Assessment of the physico-chemical and microbiological quality of household water in the Vaalharts irrigation scheme, South Africa

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ABSTRACT

Water quality in the Vaalharts region in the Northern Cape Province, South Africa, decreased over the past few years and there was a need for the microbiological and physico-chemical assessment. This problem was identified through discussions with Vaalharts Water (Vaalharts Water User Association) in 2010 when the issue of the impact of deteriorating water quality on drinking water production was raised. It was thus important to investigate concerns of the water users association pertaining to water quality issues. The aim of this study was to assess the physico-chemical and microbiological quality of household water in the Vaalharts irrigation scheme. The main residential areas were Hartswater, Pampierstad, Jan Kempdorp and Warrenton. Faecal coliforms were detected in the raw water of all the drinking water distribution systems during 2011 and 2012. No faecal coliforms were detected in the household water during 2011. This was a very positive result, because not only did the household water comply with the SANS 241 (2011) standard (0 CFU/100ml), but the purification processes were successful by removing all the *E. coli's* from the raw water. However, during March 2012 faecal coliforms were detected in the household water of Jan Kempdorp (191 CFU/100ml). This could be due to point pollution and possible breakage of faecal coliforms in the distribution system. Low amounts of total coliforms were detected in the raw water of some of the drinking water distribution systems. This could be due to high amounts of other colonies (pink and purple) growing on the m-Endo agar which suppress the growth of the metallic green sheen (total coliform) colonies. The total coliform numbers complied with the SANS 241 (2011) standard of ≤10 CFU/100ml at most of the distribution systems, except for Hartswater during July 2011 (14 CFU/100ml) and Warrenton during March 2012 (256 CFU/100ml). Heterotrophic plate count bacteria were very high in the household water of some of the distribution systems during 2011 and 2012 which exceeded the SANS 241 (2011) standard of ≤1000 CFU/ml. A large number of pigmented (yellow, orange, pink) and non-pigmented (white) colonies were isolated on R_2A agar. This can be an indication of some failure in treatment processes. Other microbiological parameters that were tested such as faecal streptococci, *Clostridia*, *Pseudomonas aeruginosa* and fungi did not indicate any danger, but there were high levels of total anaerobic bacteria in the raw water during 2011 and 2012. A high level of anaerobic bacteria was detected in the household water of Hartswater during July 2011. *Clostridia* were also present in the household water of

some of the distribution systems during 2011 and 2012. Sequencing results of the *mdh*, *lacZ* and *uidA* genes indicated that one of the isolates was identified as *Enterobacter cloacae* and the other isolates were *E. coli*. Four of the isolates were identified as *Escherichia coli* O104:H4. This is a pathogenic strain and raised concern. The physico-chemical parameters that were measured complied with the SANS 241 (2011) standards during 2011 and 2012, but some of the parameters increased gradually from 2011 to 2012. Statistical analysis indicated that physico-chemical parameters had an influence on microbiological parameters and that deteriorating raw water may have an impact on drinking water quality. Another concern currently is that there is no SANS 241 (2011) for faecal streptococci, *Clostridia*, *Pseudomonas aeruginosa*, fungi and anaerobic bacteria. These are all opportunistic pathogenic bacteria and consuming water with high levels of these bacteria may cause health problems. This study indicated good progress in the treatment processes of the distribution systems over the two years. This may be due to the feedback given to Vaalharts Water during this study regarding the water quality of the residential areas. The physico-chemical and microbiological results of the present study indicated possible biofilm formation in the distribution systems. This may have impacts on the drinking water quality of the distribution systems. It was also evident that deteriorating raw water sources may have an impact on drinking water production.

Keywords: Vaalharts irrigation scheme, microbiological water quality, physico-chemical water quality, *Escherichia coli*, drinking water production, *mdh* gene, *lacZ* gene, *uidA* gene, 16S rDNA gene.

