all the various containers following storage of water was demonstrated.

In this section, only the wide-mouthed (bucket-type) containers (plastic and metal), are compared since these appeared to be the most prone to environmental contamination. Only the *after* data are used because this is intended to demonstrate container performance including their respective biofilm profiles. The comparison is done to determine the type least exposed to contaminant build-up under the test circumstances and thereby indicating the container-type that will be recommended for use by the communities.

3.5.1 Turbidity indicating contaminant build-up

Table 3.5.1 (Appendix F) shows comparison of turbidity levels that were found in water from the bucket-type plastic and metal containers (uncovered and covered) *after* dislodging the contaminant build-up. There was no significant difference (P=0.065) between the uncovered plastic and metal containers *after* dislodging the contaminant build-up. The hypothesis was not rejected.

Figure 3.5.1 shows the results of the bucket-type plastic as compared to the bucket-type metal containers. Non-compliance of the water to accepted standards posed a risk of infection to the users.

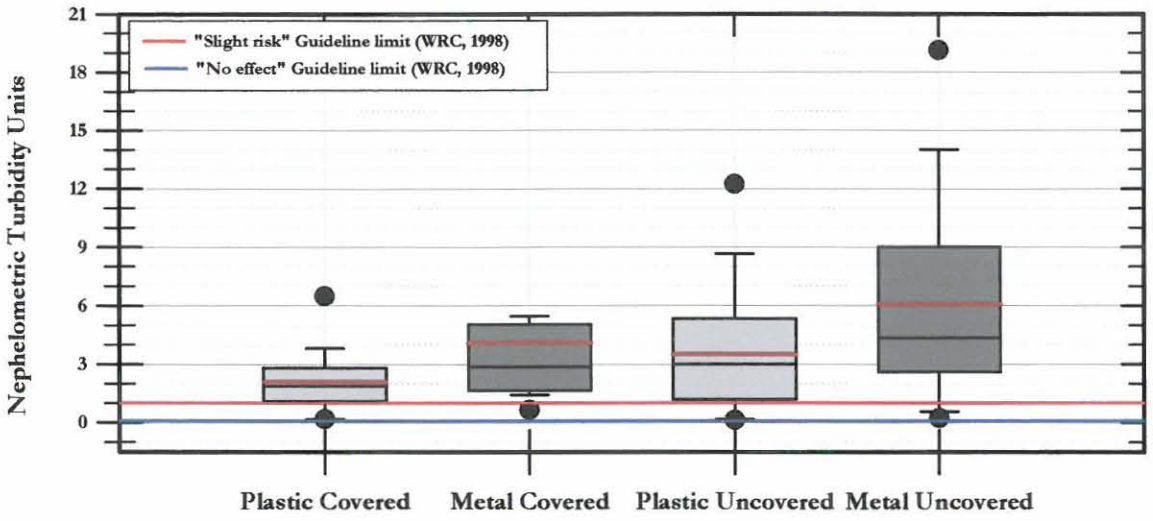

Figure 3.5.1: Comparing turbidity levels in water *after* dislodging contaminant build-up in covered and uncovered bucket-type plastic and metal containers

There was, conversely, a significant difference between the covered bucket-type plastic and metal containers *after* suspension of the contaminant build-up. Higher turbidity levels were observed in the metal than in the plastic containers. Environmental contamination (dust, etc.) might have contributed to the contamination of the metal container since the lid was not as tight-fitting as the one for the plastic container. This might have contributed to the increased contaminant build-up in the metal container. It has been reported by Sobsey (2003) that inadequately protected or poorly covered storage containers contribute to contamination of stored water by dust and other environmental contaminants.

3.5.2 Heterotrophic bacteria indicating contaminant build-up

The results are shown in Table 3.5.2 (Appendix F) and illustrated in Figure 3.5.2. There were no significant differences (P=0.375 and P=0.133) between the plastic and metal containers (both uncovered and covered). The hypotheses were accepted. This implied that there was no difference in the susceptibility of these two container types to growth and development of contaminant build-up. These results corroborated the findings of Evison and Sunna (2001), that the material of the surface has little or no effect on the development of biofilm.

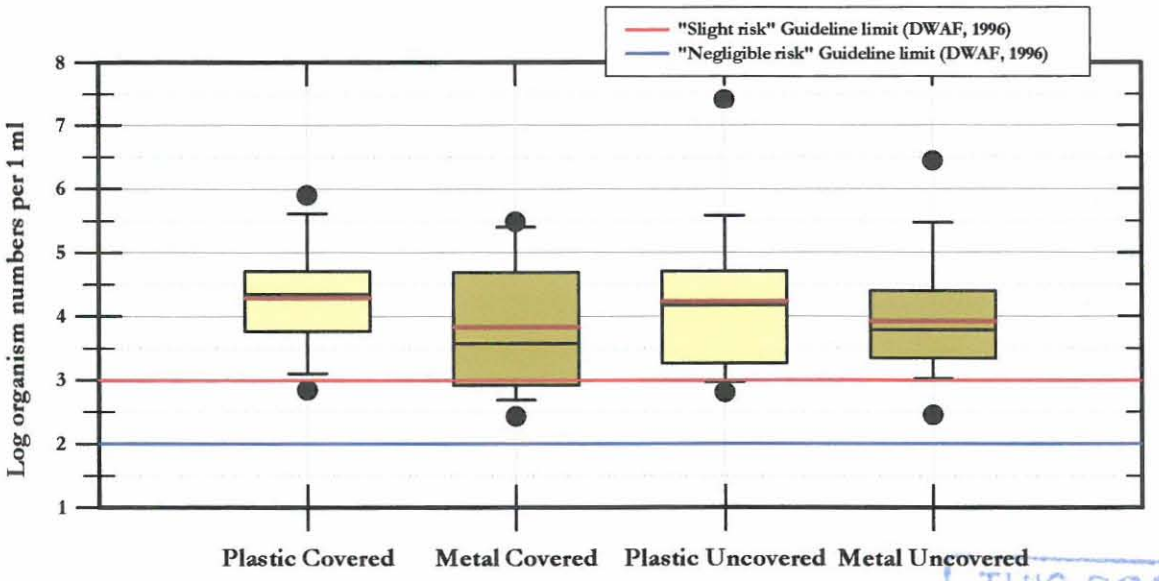

Figure 3.5.2: Comparing heterotrophic bacteria numbers in water *after* dislodging contaminant build-up in covered and uncovered bucket-type plastic and metal containers

3.5.3. Total coliforms indicating hazardous microbiological pollution

There was no significant difference (P=0.375) between the uncovered plastic and metal containers. The hypothesis was not rejected. There was, however, a significant difference (P=0.020) between the covered plastic and metal containers, leading to rejection of the hypothesis (Table 3.5.3, Appendix F). The level of total coliforms was high in the covered

plastic container compared with the Kalmbach (1997) reported that various types of plastic materials are widely used in domestic drinking water distribution systems and polyethylene has been described in a number of studies as hydrophobic material enhancing bacterial attachment and growth. The results are illustrated in figure 3.5.3.

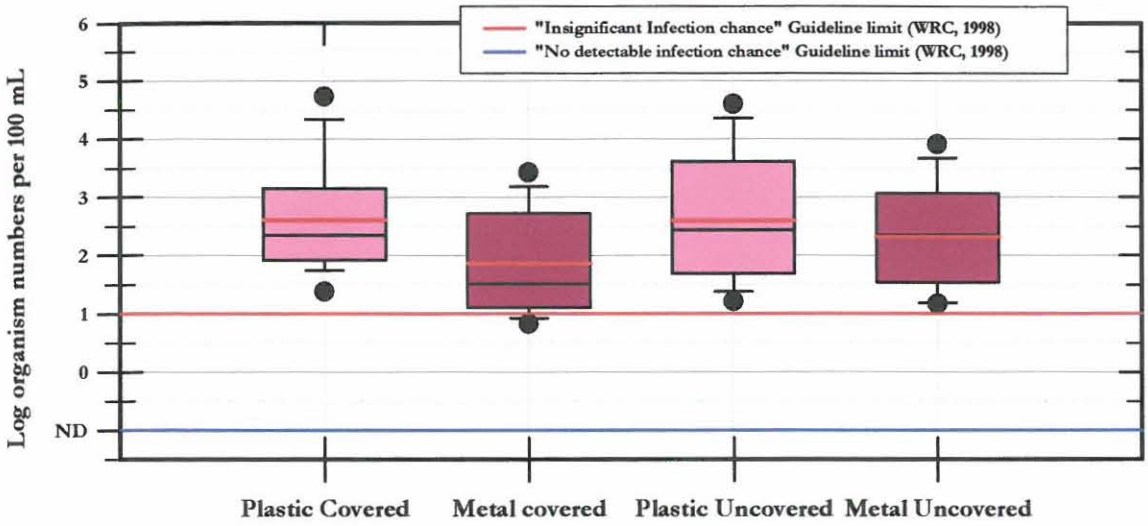

Figure 3.5.3: Comparing total coliform numbers in water *after* dislodging contaminant build-up in covered and uncovered bucket-type plastic and metal containers

3.5.4 *E coli* indicating faecal contamination

The results found after comparing the level of *E coli* in the plastic and metal containers showed that there was no significant difference (P=0.812 and P=0.266) between the plastic and metal containers (both uncovered and covered) (Table 3.5.4, Appendix F). This indicated that both container types were exposed to faecal contamination that contributed to the development of contaminant build-up. The hypotheses were not rejected. Figure 3.5.4 below shows the results.

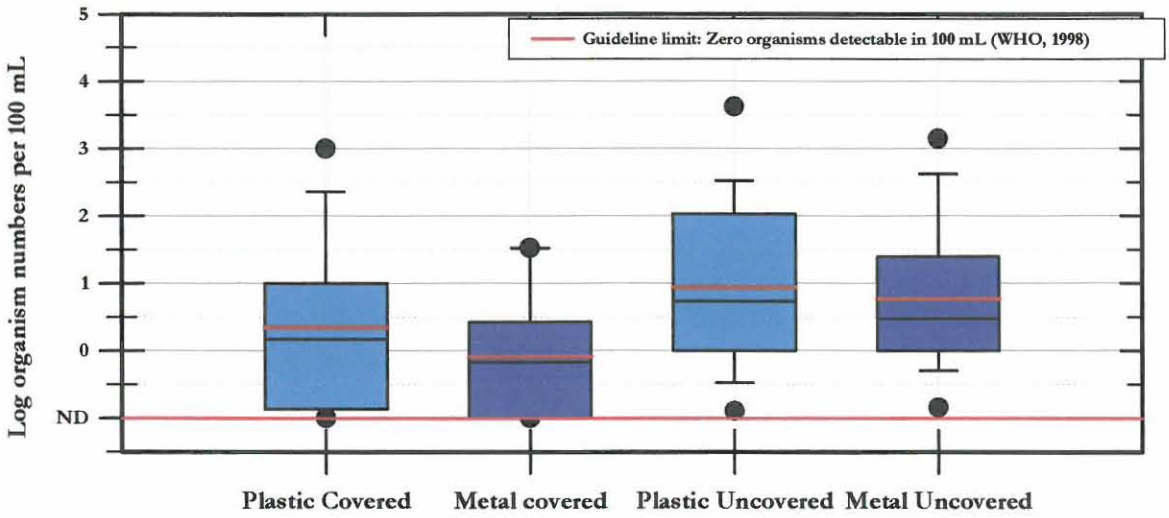

Figure 3.5.4: Comparing *E coli* numbers in water *after* dislodging contaminant build-up in covered and uncovered bucket-type plastic and metal containers

3.5.5 *C perfringens* indicating resistant microorganisms

Table 3.5.5 (Appendix F) shows a comparison of *C perfringens* results between the bucket-type plastic and metal containers. According to the results, there was no statistically significant difference between the plastic and metal containers, either uncovered or covered. The hypotheses were not rejected. The results proved that *C perfringens* were intermittently found in water. There was occasional faecal contamination to the container-stored water since these indicator bacteria are specific for resistant faecal contamination as well as for protozoan spores (Aucamp and Vivier, 1990).

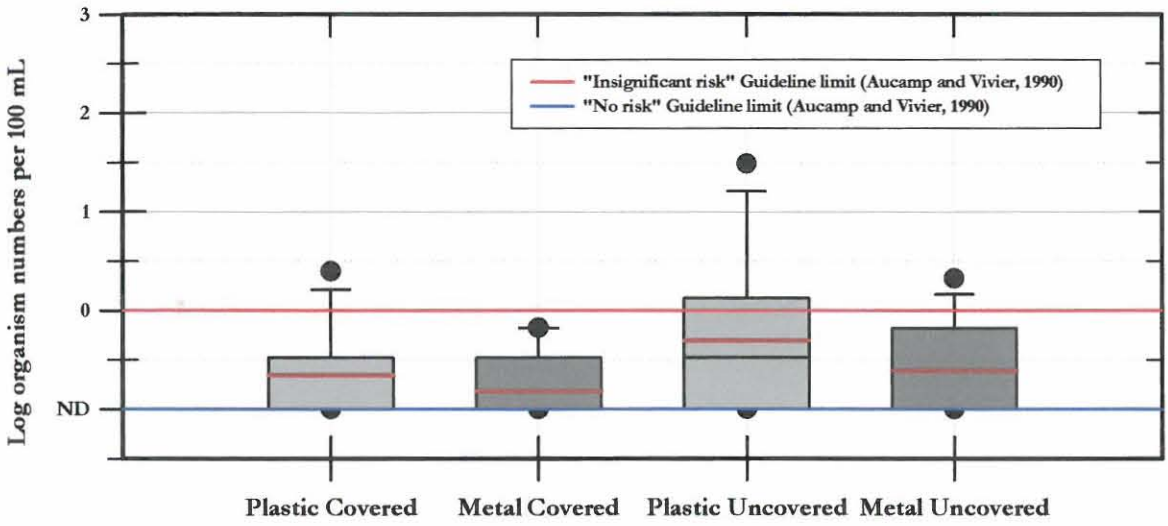

Figure 3.5.5: Comparing *C perfringens* numbers in water *after* dislodging contaminant build-up in covered and uncovered bucket-type plastic and metal containers

3.5.6 Summary

Water in the uncovered containers generally appeared to be subject to contamination from the outside environment. The level of contamination was found to be much higher in containers with maximum environmental contamination potential (uncovered) than in the containers with minimum environmental contamination potential (covered). There was no significant difference in the capacity of either the uncovered plastic or metal containers to allow for the build-up of contaminants. However, higher levels of indicators of organic pollution were found in the covered plastic than in the metal containers. Polyethylene, in plastic materials, has been described in a number of studies as hydrophobic material enhancing bacterial attachment and growth.

3.6 Plastic and metal containers (bucket-type) and plastic screw-top container

In this section, the screw-top container is included in the comparison to determine the one least exposed to contaminant build-up under the test circumstances. In this section also, the absolute worst-case scenario data were used to evaluate which container performed the best under maximum environmental exposure. The term "maximum" therefore refers to "uncovered" *after*-data in terms of the bucket-type containers and "unrinsed" *after*-data in terms of the plastic screw-top containers

3.6.1 Turbidity indicating contaminant build-up

In Table 3.6.1 (Appendix F) (Figure 3.6.1), turbidity data for various container types were compared to determine differences so that those that were least exposed or prone to promoting contaminant build-up, or the one that allowed its formation to a lesser extent, could be identified.

There was a significant difference, as expected, in the results of the three containers. The hypotheses were rejected. Results from the plastic screw-top container (unrinsed) were significantly lower than those from the uncovered plastic and metal containers. The level of turbidity was much higher in the uncovered metal and plastic containers than in the plastic screw-top container (unrinsed). From a turbidity perspective, the plastic screw-top container appeared less likely to foster contaminant build-up.

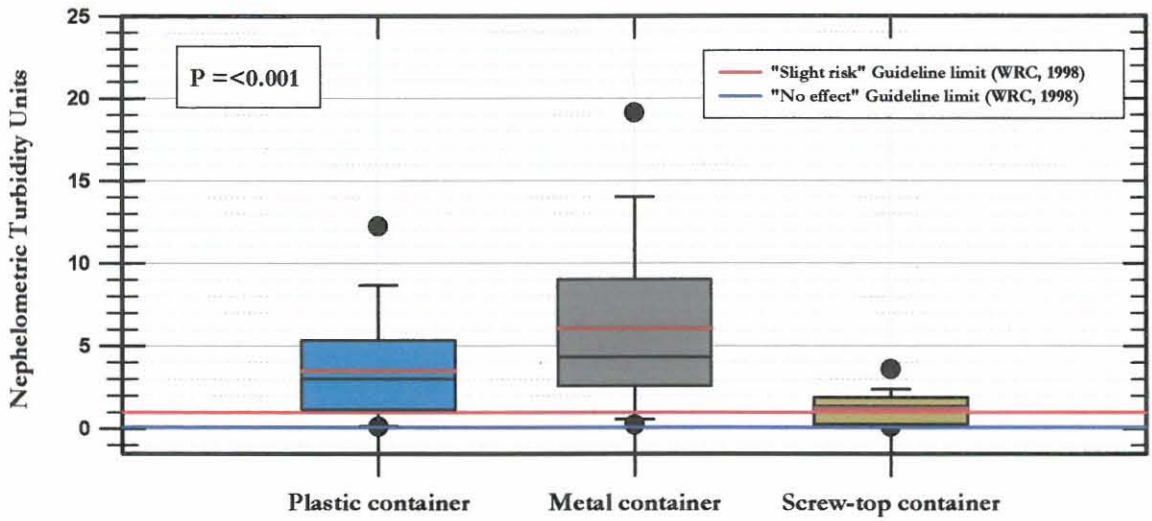

Figure 3.6.1: Comparing turbidity levels in water from uncovered bucket-type *plastic* and *metal* containers as well as unrinsed *plastic screw-top* containers *after* dislodging contaminant build-up

3.6.2 Heterotrophic bacteria inc ant build-up

The plastic screw-top container (unrinsed) was significantly different from the two uncovered bucket-type containers (plastic and metal). The plastic screw-top container had a lower heterotrophic bacteria level compared to the plastic and metal containers.

Table 3.6.2 (Appendix F). Sobsey (2003) reported that the use of containers with narrow openings for filling and dispensing protect the collected water during storage and household use. Jagals et al. (1997) also reported that contamination of stored water can be increased or decreased by the shape of the container.

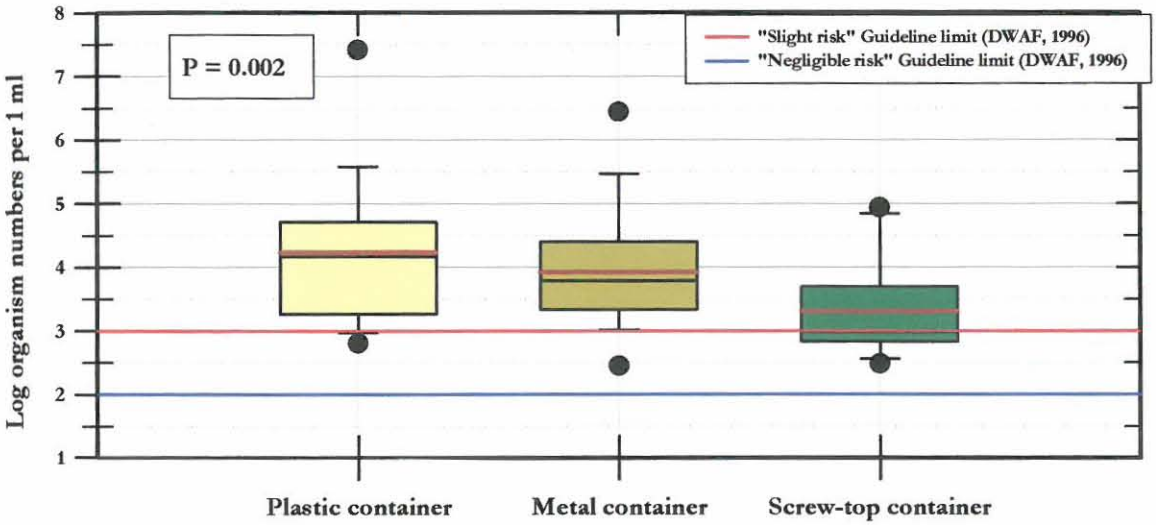

Figure 3.6.2: Comparing heterotrophic bacteria levels in water from uncovered bucket-type *plastic* and *metal* containers as well as unrinsed *plastic screw-top* containers *after* dislodging contaminant build-up

Figure 3.6.2 illustrates heterotrophic bacteria results as found in the plastic and metal containers (both uncovered) as well as the plastic screw-top containers (unrinsed) *after* dislodging the contaminant build-up.

3.6.3. Total coliforms indicating hazardous microbiological pollution

The results in Table 3.6.3 (Appendix F) show that the plastic screw-top container (unrinsed) was significantly different from the plastic and metal containers (both uncovered). The plastic screw-top container had lower total coliform levels than the other two types of containers. The plastic screw-top container was, therefore, found to be the type of container least exposed to contaminant build-up.

Differences between the plastic screw-top container (unrinsed), and the plastic and metal containers (both uncovered) are shown in Figure 3.6.3.

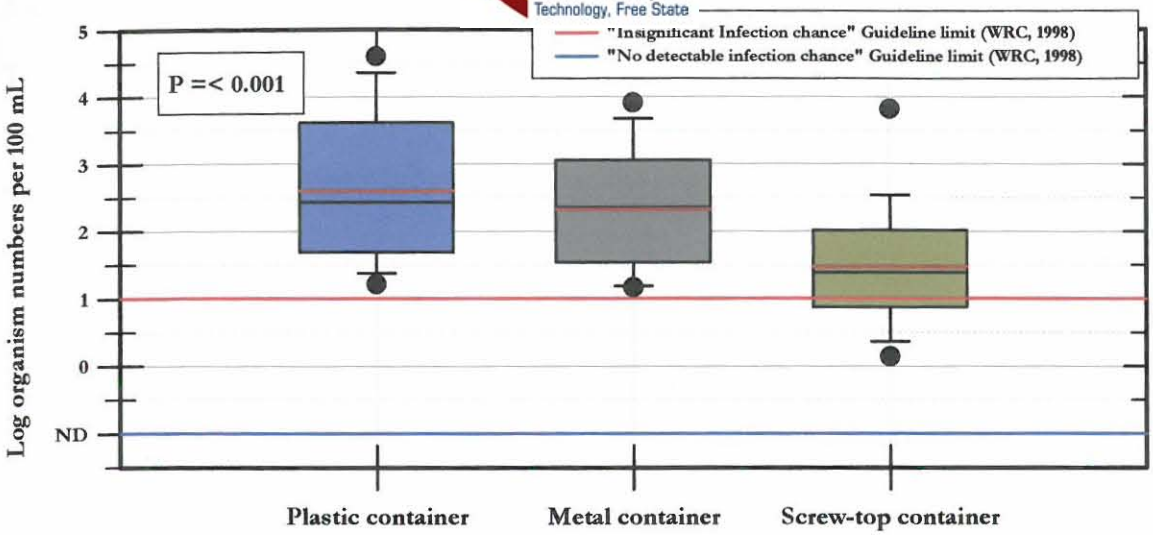

Figure 3.6.3: Comparing total coliform numbers in water from uncovered bucket-type *plastic* and *metal* containers as well as unrinsed *plastic screw-top* containers *after* dislodging contaminant build-up

3.6.4 *E coli* indicating faecal contamination

Table 3.6.4 (Appendix F) shows that there was a significant difference in the water quality sampled from the three containers. The plastic screw-top container was found to be significantly different from the other two containers. The results showed that *E coli* were detected in water from all three container types, indicating a form of faecal contamination.