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DECLARATION

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

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Guzéne O'Reilly

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Date

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CHAPTER 1
INTRODUCTION & LITERATURE REVIEW

The study area is situated in the Vaalharts region in the Northern Cape Province, South Africa. Vaalharts is located on the border between the Northern Cape- and the North West Province at the confluence of the Harts- and Vaal River and has an area of 29 181 ha. The Vaalharts irrigation scheme was established by the government in the 1930's and was managed by the Department of Water Affairs and its predecessors. In 2003 Vaalharts Water (Vaalharts Water Association) took over the management of the scheme (Van Vuuren, 2009). The main residential areas in the Vaalharts irrigation scheme include Jan Kempdorp, Pampierstad, Hartswater and Warrenton (Van Vuuren, 2009).

In discussions with the Vaalharts Water management in 2010 the issue of the impact of deteriorating water quality on drinking water production was raised. Results of a preliminary study done in 2010 (O'Reilly, 2010) of this study area indicated that even though physico-chemical parameters were not really a concern, microbiological data showed the contrary. The presence of low levels of indicator organisms in the drinking water at some of the sites was cause for concern. The results also indicated that seasonal changes may impact on the water quality. The 2010 study was a pilot study which indicated the need for the physico-chemical and microbiological assessment of the drinking water in the Vaalharts irrigation scheme.

This is the oldest irrigation scheme in South Africa (Van Vuuren, 2010) and much has been published on the effective measures to curb water wastage (Van Vuuren, 2009). However, studies about the drinking water quality of this area were not readily available in public data bases.

1.1 RESEARCH AIM AND OBJECTIVES

The aim of this study was to assess the physico-chemical and microbiological quality of household water in the Vaalharts irrigation scheme, South Africa.

Specific objectives of this study were to:

i. perform a survey of the drinking water production plants in the Vaalharts irrigation scheme.
ii. assess the physico-chemical and microbiological quality of the municipal supplied water.
iii. determine whether the municipal supplied water complies with the South African National Standards (SANS) 241:2011.
iv. determine if seasonal changes have an impact on the municipal supplied water quality.
v. perform statistical analysis of the data to determine if there is any correlation between the water prior to purification and the water after purification.

1.2 DRINKING WATER PRODUCTION

Physical, chemical and microbiological characteristics of water are properties that are used to determine the general quality of water (Schutte, 2006). One of the most important requirements for domestic water is that the water should be safe to drink (Schutte, 2006; Momba *et al.*, 2009). Studies have shown that water purification plants in South Africa may not always produce the quality and quantity of drinking water they are designed for (Momba *et al.*, 2006). This could be due to infrastructure as well as management challenges. It is known that raw water sources are polluted and may contain harmful micro-organisms which make the water unfit to drink or to use for domestic purposes (Schutte, 2006). This raw water is purified by water treatment systems and is utilized by communities to which the water is supplied. It is thus very important that the water treatment systems produce water from raw water sources that is fit for domestic use at a reasonable cost (Schutte, 2006).

1.2.1 Processes of drinking water production

Raw water is derived from lakes, rivers or reservoirs. Intake structures can either be submerged intake pipes or tower-like structures (Schutte, 2006). Water flows by gravity to the plant or through the use of pumping stations which lifts the water from the source to an adequate height. The pumping station is usually situated at the intake structure (Schutte, 2006). At the water treatment plant seven basic steps are used to purify the raw water (figure 1): coagulation, flocculation, sedimentation, stabilization, filtration, disinfection and chloramination (Obi, 2007; Rand Water, 2012b).

Water that enters the drinking water production facility firstly undergoes coagulation (figure 1). This is a process that destabilizes colloidal particles by the rapid mixing of a coagulant with the water to form small flocs (DWAF, 2002; Schutte, 2006; Rand Water, 2012b). The most common coagulants are: aluminium sulphate, ferric chloride, hydrated lime, polymeric coagulants and polyelectrolytes (Schutte, 2006). Coagulation times vary according to the design of the treatment plant, but it is a rapid process and usually takes up to 20 - 30 seconds (Rand Water, 2012b). Some coagulants, for example hydrated lime, cause an increase in the pH. The water needs to be chemically stabilized to prevent any corrosion in the distribution system (DWAF, 2002; Rand Water, 2012b).