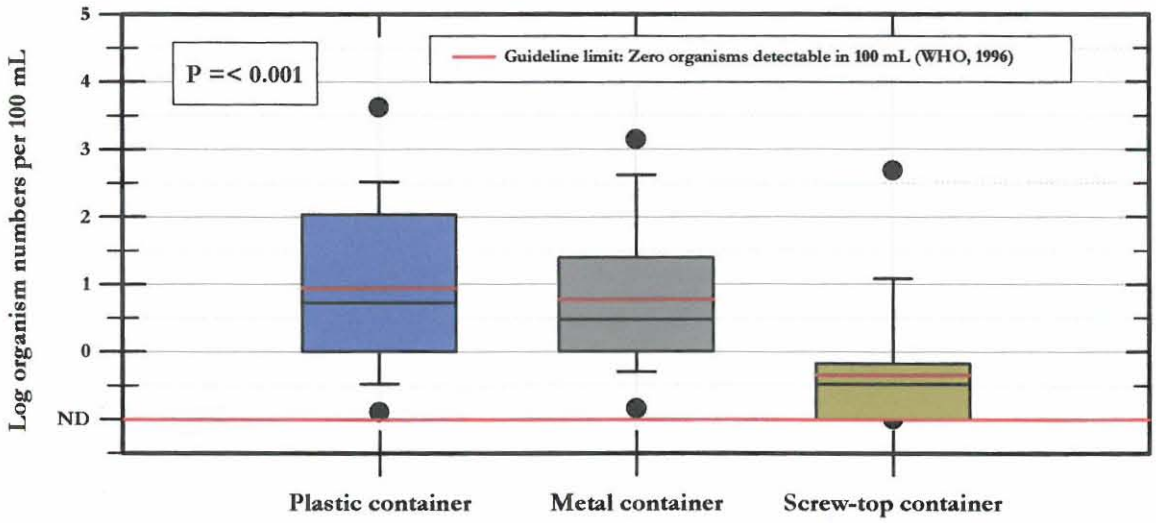

Figure 3.6.4: Comparing *E coli* numbers in water from uncovered bucket-type *plastic* and *metal* containers as well as unrinsed *plastic screw-top* containers *after* dislodging contaminant build-up

The above figure shows the differences between various water storage containers. Comparison was done on *E coli* numbers that were found *after* dislodging of the contaminant build-up.

3.6.5 *C perfringens* indicating t istant microorganisms

The results in Table 3.6.5 (Appendix F) show that the plastic screw-top container (unrinsed) was significantly different from the plastic and metal containers (both uncovered). The results signified that *C perfringens* were occasionally found in the stored water.

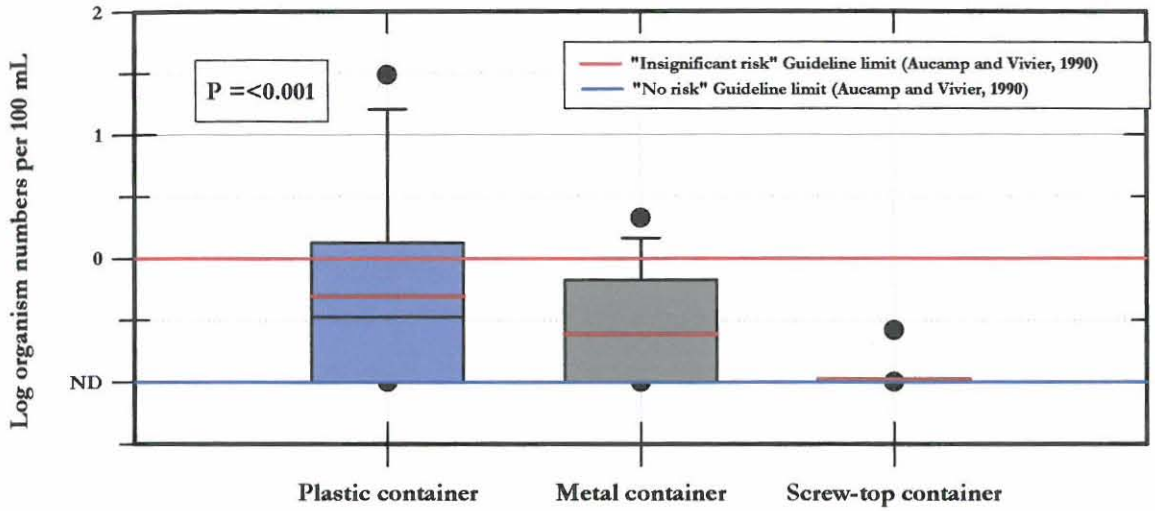

Figure 3.6.5: Comparing *C perfringens* numbers in water from uncovered bucket-type *plastic* and *metal* containers as well as unrinsed *plastic screw-top* containers *after* dislodging contaminant build-up

Figure 3.6.5 illustrates the *C perfringens* results that were used for comparison to determine the difference between various water storage containers.

3.6.6 Summary

The quality of water in the screw-top containers differed significantly from that of the water in the bucket-type plastic and metal containers. The screw-top containers were found to be the container types least prone to promoting build-up of contaminants. Therefore, plastic screw-top containers were less likely to foster contaminant build-up. Their smaller orificed mouth-top does not allow for maximum contamination, as do the other bucket-type containers.

3.7 GENERAL DISCUSSION

3.7.1 Changes in water quality due to contaminant build-up formation

The main focus of this study was to assess the occurrence of contaminant build-up associated with biofilm; to determine and compare the occurrence of health-related indicator bacteria (total coliforms, *E. coli* and *C perfringens*) and its association with indicators of contaminant build-up (turbidity and heterotrophic bacteria) that might form inside various types of drinking water storage containers.

The level of indicators of contami ell as other health-related water quality indicators was determined. According to the results, the formation of contaminant build-up altered the quality (physical and microbiological) of the stored drinking water.

The results showed that brushing dislodged particulate matter (both organic and inorganic in origin) that had accumulated, leading to increased turbidity. The results proved that a form of contamination adhered to the inside walls or settled on the bottom of the storage containers and was dislodged following scrubbing and swirling.

Dywili and Jagals (1999) reported reduced biofilm formation in containers indicated by reduced turbidity levels following an awareness campaign regarding the cleaning of containers. This campaign appeared to have influenced people's behaviour towards the cleaning containers. This was proven by the significant and sustained decrease in turbidity. However, such behavioural changes were not sufficient since the indicator bacteria levels were still above the risk limits. The same was encountered by Nala (2002) in her study to determine the impact of educational intervention on the microbiological quality of stored water. The educational intervention resulted in lower turbidity levels which indicated improved or frequent container washing and rinsing. However, heterotrophic bacteria numbers were not reduced significantly and this indicated that these bacteria were still introduced in the containers by poor hygiene and storage of water (Nala, 2002).

During this study, an increased level of indicators was found in the containers with maximum contamination potential (uncovered/un-rinsed) as compared to the one with minimum contamination potential (covered/rinsed). This supported the assumption that incidental environmental contamination (dust, etc.) might have played a role in the formation of contaminant build-up. Sobsey (2003) reported that uncovered or poorly covered storage containers and lack of protection against dust, flies and vectors, result in contamination of initially safe microbiologically safe water after its collection and storage. Again, since turbidity and heterotrophic bacteria levels in uncovered containers were still above the guideline limit *before* dislodging, it showed that there was a continuous contamination to the water during storage.

There was no significant difference between the uncovered plastic and metal containers *after* dislodging the contaminant build-up. However, there was a significant difference in the turbidity levels of water obtained from the covered plastic and metal containers. The metal container had a higher turbidity level than the plastic container. Contamination of the metal container was suspected to be as the result of a poorly fitting lid. The bucket-type plastic container had a tight-fitting lid while the metal container lid did not fit properly. This might have resulted in increased contamination build-up of the covered metal container.

Biofilms do form on the inner sidewalls of domestic water-storage containers. It was demonstrated that these could release particulates into suspension with the stored container water when disturbed. This suspension contained organic matter associated with increased concentrations of microbiological contaminants indicated by higher levels of heterotrophic bacteria in the container water. These particles therefore appeared to harbour bacteria while still adhering to the container sidewalls. The significant increase in turbidity after scrubbing the inner sidewalls of water containers, and suspending the loosened matter in the container water, supported this. It is therefore concluded that the inner walls of drinking-water storage containers can harbour the classical biofilm often described to form inside water distribution pipes.

3.7.2 Changes in water quality relating to hazardous microbiological pollution

3.7.2.1 Total coliforms

As indicators of organic pollution, total coliforms were used to indicate the hazardous microbiological pollution of water stored in the bucket-type plastic and metal containers (uncovered and covered) as well as in plastic screw-top containers.

The results showed a significant increase in the level of total coliforms *after* scrubbing the inside walls of the bucket-type containers as well as the plastic screw-top containers. In a study to detect elevated levels of coliform bacteria in a public water supply (Morbidity and Mortality Weekly Report, 1998) it was reported that high total coliform counts were due to the sloughing of coliforms bacteria that had accumulated within biofilm. The coliforms were of the same types commonly found in biofilms (MMWR, 1998). In this study it was evident that high total coliform counts that were found in container-stored water constituted part of the biofilm that had formed. The increase was much higher in the uncovered containers and the unrinsed plastic screw-top containers than in those covered and rinsed. Environmental contamination (dust, etc.) might have played a role in the formation of contaminant build-up in uncovered containers. Schultz and Ely (2000) reported that total coliforms are useful for testing drinking water where contamination by soil or organic matter is a concern. The rinsing of the screw-top container might have slowed down the building up of the contaminants since lower levels of total coliforms were found in the rinsed container than in the unrinsed one.

The increase in total coliforms (*after* scrubbing) was much higher in the plastic than in the metal containers. Kalmbach (1997) discovered that polyethylene, from plastic materials enhances bacterial attachment and growth. Once present in the biofilm, the total coliform level is

positively influenced by increased orgtrations (Camper, 1996; WSAA Report, 1996).

The unrinsed plastic screw-top container allowed lower contaminant formation/adherence compared to the bucket-type containers. Even though all the containers (covered and rinsed) were significantly different from one another, the plastic screw-top container had a lower total coliform level, indicating that they were less exposed to contamination build-up. This was observed for all the other indicators of hazardous pollution.

3.7.2.2 *Escherichia coli*

This indicator organism was used to measure the level of faecal contamination of the container-stored water. There was a significant increase in the level of *E coli after* suspension of the contaminant build-up in all the containers, except in the rinsed plastic screw-top container. *E coli* are highly specific for the faeces of humans and warm-blooded animals and are always found in faecally contaminated water (Momba et al., 1999). The results, therefore, showed that the contaminant build up that formed on the container sidewalls might have harboured pathogens that could have been sloughed into the container contents (water) leading to the use of contaminated water.

Potential contamination routes such as container hygiene between fillings, and environmental contamination during storage, might have introduced these faecal bacteria into the water. Keeping pet animals (dogs, cats etc.) within households is customary in developing settlements and these domestic animals also contribute to faecal contamination of the domestic environment (Moe et al., 1991; Jagals, 2000), which could end up in container-stored water.

There was no significant difference in the capacity of plastic and metal containers (uncovered and covered) to allow for faecal contamination. Plastic screw-top containers were found to be significantly different from the other types of containers.

3.7.2.3 *Clostridium perfringens*

These indicator bacteria were used to indicate the presence of resistant microorganisms in the stored drinking water. The results showed that *after* scrubbing the inside walls of the containers the level of *C perfringens* increased significantly. Their presence indicated occasional or intermittent pollution of stored drinking water.

There was no significant difference between the plastic and metal containers (uncovered and covered), and the plastic screw-top containers were significantly different from these two container types.

It appeared that the container-biofilm could protect and possibly propagate potentially hazardous microbiological contaminants as indicated by the numbers of total coliforms as well as *C. perfringens* spores released during the scrubbing and swirling process. The container water with suspended biofilm particles contained significantly higher levels of these bacteria than did the undisturbed container water. It appeared however, that *E. coli* were apparently not effectively supported by the layers of biofilm and probably became inactivated after a resident period in the container water.

3.7.3 The best container to use

To determine the container type least prone to contaminant build-up, the worst case scenario data sets ("*after*" data sets) for all three container types were used. There was a significant difference, as expected, in the results of the three container types (with minimum environmental contamination potential). Contamination in plastic screw-top containers (rinsed) was found to be significantly lower than that in the uncovered bucket-type containers despite the fact that these containers are difficult to wash inside. Their design appears not to allow for maximum environmental contamination and this might have contributed to their minimal exposure to contaminants.

Previous studies (Quick et al., 1996; Jagals et al., 1997; Sobsey, 2003) have documented higher levels of microbial contamination and decreased microbial quality associated with storage containers having wide openings. The use of containers with small opening, such as the screw-cap container, to facilitate dispensing but small enough to discourage or prevent introduction of contaminants by contaminated hands, utensils and other routes (e.g. dust, vectors, etc.) protect the water during storage and household use (Sobsey, 1993).

4.1 STUDY OUTCOMES AND CONCLUSION

The main focus of this study was to assess the occurrence of contaminant build-up related to biofilm, and to determine and compare the occurrence of health-related indicator bacteria (total coliforms, *E. coli* and *C perfringens*) and its association with indicators of contaminant build-up (turbidity and heterotrophic bacteria) that might form inside various types of drinking water storage containers.

To achieve the aim of the study, specific objectives were identified (Chapter 1: Introduction). The **outcomes** of the study towards attaining these objectives are summarised below:

- The level of turbidity and heterotrophic bacteria validated the occurrence of contaminant build-up in various types of drinking water storage containers. There was a significant increase (*before* results < *after* results) in the levels of these indicators of contaminant build up *after* scrubbing the inner sidewalls and swirling of the containers. This increase was attributed to biofilm and other environmentally introduced particulates that were released and suspended in the container water contents.
- The levels of other health-related indicator organisms (total coliforms, *E coli* and *C perfringens*) increased significantly *after* the suspension of contaminant build-up. This showed a strong association between these indicators and those of contaminant build-up. It is therefore evident that biofilm consists of organic or inorganic surface deposits made up of microorganisms, microbial products and debris.
- The level of contamination was found to be much higher in containers with maximum environmental contamination potential (uncovered/unrinsed), as expected, than in the containers with minimum environmental contamination potential (covered/rinsed). This contamination might have resulted from dust and other environmental pollutants that found their way into the containerised water. Higher levels of microbial contamination and decreased water quality were associated with storage containers having wide openings (e.g. bucket-type containers) and those that are inadequately protected (uncovered or poorly covered).
- The container type least prone to contaminant build-up was determined. The "*after*" data sets (worst case scenario data sets) were used for this purpose. The containers (bucket-type plastic and metal containers, and screw-top containers) were found to be significantly different from one another. The screw-top containers were found to be the

container types least suscept build-up of contaminants. The most contaminated types were assumed to be the most likely to foster contaminant build-up.

There was a significant difference in water quality (turbidity levels) obtained from the covered bucket-type plastic and metal containers. Most contamination was found in metal rather than in the plastic container. There was no difference in the quality of water (heterotrophic bacteria) obtained from the bucket-type containers (both uncovered and covered). This might have been as a result of the slip-up during sampling since the lids for the metal containers were not tight fitting compared to the plastics. Environmental contamination might have contributed to the elevated levels of contamination in these containers. Higher levels of indicators of organic pollution (total coliform bacteria) were found in the plastic than in the metal container. Polyethylene (from the plastic material) has been described in a number of studies as hydrophobic material enhancing bacterial attachment and growth.

From the study outcomes, it is clear that the combined roles of safe water and adequate hygiene and sanitation are likely to achieve the greatest improvement in water quality and reduction of water-related diseases, compared to just intervention. However, it is now evident that improving household water collection and storage is one option for achieving a beneficial health effect. Household water collection and storage deserve due consideration in the prioritization and implementation of water, sanitation and hygiene measures for use at household, community and regional levels.

4.2 RECOMMENDATIONS

It is crucial that the public should understand the importance of water safety to the quality of life. Particularly in developing countries, it is critical to educate the population about good hygiene, maintenance of water delivery systems and safe storage of water in the household. Improving and protecting the microbial quality and reducing the risk of infection to consumers of collected water stored in households requires alternative or interim strategies and approaches that can be implemented effectively, quickly and affordably.

Waiting for the provision of piped, microbiologically safe community water systems to many people lacking such services is an inappropriate response to the basic need for safer drinking water that can be met by available technologies. Effective measures are needed immediately to provide at-risk populations with safer water at the household level until the long-term goal of providing safe, piped, community water supplies can be achieved. The most guaranteed household water storage methods and their implementation strategies to achieve sustainability in

the provision of safe water includ nied by efforts to address social and economic aspects such as education, behaviour and belief modification.

4.2.1 Educational, Behavioural and Related Socio-Cultural Considerations for Household Storage Methods and Techniques

Since numerous studies have shown that the introduction of water treatment technology without consideration of the socio-cultural aspects of the community, and without behavioural, motivational, educational and participatory activities within the community, is unlikely to be successful or sustainable, initiatives in water, hygiene and sanitation must include:

- education,
- community participation; and
- behaviour modification

4.2.1.1 Education

A comprehensive hygiene education programme is essential to ensure that the communities are aware of the importance of water quality and its relation to health. Such a programme can include:

- the advantages and reasons for regular cleansing of drinking water storage containers used for storage and conveyance.
- the promotion of hand-washing with soap after a visit to the toilet, and before preparing food and eating.

4.2.1.2 Community Participation and Hygiene behaviour

Access to water and sanitation must be accompanied by promotion of hygiene behaviour since health benefits from these programmes will not be fully realised if hygiene behaviour is not improved. There are many stages in the collection, storage and handling at which drinking water can be contaminated. Health improvements can be achieved through the promotion of personal and domestic hygiene.

A number of systems have been developed and successfully implemented for this purpose. One of the most widely used and successful of these is termed PHAST, which stands for Participatory Hygiene and Sanitation Transformation. It is an adaptation of the SARAR (Self-esteem, Associative strengths, Resourcefulness, Action-planning and Responsibility) method of participatory learning. PHAST promotes health awareness and understanding among all members of a community or society in order to change hygiene and sanitation behaviours. It encourages participation, recognizes and encourages self-awareness and innate abilities, encourages group participation at the grassroots level, promotes concept-based learning as a

group process and attempts to li ning to group decision-making about solutions and plans of action for change and improvement of the current situation. It encourages internally-derived decisions and both material and financial investment of the community to affect change.

4.2.2 Drinking water collection and household storage

Poor hygienic water handling and storage practices are common sources of water contamination. Water collected for domestic use often becomes contaminated by unsafe consumer storage and handling practices at the household level. Key factors in the provision of safe household water include the conditions and practices of water collection and storage as well as the choice of water collection and storage containers.

The following are the proposed household handling and storage approaches that can be practised:

- Since higher levels of microbial contamination and decreased microbial quality are associated with storage containers having wide openings, drinking water should be collected in containers with smaller fill openings or screw-cap openings, to facilitate dispensing but small enough to discourage or prevent introduction of contaminants by contaminated hands, utensils and other routes (e.g dust, vectors etc.).
- The container should be portable; based on the capacity, weight, presence of handles and flat bottomed for ease of storage; and should be composed of easily cleaned material (preferably plastic).
- Locally available storage containers or used beverage containers are low in cost and are readily available. However, care is needed in the choice of the containers for collection and storage of drinking water. Containers that were used for hazardous substances should never be used to collect and store water since residuals may affect the water quality.
- Water storage containers should be thoroughly washed (with boiling water and soap) before use and regularly cleaned (washed with every filling). Re-used containers should preferably be disinfected.
- Long periods of water storage should be avoided since it is evident that biofilm can develop following storage of water for any length of time.
- Extraction of water from the container should be such that contamination is minimised (avoid touching water or creation of excessive floating dust in the house). The water should be poured rather than extracted with a cup as contaminated extraction cups may introduce contaminants into the water and consequently worsen its quality.

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INDICATOR ANALYSIS APPROACH

Equipment, methods and techniques

MEMBRANE FILTRATION

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

1. EQUIPMENT

1.1 Filter and vacuum assembly

- Three Millipore® three-place PVC manifolds.
- Nine glass 47mm diameter Millipore® filter holder sub assemblies comprising:
    - Glass funnels (± 250 capacity).
    - Fritted glass base support for filter membrane.
    - Clamp (for securing funnel on base after loading filter membrane).
- Two sets of one-litre vacuum filter glass flasks (for trapping moisture before vacuum pump).
- Two EDWARDS® 1.5 Two-Stage 220/240 V 50/60 Hz vacuum/pressure pumps.
- Silicone rubber tubing for connecting the assembly.

1.2 MEMBRANE FILTERS

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

1.3 PIPETTES

Adjustable pipettes (Finn®) with sterile disposable tips for pipetting 1 mℓ and smaller volumes of sample were used. Errors in calibration were checked not to exceed 2.5%. Large volumes were dispensed with standard graduated glass pipettes.

1.4 INCUBATORS

1.4.1 Fan-induced incubators with circulating air were used. Temperatures varied within 0.5° accuracy, especially within stacks of incubated plates.

1.4.2 Water baths (25 ℓ) with unifo ating elements in the steel inner jacket to ensure constant temperature distributions were used. These baths were equipped with gabled covers to aid temperature maintenance within 0.2°C of setting.

2. METHODS and TECHNIQUES

2.1 Sterilisation

Each glass assembly was separately wrapped in tinfoil and sterilised before each completed session of filter plating.

Steam sterilisation of equipment was done in an autoclave 121°C/ 15 psi for 15 minutes.

Dry sterilisation was also done on equipment, in an oven at 180°C for 10 minutes. This sterilisation was done between each sample filtration session.

Forceps were decontaminated by immersion in alcohol and flamed before every filter handling.