The water is then allowed to flocculate (figure 1). Flocculation is the aggregation of the small flocs formed during the coagulation step to form larger rapid-settling flocs (DWAF, 2002; Schutte, 2006; Rand Water, 2012b). The flocculation process involves the stirring of water, to which a coagulant has been added, at a slow rate. Aggregation of the small flocs takes place to form larger flocs which settles to the bottom of the tank (Schutte, 2006). The flow velocity at this stage must be at a suitable rate to ensure that floc formation takes place. If the flow velocity is too high, the aggregates may break up and settling of the broken flocs will be incomplete (Schutte, 2006). After flocculation the sedimentation process takes place (figure 1).

Sedimentation is the process where the aggregated flocs formed during coagulation and flocculation settles to the bottom of the tank by gravity. The flocs collect as sludge at the bottom of the tank and must be removed regularly (DWAF, 2002; Schutte, 2006; Rand Water, 2012b). There are various designs for sedimentation tanks. Rectangular sedimentation tanks are usually used at large conventional treatment plants where water enters one side of the tank and leaves at the other side (Schutte, 2006). Smaller water treatment plants normally use circular tanks with flat or cone shaped bottoms (Schutte, 2006). Rand Water uses horizontal flow tanks with retention times of four hours (Rand Water, 2012b). Water with a turbidity of 5 NTU is considered to be acceptable for filtration (Rand Water, 2012b). Water leaves the sedimentation tank through troughs situated at the top of the tank (figure 1).

From the sedimentation tanks the water overflows and is filtered (figure 1) to remove the remaining flocculated particles (Schutte, 2006; Rand Water, 2012b). Sand filtration is

the most conventional method used by water treatment plants. Slow sand filtration has been used for over 200 years in drinking water treatment (Langenbach *et al.*, 2010). The water is filtered through a sand bed allowing colloidal matter and microorganisms to form a layer on the granules and thus removing colloidal matter, microorganisms and colour from the water (Schutte, 2006). Slow sand filtration is simple and economical to construct, operate and maintain and does not need any chemicals or energy to operate (Langenbach *et al.*, 2010).

The last step in the water treatment process is disinfection (figure 1). This is a very important step in water treatment as it kills or inhibits pathogenic organisms that may be present in the water. Primary disinfection is usually achieved by using chlorine, but ultraviolet radiation, ozone and chlorine dioxide can also be used (DWAF, 2002; Schutte, 2006; Rand Water, 2012b). Chlorine only remains active in the water for 6 – 8 hours (Rand Water, 2012b). Chloramination may be used as a primary or secondary disinfectant (Schutte, 2006). Chloramination is the process where ammonia converts the free chlorine residual to chloramines. When in this form, chlorine is less reactive and lasts longer in the distribution system (Schutte, 2006; Rand Water, 2012b). After disinfection the final water is transported to storage tanks from where it is distributed.

1.2.2 Advanced treatment processes

More advanced processes such as desalination, fluoridation, reverse osmosis and activated carbon are options available to water treatment plants for the removal of specific substances. Desalination is a process which removes dissolved salts from the water by making use of distillation, membrane processes and ion exchange (Schutte, 2006). Fluoridation is where sodium chloride, sodium silicofluoride or hydrofluosilicic acid is added to the water to ensure optimum fluoride level for the prevention of dental caries (Schutte, 2006). Reverse osmosis is a general desalination process used for the removal of dissolved substances, including nitrate and arsenic (Schutte, 2006). The smallest dissolved ions (0.1 nm) can be removed and therefore reverse osmosis also removes bacteria and viruses. As a result reverse osmosis produces water of extremely good quality (Schutte, 2006). Taste and odour causing compounds as well as various metals can be removed by the activated carbon process (Schutte, 2006).