2.2 Phosphate buffer

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

Sterile working solutions of buffer were made up by adding 1.25 mℓ of phosphate solution (34g KH_2PO_4/500 mℓ distilled water) and 5 mℓ of magnesium chloride solution (81.1g $MgCl_2.6H_2O$/ 1000 mℓ distilled water) to 1 litre of reagent grade water.

The phosphate buffer was steam sterilised in an autoclave at 121°C/ 15 psi for 15 minutes.

2.3 Dilutions

Water samples were diluted in such a way that the counts of between 20 to 60 (Standard Methods, 1998) colonies per plate were achieved. To ensure homogenous mixture and organism distribution in the water sample, samples were shaken vigorously.

To achieve the ideal colony range the following dilutions were followed:

- Heterotrophic plate counts (-1,-2,-3 and –4 mℓ)
- Chromocult® coliform (100, 10, 0, -1, -2 mℓ)
- Clostridium perfringens (Undiluted sample applications, 100 and 10 mℓ)

Undiluted sample application ranged from 0 (1 mℓ) to 100. Sample dilution of 0 and 10 were pipetted onto the filter.

Dilution procedure:

- Sterilised (Autoclaved 121°C psi for 15 minutes) test tubes filled with 9 mℓ volume of sterile buffer solution.

- 1 mℓ extractions from various d the water samples were aseptically taken and transferred to a prepared 9 mℓ volume of buffer solution to complete a 10^{-1} diluted sample.
- 1 mℓ was aseptically taken from the 10^{-1} dilute to another 9 mℓ volume of sterile phosphate buffer to provide a 10^{-2} dilution. Subsequent dilutions were followed in a similar manner.

All the samples were filtered in triplicates (three filters) per dilution.

3. FILTERING TECHNIQUE

A complete set of filter and vacuum assembly was used. Vacuum was created by the electric vacuum pump evacuating through a dual moisture trap system comprising 1 litre capacity vacuum flask.

Each glass assembly was separately wrapped in tinfoil and sterilised (autoclaving at 121°C psi for 15 minutes) before each filter plating session. During filtration sessions, constant sterilisation and decontamination of the glass sub-assemblies was done to avoid cross contamination. This was done by placing the glass sub-assemblies in an oven, at 180°C for 5 to 10 minutes.

Sterile phosphate buffer was used for diluting samples and rinsing the funnels after filtration (Standard Methods, 1998). To eliminate contamination between dilutions, filter plating was done in decreasing dilution order.

Membranes were loaded, grid side up, onto the fritted glass support base of the funnel holder with sterile forceps and the funnel clamped onto the filter base.

The water samples were re-mixed by shaking the whirlpack for several seconds. 10 to 30 mℓ of sterile buffer solution was poured into the funnel and a given volume of sample pipetted into the buffer with an adjustable pipette.

The diluted samples were filtered within 10 to 20 minutes to avoid inactivation or multiplication of microorganisms in the dilution.

The manifold unit was slightly swirled, while applying the vacuum, to ensure uniform suspension of sample in the volume of buffer during filtering. The funnel walls were then rinsed (at least three times) with sterile phosphate buffer.

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

The incubation times for each of the bacteriological indicator organisms are described in Appendix B.

4. Pour plate Method

- The procedure was conducted within a laminar flow cabinet.

- Sample dilutions of 0.3 mℓ were [Central University of Technology, Free State] selective medium.
- The prepared plates were then inverted and incubated aerobically at 37°C for 48 hrs (Standard Methods, 1998).
- All the visible colonies on the plates were counted as heterotrophic bacterial colonies and expressed in microorganisms per 1 mℓ.

5. Counting

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

$$\frac{[(\text{Sum organisms } 1^{st} \text{ filter} + 2^{nd} \text{ filter} + 3^{rd} \text{ filter}) / 3] \times 100}{\text{Sample size (Volume)}}$$

$$\text{Sample dilute}$$

This formula was programmed in a Microsoft Excel® spreadsheet and the following entered:

i. The counts from each of the three membrane filters in the petri dish.
ii. The sample volume (maximum 1 mℓ for undiluted samples).
iii. The dilutions expressed as 0.1; 0.01 etc. (minimum 1 mℓ for undiluted samples).

Counts for heterotrophic bacteria were calculated and expressed per 1 mℓ and those of total coliforms, *E coli* and *Clostridium perfringens* were calculated and expressed as number of organisms per 100 mℓ.

INDICATOR ANALYSIS APPROACH

Media, Reagents and Procedures

1. HETEROTROPHIC BACTERIA

1.1 Enumeration by means of a non-selective medium

Heterotrophic bacteria were enumerated by means of a pour plate technique (Appendix A).

Preparation of the culture media (Merck, 1996):

Ingredients:

Peptone	3.0 g
Soluble casein	0.5 g
K_2HPO_4	0.2 g
$MgSO_4$	0.05 g
$FeCl_3$	0.001 g
Agar bacteriological	15 g

All the ingredients were added to 500 mℓ of distilled water and the pH was adjusted to 7.2 before autoclaving.

The mixture was gently boiled to dissolve the powder and autoclaved at 121°C for 15 minutes.

The media was cooled to 50°C and poured over 0.3 aliquots of sample dilution into 90 mm petri dishes.

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

Identification: All visible colonies on the plates were counted as heterotrophic bacteria.

2. THE COLIFORMS

2.1 TOTAL COLIFORMS

2.1.1 Enumeration by means of chromogenic substrate agar

Total coliforms were enumerated on Chromocult® Coliform Agar for the simultaneous detection of coliforms and *E coli* in water samples (Merck, 1996) with the membrane filter technique.

Preparation of Chromocult® Coliform Agar (Merck, 1996):

- 26.5 g of powder was suspended in 1 litre of distilled water.

- The mixture was gently heated until the powder was totally dissolved.
- The medium was cooled to 40-50°C and the Cefsulodin solution (10 mg in 2 mℓ of distilled water) was added to the 1 litre of medium by gently shaking to homogenise. Cefsulodin was added to knockout the flora, especially *Pseudomonas spp.* and *Aeromonas spp.*
- This medium does not require 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°C. Unused plates were discarded when contaminated or after six months.

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

Identification: Colonies appeared in various shades of salmon to red.

Confirmation: API® 20E (bioMérieux®) (Appendix C).

2.2 *ESCHERICHIA COLI*

2.2.1 Enumeration by means of chromogenic substrate agar

Escherichia coli were enumerated on Chromocult® Coliform Agar for the simultaneous detection of coliforms and *E coli* in water samples (Merck, 1996) with the membrane filter technique.

The same procedure as with total coliforms was used.

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

Identification: Colonies appeared in various shades of dark blue-to-violet (Merck, 1996).

Confirmation: API® 20E (bioMérieux®) (Appendix C).

3. *CLOSTRIDIUM PERFRINGENS*

3.1 Enumeration by means of Perfringens (OPSP) Agar

Clostridium perfringens was enumerated by means of the membrane filtration technique (Appendix A) using supplemented Perfringens (OPSP) Agar (Oxoid Corporation, 1990). Enumeration was done in triplicates on 90 mm petri dishes.

Preparation of the Perfringens (OPSP) Agar (Oxoid Corporation, 1990)

- 22.8 g of the powder was added to 500 mℓ distilled water and the mixture gently boiled to dissolve the powder.
- The mixture was then autoclaved at 121°C for 15 minutes.

- After cooling to 50°C, rehydrate (SR76) and B (SR77) were added to the medium. This was done to give a high degree of selectivity and specificity for *Clostridium perfringens*.
- 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 when contaminated or after 2 weeks.

Pasteurisation: Samples (presumably containing *Clostridium perfringens* spores) were pasteurised in a water bath at 75 - 80°C for 10 minutes (Oxoid Corporation, 1990). Pasteurisation does not harm the spores of *Clostridium perfringens* but only inhibits the growth of the background flora.

Incubation: The plates were inverted and incubated anaerobically in an incubator at 37°C for 48 hours. Oxoid gas generating kits producing atmosphere 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 were confirmed on Rapid ID® 32A galleries (bioMérieux®). (Appendix C).

ANALYTICAL QUALITY CONTROL

1. QUALITY CONTROL PROCEDURES

A laboratory quality assurance program is the integration of intra- and inter-laboratory quality control (QC), standardization and management practices into a formally documented program with clearly defined responsibilities and duties to ensure that the data are of a required type, quality and quantity. An effective quality assurance program will confirm the quality of results and increase confidence in the data (Standard Methods, 1989).

To ensure accuracy of the results obtained during this study, a quality assurance programme was established. Strict quality control procedures, recommended by the Standard Methods (1998) were followed.

Membrane Filter test: To check the sterility of the media, filters, glassware and equipment, sterile water was used as a sample during each sample series analysis.

Positive and negative control cultures: The medium was checked by testing for known positive and negative control cultures for the indicator organisms being tested.

1.1 Control cultures for the microbiological tests

Total coliforms, *E coli* and *Clostridium perfringens* positive and negative control cultures were acquired from the South African Bureau of Standard (SABS).

1.1.1 Total coliforms - Stock cultures of *Enterobacter aerogenes* and *Citrobacter freundii* (positive control culture) and *Staphylococcus aureus* (negative control cultures) were made up (Standard Methods, 1998; Merck, 1996; bioMèrieux, 1996).

1.1.2 *E coli* - Stock cultures of *E coli* (positive control culture), *Enterobacter aerogenes* and *Citrobacter freundii* (negative control cultures) were made up (Standard Methods, 1998; Merck, 1996; bioMèrieux, 1998).

1.1.3 *C. perfringens* - Stock cultures of *C. perfringens* (positive control culture) and *C. bifermentans* (negative control culture) were made up (Oxoid Corporation, 1990; bioMèrieux, 1994).

1.2 Procedure for medium

Volumes of 1 mℓ of the positive and negative stock culture solutions were filtered through the membrane filters. The membranes were then placed on petri dishes containing various selective growth media. A specific medium was checked at least once a month, for the duration of the project.

The specific colony colour identification and distinction was standardised by the analyst group and used to identify various indicator organisms tested for on different media. It was ensured that the same colour, including various shades, was seen and understood by everyone.

2 Colony verification

This was done due to the great range of species and sub-species often to be found in a single indicator organism group as well as in the massive number of non-indicator groups. Many of these non-indicator organisms may find the specific medium accommodating and therefore manifest in the colours prescribed for the analyst for identification.

According to the Standard Methods (1998), at least 10 colonies should be picked randomly per month and verified from known positive samples.

Between 12% and 40% of the entire target colonies cultured on various media were randomly selected for colony confirmation. Plate count agar was used for streaking and growing single colonies (Standard Methods, 1998). Any colouration caused by the selectivity of the medium was therefore removed from the selected colonies. This was done to eliminate all possible interference with the functioning of the API test strips.

Sterile swabs were used to pick up the colonies. This was done to exclude possible interference from metal inoculum needles with some of the tests used in the strips.

The strips were inoculated, incubated and analysed according to the prescriptions contained in the manual provided with the identification kit (bioMerieux®, 1998). The numbers of false positive organisms were established in order to calculate the detected indictor numbers accurately (Standard Methods, 1998).

3 Confirmation procedure: Coliforms

To obtain pure colonies, the coliforms were colonies were picked up from the membranes with inoculum needles and streaked out on the same selective medium and incubated at the prescribed temperature. Single colonies on the selective media were then streaked out and grown on Plate Count Agar to strip the colonies of their colour. This was the last step of the process in which the colonies were touched with the metal eye of an inoculum needle. Further removal of the isolated colony from the Plate Count Agar to be used for identification on the

API strip was done with sterile swabs to avoid interferences from the metal eye of an inoculum needle with the oxidase test on the API strip.

3.1 Chromocult Coliform® Agar

Salmon to red (Total coliform on Chromocult® Coliform Agar) and deep blue to violet (*E coli* on Chromocult® Coliform Agar) colonies were selected. The colony morphology was carefully noted and included colour, size, shape, composition and edge appearance. A note was made of the number of colonies counted from each particular plate (membrane) as well as the number taken for verification by the API® 20E identification system.

3.2 API® 20E Multi-test galleries (bioMerieux®)

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 data base. These systems can be used to identify a substantial number of species that included the most important species used in this study.

3.3 Preparation of the innoculum

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

3.3.1 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.

3.3.2 Reading the strips

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

3.3.3 Identification

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

3.3.4 Quality control (QC)

Several QC tests were done on 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.*

4 Confirmation procedure: *ngens*

4.1 Perfringens (OPSP) Agar

Dark brown to black (*Clostridium perfringens* on perfringens (OPSP) agar from Oxoid) colonies were selected. Colony morphology was carefully noted and included colour, size, shape, composition, and edge appearance. 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 strips. The colonies were emulsified according to prescription and the emulsion flooded onto Columbia sheep blood agar (Oxoid®) plates. The plates were incubated anaerobically at 37°C for 24-48 hours.

4.2 Rapid ID® 32A Multi-test galleries (bioMerieux, 1994)

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

4.3 Preparation of the inoculum

Homogeneous bacterial suspensions of the harvested colonies from the blood plates were made according to the prescriptions contained in the manual provided with the commercial identification kit (bioMérieux®, 1994).

4.4 Inoculation of strips

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

4.5 Reading the strips

After incubation, the spontaneous colour reaction from each strip was recorded. Reagents were added to the prescribed tubes and the colour reaction noted. All these recording were done on results sheet provided with the kit.

4.6 Identification

The pattern of each of the reactions obtained was hand-coded, on the results 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.

4.7 Quality Control (QC)

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

STATISTICAL STRATEGIES

1. DATA DESCRIPTION

According to the Standard Methods (1998), microbiological water resource data generally have substantial variations which cause the data not to be normally distributed around the mean for the particular data set. Helsel and Hirsch (1995) reported that data analysed by water resource scientists often have these characteristics:

I. A lower bound of zero – no negative values are possible

II. Presence of "outliers". These are observations considerably higher or lower than most of the data. This occurs infrequently but regularly. Outliers on the high side are more common in water resource data.

III. Positive skewness, due to points I and II. Skewness can be expected when outlying values occur only in one direction. For positive skewness, the mean exceeds more than 50 % of the data.

IV. Non-normal distribution of the data due to points I – III above. While many statistical tests assume that data follow a normal distribution, water resource data often do not.

V. Data reported only below or above some threshold (censored data).

VI. Seasonal patterns. Values tend to be high or lower in certain seasons of the year.

VII. Autocorrelation. Consecutive observations under similar circumstances tend to be strongly correlated with each other. An example of the most common kind of autocorrelation in water resources is that high numbers of microbiological indicator organisms will tend to follow high numbers of microbiological indicator organisms in circumstances such as intermittent high volumes of intensive rainfall.

VIII. Dependence on other uncontrolled variables.

2. CENTRAL TENDENCY (MEASURES OF LOCATION)

The mean or median is usually the most used measure of location.

2.1 Mean

The mean is computed as the sum of all the data values (X_i), divided by the sample size (n). The arithmetic mean values of the colony counts on each membrane, per sample, were calculated because of the predominantly symmetrical distributions of the colonies per triplicate set (the

formula in Appendix A). The mean is influenced by outlying data and is therefore not used as a measure of central value for the untransformed data obtained. Since microbiological data may vary to a great extent (outliers) in the same sample, the mean is strongly influenced and the estimates may therefore not be realistic.

2.2 Geometric mean

The geometric mean is the mean of the logarithms, transformed back to the original unit. It is the preferred best estimate of central tendencies of the untransformed microbiological data (Standard Methods, 1998). However, for transformed data (logs), the mean was used.

2.3 Median

The median is the measure of the central value of the distribution when the data are ranked in order of magnitude. The median is the 50th percentile of the ranked data set. It is the only measure of location that is minimally affected by the magnitude of a single observation such as an outlier (Helsel and Hirsch, 1995).

3. OUTLIERS

These are observations whose values are quite different from other values in the data set (Helsel and Hirsch, 1995).

Outliers have generally one of three causes:

I. A measurement or recording error

II. An observation from a population not similar to most of the data

III. A rare event from a single population that is quite skewed.

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

I. Erroneous/incorrect entering of data into calculation programmes

II. Copying, decimal points or other obvious errors

III. Comparing the outlying tendency with the other indicators enumerated from the same sample to see if a similar event occurred.

When no errors were detected, the outliers where kept in the sets as they presented real events in the sampling and analysis routine such as higher pollution in the particular water type at the time of the season.

4. HYPOTHESIS

Statistical tests are quantitative methods to determine whether hypotheses can be substantiated or whether they must be modified or rejected outright.

Hypotheses were formulated (Chapter 2: Methodology, Section 2.8) for various sections (Chapter 3: Results and Discussion).

4.1 α- value

The significance level (α- value) is the probability of incorrectly rejecting the null hypothesis. The significance value for this study is set at default 5% and 0.05 is the statistical tradition that was followed for the significant value.

4.2 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).

4.3 Expected outcomes

These were the anticipated results before the factual outcomes of the study analysis.

5. MINIMAL SAMPLE SIZE

The minimal sample sizes for statistical significant differences were determined before each series of experiments commenced.

One should determine approximately how big the sample size has to be in order to detect an impact or statistical difference at a specified level. The larger the sample size, the greater the power of relevant test applied (Helsel and Hirsch, 1995; SigmaStat®, 1997).

5.1 Sample size and ANOVA

Crude initial estimates of 15 samples for each microorganism group used for each water category were based on the minimum number of samples prescribed by Standard Methods (1998) for an intra-laboratory proficiency programme. According to Helsel and Hirsch (1995) the larger the sample size the more accurate the relevant test applied.

After assessing the first 15 samples, the mean differences of each (n = 15) of the data sets 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. The data in the sets used for this study were non-parametric. However, to determine the minimum sample size, the normality of

the data is generally ignored and determined (Helsel and Hirsch, 1995; SigmaStat®, 1997).

- Sensitivity of the test (desired power). The power is the probability that the correlation coefficient quantifies an actual association. According to Helsel and Hirsch (1995), sensitivities in water resource 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-α confidence (i.e 95% confidence when $\alpha = 0.05$).
- 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 $\alpha \leq 0.05$).

6. NORMALITY OF DATA

According to Helsel and Hirsch (1995), serious problems can be experienced when statistical procedures are used assuming symmetry or linearity. Microbiological water-quality data distributions are often not symmetrical. Organism counts often have a skewed distribution because of more low counts than high counts (Standard Methods, 1998). Incorrect statements can therefore be made when statistical procedures, which assume normality, are used. Non-parametric tests were used throughout, in this study, to analyse the data.

7. DATA TRANSFORMATION

For this study, data sets were transformed to their logarithms, which produced more symmetrical data. Transformations of data could be used to produce data that would display normal distribution characteristics (Standard Methods, 1998). Transformations are used to make data more linear, symmetric and more consistent in variance (Helsel and Hirsch, 1995).

8. ANALYSIS OF VARIANCE (ANOVA)

ANOVA includes a series of parametric tests done on the assumption that the data concerned are normally distributed around the mean with similar variance. Equal variance test results display whether or not 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 (Helsel and Hirsch, 1995).

8.1 Non-parametric tests

Where parametric test methods lose considerable power to detect differences in non-normal data, non-parametric testing displays considerable power in non-normal as well as normal data testing and display (Helsel and Hirsch, 1995).

The following non-parametric tests ' nd Hirsch, 1995):

- Rank-sum test. This 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 hypothesis, the non-parametric Mann-Whitney Rank sum test was used. The test was selected because no assumption about the normality of the data was needed, and again, it could determine whether data from each of the two groups came from the same population.
- Signed Rank test. This is a non-parametric test that was applied on paired data sets. In the context of this study, the Wilcoxon Signed Rank Test was used to test for variance in the paired before and after data sets to test for any significant changes in water quality.
- Kruskal-Wallis ANOVA on Ranks. This is a non-parametric test that was used to compare data of more that two groups. It compares results from several different experimental groups that may be affected by a single factor. Rank transformation of data implies that the original data are placed by ranks, which omits substantial variance and error from the multiple comparison procedure.

  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 zero 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.
- Multiple Comparison Test (MCT). When more than two samples are compared, the interest is not only whether the indicator organism numbers determined at each point differed, but also which differed from the others. Therefore the Multiple Comparison tests were applied where significant differences were encountered. Tukey's MCT was used to identify any significantly different groups after using the Kruskal-Wallis test to identify the significantly different groups in data sets of equal sizes. Dunn's MCT was used when the sample sizes of the groups were not of equal size. Q test statistic indicates the number of means spanned in the comparison *p*. The larger the Q, the more acceptable the conclusion that the difference of 2 groups being compared is statistically significant. If the *P* is greater than 0.05, it cannot be confidently concluded that there is a statistically significant difference between the means of the two groups compared.

9. DATA PRESENTATION

Data were plotted in box plot graphs to provide visual summaries and describe essential information more quickly. According to Helsel and Hirsch (1995), box plots provide the clearest visual summaries of the following:

- The centre of the data, the m red measure of central tendency for the data because it is resistant to the effects of the outliers and tends to indicate a more sensible central point in data.
- The variation of spread (interquartile range) indicates the spread of data between the 25th and 75th percentile. The closer the data are clustered to the median within the interquartile range, the less variation there is in the data.
- The skewness (also referred to as the quartile skew) is represented by the relative size of the box halves. The smaller the upper quartile skews, the more positive the data are skewed.
- The caps whiskers on the lines protruding above and below the box indicate the 95th and 5th percentiles.

STORAGE CONTAINER TYPES

Figure 1: A bucket-type plastic container used to store water

Figure 2: Bucket-type galvanised metal container used to store water

Figure 3: Plastic screw-top containers used to store water

Figure 4: Bucket-type plastic container (control) used to store water

Figure 5: The wide-mouthed, bucket-type plastic and galvanised metal containers used.