The use of membrane filtration (microfiltration, ultrafiltration and nanofiltration) has increased over the past decade (Zularisam *et al.*, 2006). This is mainly due the high level removal of bacteria, viruses and protozoa cysts such as *Giardia* and *Cryptosporidium* (Guo *et al.*, 2010; Kommineni *et al.*, 2010). Some of the advantages of using membrane filtration include easier maintenance, lower energy consumption and extremely good quality water produced (Zularisam *et al.*, 2006). These advanced processes may be implemented individually or as a package, depending on the need of the water treatment plant.

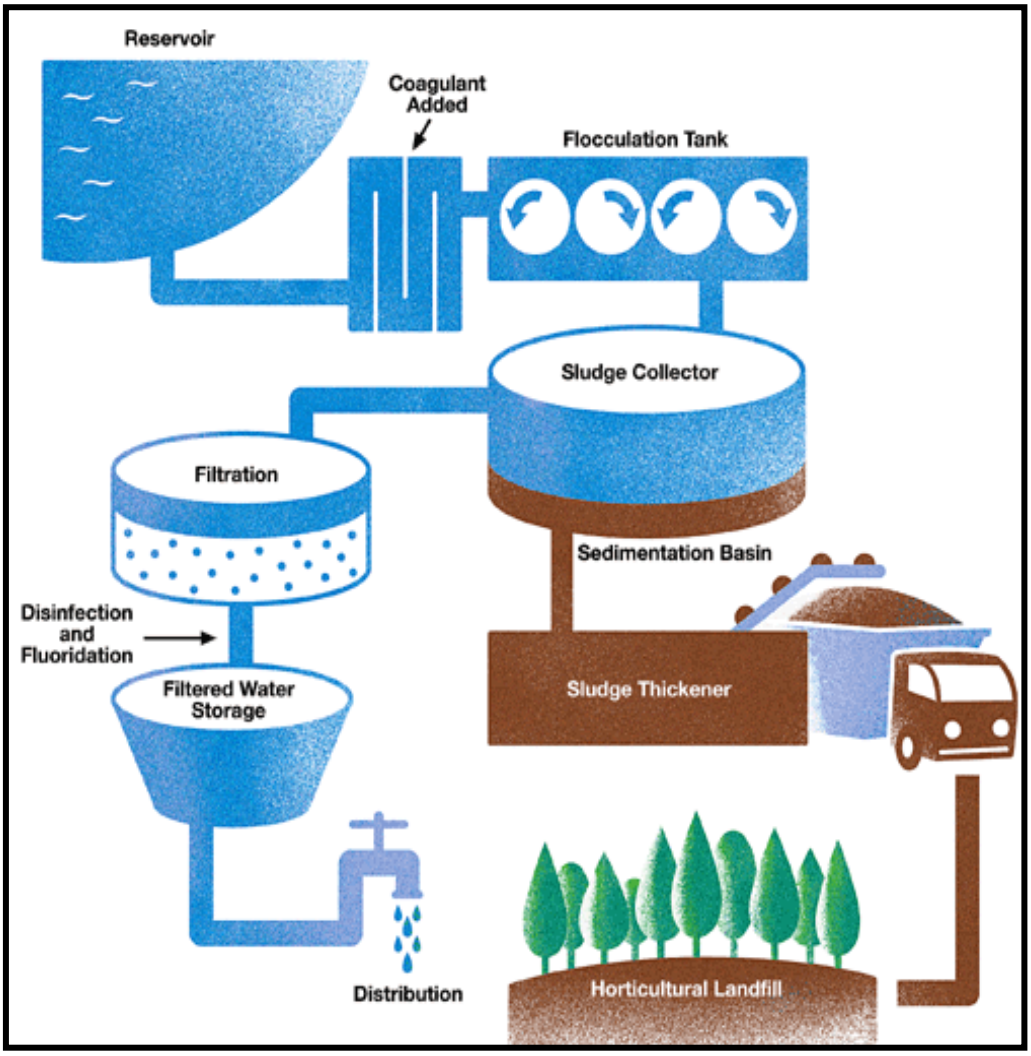

Figure 1: Diagram indicating the drinking water treatment process. (Source: SA Water: http://www.sawater.com.au/SAWater/Education/OurWaterSystems/Treating+Water.htm Date of access: 25 Oct. 2012).

1.2.3 Management

Another important aspect in drinking water production is the management of the water treatment plant. The water treatment plant needs to be managed properly to ensure

safe and good quality water is produced to consumers and to ensure optimal utilisation of the resources (water, money and manpower) (Schutte, 2006). Water quantity, quality and cost management are three important management aspects to ensure successful drinking water production. The principle objective of a water treatment plant is therefore to consistently produce water to the consumer which is fit for domestic use at a reasonable cost (Schutte, 2006).

The final water (drinking water) produced by the water treatment plant should comply with the South African National Standard (SANS) 241:2011 for drinking water. SANS 241:2011 provides the South African limits of the microbiological, physical, aesthetic and chemical determinands to which the drinking water should comply with at the point of delivery (DWA, 2012).

1.3 WATER TREATMENT PLANTS IN THE VAALHARTS IRRIGATION SCHEME

There are three small towns in the Vaalharts irrigation scheme and each has its own drinking water production facility. These facilities are operated and managed by the separate municipalities, except for the Pampierstad facility that is operated and managed by Sedibeng water. However, the bulk water supplier is Vaalharts Water. Water from the Vaal River is the source water for all these plants. One of the towns on the border of the irrigation scheme, Warrenton, was also included in the study. During 2011 a survey of the four water treatment plants (Hartswater, Pampierstad, Jan Kempdorp and Warrenton) was conducted. The survey was based on the regulations for the registration of waterworks and process controllers (South Africa, 2006). Information about the design parameters, operating procedures, control processes, special processes, microbiological analysis, population size, distribution materials and historical information of each water treatment plant were recorded. The data presented were provided by the management of the plants and therefore no references are provided in this section.

Table 1, 2 and 3 indicates the information obtained of the water treatment plants during the survey. Pampierstad is the oldest water treatment plant and has the smallest water treatment capacity (2501 – 7500 kl/day). Hartswater and Warrenton have the highest water treatment capacity (> 25 000 kl/day) (table 1). All four water treatment plants record their readings and daily flow and stock taking is calculated (table 2). All the water

treatment plants use chlorine gas as disinfectant, except for Hartswater which uses chlorine (table 3). Hartswater and Jan Kempdorp are the only two water treatment plants which stabilizes the water after sedimentation (table 3). Pampierstad and Jan Kempdorp water works are the only two to use jar tests to maintain optimum dosing.

None of the water treatment plants perform advanced processes such as fluoridation, reverse osmosis, activated carbon and softening of the water. The microbiological analysis of the water of all four water treatment plants are done by accredited laboratories. Distribution materials of the water treatment plants differ, but asbestos pipes are present in some areas of all four distribution systems (table 1). The pipe material of a distribution system is important, because it plays an important role in the proliferation of biofilms which attach to the inside surface of the pipe (Zhou *et al.*, 2009). The effect of various pipe materials on biofilm formation in chlorinated and combined chlorine-chloraminated water systems were studied by Momba and Makala (2004). Their study concluded that cement based pipes (cement and asbestos) support less fixed bacteria than plastic-based material (such as polyvinyl chloride (PVC)).

Table 1: Design parameters of the water treatment plants.

		Population size	Age of WTP	Water treatment capacity	Final water storage capacity (during peak time)	Piping material
Design parameters	**Hartswater WTP**	± 27 000	± 30 years	> 25 000 kl/day	> 60 hours	PVC & asbestos
	Pampierstad WTP	± 50 000	More than 30 years	2501 – 7500 kl/day	> 60 hours	Asbestos
	Jan Kempdorp WTP	± 30 000	± 20 years	7501 – 25 000 kl/day	30 - 60 hours	PVC, asbestos & galvanized steel
	Warrenton WTP	± 18 000	± 14 years	> 25 000 kl/day	< 36 hours	Mostly asbestos

WTP: Water treatment plant; **kl/day**: kilolitre per day

Table 2: Control processes of the water treatment plants.