Figure 6: The most commonly used containers for water collection

Figure 7: The most common method of water collection

Figure 8: The most common method of water collection

CHAPTER 3: TABLES

3.1 Bucket-type plastic containers

Table 3.1.1: Turbidity levels in water from the bucket-type plastic containers

Contaminant build-up potential	Results in NTU	Before dislodging	After dislodging	Wilcoxon Signed Rank Test on Paired data
Maximum (Uncovered)	n Mean 95th Percentile Min Max	23 0.6 4.86 0.11 5.30	24 1.43 10.25 0.13 13.10	Significant increase (P≤0.001) H_0 Rejected After > Before
Minimum (Covered)	n Mean 95th Percentile Min Max	24 0.5 4.1 0.11 5.04	24 0.66 5 0.17 7.34	Significant increase (P≤0.001) H_0 Rejected After > Before
Mann-Whitney Rank Sum Test		Increase NOT significant (P = 0.126) H_0 NOT rejected	Increase NOT significant (P = 0.198) H_0 NOT rejected	

n = number; Min = minimum; Max = maximum;

Table 3.1.2: Heterotrophic bacteria numbers in water from the bucket-type plastic containers

Contaminant build-up potential	Organism numbers per 1 mℓ	Before dislodging	After dislodging	Wilcoxon Signed Rank Test on Paired data
Maximum (Uncovered)	n GeoMean 95th Percentile Min Max	23 4,570 214,650 200 409,000	23 17,000 2.54×10^7 589 7.17×10^7	Significant increase (P≤0.001) H_0 Rejected After > Before
Minimum (Covered)	n GeoMean 95th Percentile Min Max	24 7.40×10^3 594,000 256 874,000	24 1.94×10^4 603,000 633 946,000	Significant increase (P≤0.001) H_0 Rejected After > Before
Mann-Whitney Rank Sum Test		Increase NOT significant (P = 0.413) H_0 NOT rejected	Increase NOT significant (P = 0.489) H_0 NOT rejected	

n = number; GeoMean = geometric mean; Min = minimum; Max = maximum;

Table 3.1.3: Total coliform numbers in water from the bucket-type plastic containers

Contaminant build-up potential	Organism numbers per 100 mℓ	Before dislodging	After dislodging	Wilcoxon Signed Rank Test on Paired data
Maximum (Uncovered)	n GeoMean 95th Percentile Min Max	23 101 16,845 10 17,300	23 394 31,300 15 44,300	Significant increase (P≤0.001) H_0 Rejected After > Before
Minimum (Covered)	n GeoMean 95th Percentile Min Max	24 121 37,610 14 64,700	24 404 38,720 19 67,700	Significant increase (P≤0.001) H_0 Rejected After > Before
Mann-Whitney Rank Sum Test		Increase NOT significant (P = 0.242) H_0 NOT rejected	Increase NOT significant (P = 0.790) H_0 NOT rejected	

n = number; Geomean = geometric mean; Min = minimum; Max = maximum;

Table 3.1.4: *E coli* numbers in w et-type plastic containers

Contaminant build-up potential	Organism numbers per 100 mℓ	Before dislodging	After dislodging	Wilcoxon Signed Rank Test on Paired data
Maximum (Uncovered)	n	23	23	Significant increase (P≤0.001)
	GeoMean	2.63	8.61	H_0 Rejected
	95th Percentile	2.847	2.912	After > Before
	Min	1	1	
	Max	7.670	7.670	
Minimum (Covered)	n	24	24	Significant increase (P≤0.001)
	GeoMean	0.662	2.11	H_0 Rejected
	95th Percentile	443	724	After > Before
	Min	1	1	
	Max	700	1,230	
Mann-Whitney Rank Sum Test		Increase NOT significant (P = 0.083) H_0 NOT rejected	Increase NOT significant (P = 0.142) H_0 NOT rejected	

n = number; Geomean = geometric mean; Min = minimum; Max = maximum;

Table 3.1.5: *C perfringens* numbers in water from the bucket-type plastic containers

Contaminant build-up potential	Organism numbers per 100 mℓ	Before dislodging	After dislodging	Wilcoxon Signed Rank Test on Paired data
Maximum (Uncovered)	n	23	23	Significant increase (P≤0.001)
	GeoMean	0.14	0.39	H_0 Rejected
	95th Percentile	6.8	25.5	After > Before
	Min	1	1	
	Max	12	34	
Minimum (Covered)	n	24	24	Significant increase (P = 0.004)
	GeoMean	0.021	0.12	H_0 Rejected
	95th Percentile	0.87	2.2	After > Before
	Min	1	1	
	Max	2	3	
Mann-Whitney Rank Sum Test		Increase NOT significant (P = 0.181) H_0 NOT rejected	Increase NOT significant (P = 0.241) H_0 NOT rejected	

n = number; Geomean = geometric mean; Min = minimum; Max = maximum

3.2 Bucket-type metal containers

Table 3.2.1: Turbidity levels in water from the bucket-type metal containers

Contaminant build-up	Results in NTU	Before dislodging	After dislodging	Wilcoxon Signed Rank Test on Paired data
Maximum (Uncovered)	n	25	25	Significant increase (P≤0.001)
	Mean	2.12	6.08	H_0 Rejected
	95th Percentile	4.66	16.15	After > Before
	Min	0.13	0.1	
	Max	5.1	21.1	
Minimum (Covered)	n	19	19	Significant increase (P≤0.001)
	Mean	1.61	4.1	H_0 Rejected
	95th Percentile	3.17	14.83	After > Before
	Min	0.2	0.65	
	Max	3.62	22.5	
Mann-Whitney Rank Sum Test		Increase NOT significant (P = 0.407) H_0 NOT rejected	Increase NOT significant (P = 0.145) H_0 NOT rejected	

n = number; Min = minimum; Max = maximum

Table 3.2.2: Heterotrophic bacte er from the bucket-type metal containers

Contaminant build-up potential	Organism numbers per 1 ml	Before dislodging	After dislodging	Wilcoxon Signed Rank Test on Paired data
Maximum (Uncovered)	**n**	25	25	Significant increase ($P \leq 0.001$)
	GeoMean	2,870	8,400	H_0 Rejected
	95th Percentile	48,375	1.90×10^6	After > Before
	Min	56	167	
	Max	90,300	6.54×10^6	
Minimum (Covered)	**n**	19	19	Significant increase ($P \leq 0.001$)
	GeoMean	1,810	6,770	H_0 Rejected
	95th Percentile	68,215	2.78×10^5	After > Before
	Min	244	267	
	Max	103,000	301,000	
Mann-Whitney Rank Sum Test		Increase NOT significant ($P = 0.281$) H_0 NOT rejected	Increase NOT significant ($P = 0.687$) H_0 NOT rejected	

n = number; GeoMean = geometric mean; Min = minimum; Max = maximum;

Table 3.2.3: Total coliform numbers in water from the bucket-type metal containers

Contaminant build-up potential	Organism numbers per 100 ml	Before dislodging	After dislodging	Wilcoxon Signed Rank Test on Paired data
Maximum (Uncovered)	**n**	25	25	Significant increase ($P \leq 0.001$)
	GeoMean	63.4	210	H_0 Rejected
	95th Percentile	3,273	6,598	After > Before
	Min	6	15	
	Max	3,400	9,200	
Minimum (Covered)	**n**	19	19	Significant increase ($P \leq 0.001$)
	GeoMean	15.6	72.4	H_0 Rejected
	95th Percentile	1,564	2,122	After > Before
	Min	1	7	
	Max	1,870	2,630	
Mann-Whitney Rank Sum Test		Significant increase ($P = 0.021$) H_0 rejected	Increase NOT significant ($P = 0.072$) H_0 NOT rejected	

n = number; Geomean = geometric mean; Min = minimum; Max = maximum;

Table 3.2.4: *E coli* numbers in water from the bucket-type metal containers

Contaminant Build-up potential	Organism numbers per 100 ml	Before dislodging	After dislodging	Wilcoxon Signed Rank Test on Paired data
Maximum (Uncovered)	**n**	25	25	Significant increase ($P \leq 0.001$)
	GeoMean	0.8	5.79	H_0 Rejected
	95th Percentile	118	908	After > Before
	Min	1	1	
	Max	270	2,200	
Minimum (Covered)	**n**	19	19	Significant increase ($P \leq 0.001$)
	GeoMean	0.10	0.72	H_0 Rejected
	95th Percentile	2,402	33.3	After > Before
	Min	1	1	
	Max	3	34	
Mann-Whitney Rank Sum Test		Significant increase ($P = 0.049$) H_0 rejected	Significant increase ($P = 0.007$) H_0 rejected	

n = number; Geomean = geometric mean; Min = minimum; Max = maximum

Table 3.2.5: *C perfringens* numb ne bucket-type metal containers

Contaminant build-up potential	Organism numbers per 100 ml	Before dislodging	After dislodging	Wilcoxon Signed Rank Test on Paired data
Maximum (Uncovered)	**n**	25	25	Significant increase (P = 0.002) H_0 Rejected After > Before
	GeoMean	0.03	0.14	
	95th Percentile	0.5	1.84	
	Min	1	1	
	Max	1	3	
Minimum (Covered)	**n**	19	19	Increase NOT significant (P = 0.063) H_0 NOT Rejected After = Before
	GeoMean	0	0.05	
	95th Percentile	0.1	0.67	
	Min	1	1	
	Max	1	1	
Mann-Whitney Rank Sum Test		Increase NOT significant (P = 0.262) H_0 NOT rejected	Increase NOT significant (P = 0.235) H_0 NOT rejected	

n = number; Geomean = geometric mean; Min = minimum; Max = maximum

3.3 Plastic Screw-top containers

Table 3.3.1: Turbidity levels in water from the plastic screw-top containers

Contaminant build-up	Results in NTU	Before dislodging	After dislodging	Wilcoxon Signed Rank Test on Paired data
Maximum (Unrinsed)	**n**	22	22	Significant increase (P=0.002) H_0 Rejected After > Before
	Mean	1.1	1.22	
	95th Percentile	2.8	3.1	
	Min	0.1	0.1	
	Max	3.41	3.78	
Minimum (Rinsed)	**n**	15	15	Significant increase (P≤0.001) H_0 Rejected After > Before
	Mean	1.1	1.33	
	95th Percentile	1.55	2.49	
	Min	0.42	0.43	
	Max	1.58	2.62	
Mann-Whitney Rank Sum Test		Increase NOT significant (P = 0.578) H_0 NOT rejected	Increase NOT significant (P = 0.599) H_0 NOT rejected	

n = number; Min = minimum; Max = maximum

Table 3.3.2: Heterotrophic bacteria numbers in water from the plastic screw-top containers

Contaminant build-up potential	Organism numbers per 1 ml	Before dislodging	After dislodging	Wilcoxon Signed Rank Test on Paired data
Maximum (Unrinsed)	**n**	22	22	Significant increase (P≤0.001) H_0 Rejected After > Before
	GeoMean	836	2,060	
	95th Percentile	26,872	84,360	
	Min	100	300	
	Max	61,300	86,400	
Minimum (Rinsed)	**n**	15	15	Significant increase (P≤0.001) H_0 Rejected After > Before
	GeoMean	466	1,450	
	95th Percentile	2,648	26,600	
	Min	111	200	
	Max	2,920	27,600	
Mann-Whitney Rank Sum Test		Increase NOT significant (P = 0.130) H_0 NOT rejected	Increase NOT significant (P = 0.386) H_0 NOT rejected	

n = number; GeoMean = geometric mean; Min = minimum; Max = maximum;

Table 3.3.3: Total coliform numb[illegible]e plastic screw-top containers

Contaminant build-up potential	Organism numbers per 100 ml	Before dislodging	After dislodging	Wilcoxon Signed Rank Test on Paired data
Minimum (Rinsed)	**n**	15	15	Significant increase ($P \leq 0.001$) H_0 Rejected After > Before
	GeoMean	3.87	10.5	
	95th Percentile	210	235	
	Min	1	1	
	Max	257	257	
Maximum (Unrinsed)	**n**	22	22	Significant increase ($P \leq 0.001$) H_0 Rejected After > Before
	GeoMean	6.25	29.6	
	95th Percentile	262	4,400	
	Min	1	2	
	Max	467	10,300	
Mann-Whitney Rank Sum Test		Increase NOT significant ($P = 0.345$) H_0 NOT rejected	Increase NOT significant ($P = 0.104$) H_0 NOT rejected	

n = number; Geomean = geometric mean; Min = minimum; Max = maximum;

Table 3.3.4: *E coli* numbers in water from the plastic screw-top containers

Contaminant build-up potential	Organism numbers per 100 ml	Before dislodging	After dislodging	Wilcoxon Signed Rank Test on Paired data
Minimum (Rinsed)	**n**	15	15	Increase NOT significant ($P = 0.063$) H_0 NOT Rejected After = Before
	GeoMean	0.02	0.16	
	95th Percentile	0.78	13.75	
	Min	1	1	
	Max	1	18	
Maximum (Unrinsed)	**n**	22	22	Significant increase ($P = 0.002$) H_0 Rejected After > Before
	GeoMean	0.09	0.35	
	95th Percentile	15.58	346	
	Min	1	1	
	Max	38	833	
Mann-Whitney Rank Sum Test		Increase NOT significant ($P = 0.231$) H_0 NOT rejected	Increase NOT significant ($P = 0.412$) H_0 NOT rejected	

n = number; Geomean = geometric mean; Min = minimum; Max = maximum

Table 3.3.5: *C perfringens* numbers in water from plastic screw-top containers

Contaminant build-up potential	Organism numbers per 100 ml	Before dislodging	After dislodging	Wilcoxon Signed Rank Test on Paired data
Minimum (Rinsed)	**n**	16	16	Increase NOT significant ($P = 1.000$) H_0 NOT Rejected After = Before
	GeoMean	0	0.1	
	95th Percentile	0.1	0.1	
	Min	1	1	
	Max	1	1	
Maximum (Unrinsed)	**n**	23	23	Increase NOT significant ($P = 1.000$) H_0 NOT Rejected After = Before
	GeoMean	0	0.005	
	95th Percentile	0.1	0.19	
	Min	1	1	
	Max	1	1	
Mann-Whitney Rank Sum Test		Increase NOT significant ($P = 0.988$) H_0 NOT rejected	Increase NOT significant ($P = 0.828$) H_0 NOT rejected	

n = number; Geomean = geometric mean; Min = minimum; Max = maximum

3.4 Control container

Table 3.4.1: Turbidity levels in water from the control bucket-type plastic container

Contaminant build-up	Results in NTU	Before dislodging	After dislodging	Wilcoxon Signed Rank Test on Paired data
(Unwashed)	n	26	26	Significant increase ($P \leq 0.001$)
	Mean	1.1	2.1	H_0 Rejected
	95th Percentile	2.86	7.1	After > Before
	Min	0.15	0.22	
	Max	3.33	8.32	

n = number; Min = minimum; Max = maximum

Table 3.4.2: Heterotrophic bacteria numbers in water from the control bucket-type plastic container

Contaminant build-up potential	Organism numbers per 1 ml	Before dislodging	After dislodging	Wilcoxon Signed Rank Test on Paired data
(Unwashed)	N	26	26	Significant increase ($P \leq 0.001$)
	GeoMean	6,500	18,100	H_0 Rejected
	95th Percentile	2.52×10^5	1.3×10^6	After > Before
	Min	144	656	
	Max	297,000	5.24×10^6	

n = number; GeoMean = geometric mean; Min = minimum; Max = maximum;

Table 3.4.3: Total coliform numbers in water from the control bucket-type plastic container

Contaminant build-up potential	Organism numbers per 100 ml	Before dislodging	After dislodging	Wilcoxon Signed Rank Test on Paired data
Unwashed	n	26	26	Significant increase ($P \leq 0.001$)
	GeoMean	0.63	3.17	H_0 Rejected
	95th Percentile	10	26.18	After > Before
	Min	1	1	
	Max	11	50	

n = number; Geomean = geometric mean; Min = minimum; Max = maximum;

Table 3.4.4: *E coli* numbers in water from the control bucket-type plastic container

Contaminant build-up potential	Organism numbers per 100 ml	Before dislodging	After dislodging	Wilcoxon Signed Rank Test on Paired data
Unwashed	n	26	26	Significant increase ($P = 0.014$)
	GeoMean	0.02	0.14	H_0 Rejected
	95th Percentile	0.4	1.8	After > Before
	Min	1	1	
	Max	1	5	

n = number; Geomean = geometric mean; Min = minimum; Max = maximum

Table 3.4.5: *C perfringens* numbers in water from the control bucket-type plastic container

Contaminant Build-up potential	Organism numbers per 100 ml	Before dislodging	After dislodging	Wilcoxon Signed Rank Test on Paired data
Unwashed	n	26	26	Increase NOT significant ($P = 0.500$)
	GeoMean	0	0.010	H_0 NOT Rejected
	95th Percentile	0.1	0.33	After = Before
	Min	1	1	
	Max	1	1	

n = number; Geomean = geometric mean; Min = minimum; Max = maximum

Table 3.5.1: Comparing turbidity levels in water from the bucket-type plastic and metal containers

Contaminant build-up potential	Results in NTU	Plastic bucket	Metal bucket	Mann-Whitney Rank Sum test
Maximum (Uncovered)	n	23	25	Differences NOT significant P = 0.065 H_0 NOT Rejected
	Mean	1.43	6.08	
	95th Percentile	10.25	16.15	
	Min	0.13	0.1	
	Max	13.1	21.1	
Minimum (Covered)	n	24	19	Significant difference P = 0.031 H_0 Rejected
	Mean	0.66	4.09	
	95th Percentile	4.92	14.83	
	Min	0.17	0.65	
	Max	7.34	22.5	

n = number; Min = minimum; Max = maximum

Table 3.5.2: Comparing heterotrophic bacteria numbers in water from the bucket-type plastic and metal containers

Contaminant build-up potential	Organism numbers per 1 ml	Plastic bucket	Metal bucket	Mann-Whitney Rank Sum test
Maximum (Uncovered)	n	23	25	Differences NOT significant P = 0.375 H_0 NOT Rejected
	GeoMean	17,000	8,400	
	95th Percentile	2.54×10^7	1.9×10^6	
	Min	589	167	
	Max	7.17×10^7	6.54×10^6	
Minimum (Covered)	n	24	19	Differences NOT significant P = 0.133 H_0 NOT Rejected
	GeoMean	1.94×10^4	6,770	
	95th Percentile	603,000	278,050	
	Min	633	267	
	Max	946,000	301,000	

n = number; Geomean = geometric mean; Min = minimum; Max = maximum

Table 3.5.3: Comparing total coliform numbers in water from the bucket-type plastic and metal containers

Contaminant build-up potential	Organism numbers per 100 ml	Plastic bucket	Metal bucket	Mann-Whitney Rank Sum test
Maximum (Uncovered)	n	23	25	Differences NOT significant P = 0.380 H_0 NOT Rejected
	GeoMean	394	210	
	95th Percentile	31,300	6,598	
	Min	15	15	
	Max	44,300	9,200	
Minimum (Covered)	n	24	19	Significant difference P = 0.020 H_0 Rejected
	GeoMean	404	72.4	
	95th Percentile	38,720	2,122	
	Min	19	7	
	Max	67,700	2630	

n = number; Geomean = Geometric mean; Min = minimum; Max = maximum

Table 3.5.4: Comparing *E co* from the bucket-type plastic and metal containers

Contaminant build-up potential	Organism numbers per 100 ml	Plastic bucket	Metal bucket	Mann-Whitney Rank Sum test
Maximum (Uncovered)	n	23	25	Differences NOT significant P = 0.812 H_0 NOT Rejected
	GeoMean	8.61	5.79	
	95th Percentile	2,912	908	
	Min	1	1	
	Max	7,670	2,200	
Minimum (Covered)	n	24	19	Differences NOT significant P = 0.266 H_0 NOT Rejected
	GeoMean	2.11	0.72	
	95th Percentile	724	33.3	
	Min	1	1	
	Max	1,230	34	

n = number; Geomean = Geometric mean; Min = minimum; Max = maximum

Table 3.5.5: Comparing *C perfringens* numbers in water from the bucket-type plastic and metal containers

Contaminant build-up potential	Organism numbers per 100 ml	Plastic bucket	Metal bucket	Mann-Whitney Rank Sum test
Maximum (Uncovered)	n	23	25	Differences NOT significant P = 0.347 H_0 NOT Rejected
	GeoMean	0.39	0.14	
	95th Percentile	25.5	1.84	
	Min	1	1	
	Max	34	3	
Minimum (Covered)	n	24	19	Differences NOT significant P = 0.351 H_0 NOT Rejected
	GeoMean	0.12	0.05	
	95th Percentile	2.2	0.67	
	Min	1	1	
	Max	3	1	

n = number; Geomean = Geometric mean; Min = minimum; Max = maximum

3.6 Plastic, Metal and Screw-top containers

Table 3.6.1: Comparing turbidity data for all three container types

Contaminant build-up potential	Results in NTU	Plastic screw top containers PSC	Plastic container (bucket-type) PC	Metal containers (bucket-type) MC	ANOVA on Ranks Kruskal-Wallis test
Maximum	n	22	23	25	PSC significantly different from: MC and PC (P<0.05)
	Mean	1.22	1.43	6.08	
	95th Percentile	3.05	10.25	16.15	
	Min	0.1	0.13	0.1	
	Max	3.8	13.1	21.1	

n = number; Min = minimum; Max = maximum

Table 3.6.2: Comparing heterotrophic bacteria data for all three container types

Contaminant build-up potential	Organism numbers per 1 ml	Plastic screw top containers PSC	Plastic container (bucket-type) PC	Metal containers (bucket-type) MC	ANOVA on Ranks Kruskal-Wallis test
Maximum	n	22	23	25	PSC significantly different from PC and MC (P<0.05)
	GeoMean	2,060	17,000	8,400	
	95th Percentile	84,360	2.54×10^7	1.9×10^6	
	Min	300	589	167	
	Max	86,400	7.17×10^7	6.54×10^6	

n = number; Geomean = geometric mean; Min = minimum; Max = maximum

Table 3.6.3: Comparing total col ree container types

Contaminant build-up potential	Organism numbers per 100 ml	Plastic screw top containers PSC	Plastic container (bucket-type) PC	Metal containers (bucket-type) MC	ANOVA on Ranks Kruskal-Wallis test
Maximum	n	22	23	25	PSC significantly different from PC and MC (P<0.05)
	GeoMean	29.6	394	210	
	95th Percentile	4,400	31,300	6,598	
	Min	2	15	15	
	Max	10,300	44,300	9,200	

n = number; Geomean = geometric mean; Min = minimum; Max = maximum

Table 3.6.4: Comparing *E coli* data for all three container types

Contaminant build-up potential	Organism numbers per 100 ml	Plastic screw top containers PSC	Plastic container (bucket-type) PC	Metal containers (bucket-type) MC	ANOVA on Ranks Kruskal-Wallis test
Maximum	n	22	23	25	PSC significantly different from PC and MC (P<0.05)
	GeoMean	0.35	8.61	5.79	
	95th Percentile	346	2,912	908	
	Min	1	1	1	
	Max	833	7.670	2.200	

n = number; Geomean = geometric mean; Min = minimum; Max = maximum

Table 3.6.5: Comparing *C perfringens* data for all three container types

Contaminant build-up potential	Organism numbers per 100 ml	Plastic screw top containers PSC	Plastic container (bucket-type) PC	Metal containers (bucket-type) MC	ANOVA on Ranks Kruskal-Wallis test
Maximum	n	23	23	25	PSC significantly different from PC and NOT from MC (P<0.05)
	GeoMean	0.005	0.39	0.14	
	95th Percentile	0.18	25.5	1.84	
	Min	1	1	1	
	Max	1	34	3	

n = number; Geomean = geometric mean; Min = minimum; Max = maximum