		Water losses	Water management	Pumping	Level	Maintenance	Laboratory service	Administration
Control Processes	**Hartswater WTP**	On works only	Different reservoirs	Gravitation	Indicators	Specialised - by operators	Reading of instrumentation by operators	Readings are recorded.
	Pampierstad WTP	None	Different reservoirs	Gravitation & pumping	Telemetrically	Basic - by operators	Reading of instrumentation by operators	Readings are recorded.
	Jan Kempdorp WTP	On works only	Different reservoirs	Gravitation & pumping	Indicators	Basic - by operators	Reading of instrumentation by operators	Readings are recorded.
	Warrenton WTP	On works only	Different reservoirs	Gravitation & pumping	Indicators	Basic - by operators	Reading of instrumentation by operators	Readings are recorded.

WTP: Water treatment plant.

Table 3: Operating procedures of the water treatment plants.

		Raw water quality	Chemical dosing		Desludging	Filter backwash
			Chemical	pH control		
Operating procedures	**Hartswater WTP**	Monthly adjustments	Disinfection + 1 flocculation chemical	Yes	Automatically	Automatically (timer)
	Pampierstad WTP	Seasonal adjustments	Disinfection + 1 flocculation chemical	No	Manually	Optimised
	Jan Kempdorp WTP	Seasonal adjustments	Disinfection + 1 flocculation chemical	Yes	Manually	Manually (fixed time schedule)
	Warrenton WTP	No adjustments	Disinfection + 1 flocculation chemical	No	Manually	Manually (fixed time schedule)
		Settling process	**Stabilization of pH**		**Disinfection**	**Recirculation**
	Hartswater WTP	Controlled	Automatic dosing		Chlorine	Controlled with adjustments
	Pampierstad WTP	Uncontrolled	No stabilization		Chlorine gas	Automatic adjustments
	Jan Kempdorp WTP	Controlled	Manual dosing		Chlorine gas	Uncontrolled with adjustments
	Warrenton WTP	Uncontrolled	No stabilization		Chlorine gas	No recirculation

WTP: Water treatment plant.

1.4 VAAL RIVER WATER QUALITY

Domestic water is derived from the Vaal River and this water is purified to provide drinking water to the communities in the Vaalharts region. It is important to have an understanding about the quality of the water in Vaal River, because this will reflect on the quality of water after purification, that is, the household water of the Vaalharts region. Quality of the water from the Vaal River that is channelled into the Vaalharts irrigation system has been gradually deteriorating over the past few decades (Le Roux *et al*., 2007). This is mainly due to anthropogenic activities in the upper, middle and lower Vaal catchment areas (Braune & Rogers, 1987). According to water quality status assessments, a wide range of problems have been identified with regard to the water quality of the Vaal River. Some of the issues are found across the entire Vaal River, while other issues are more localised (DWAF-DNWRP, 2009b).

The Vaalharts weir on the Vaal River supply large quantities of water to the Vaalharts irrigation scheme in the Harts River catchment. Even though the Upper Vaal catchment has fairly good quality water, high levels of Total Dissolved Solids (TDS) are found in the Middle Vaal and Lower Vaal catchments downstream from the Harts River confluence (DWAF-DNWRP, 2009a). Irrigation return flows carried by the Harts River cause the increase in TDS levels at the confluence (DWAF-DNWRP, 2009a). Increasing levels of Total Dissolved Solids (TDS) in the Vaal River not only have an impact on industrial and agricultural use of water, but on domestic use as well (DWA, 2007). Nutrient enrichments in the Vaal River cause an increase in the treatment of the water for drinking purposes, which is an expensive problem (DWAF-DNWRP, 2009a).

The occurrence of microbiological contaminants in the Vaal River at localised points is also of concern (DWAF-DNWRP, 2009a). Even though microbiological data of the Vaal River is only available at localised points of the Vaal Barrage, data obtained from Midvaal Water indicated an increase in heterotrophic bacteria from 2003 to 2006 (DWAF-DNWRP, 2009a). Vaalharts forms part of the lower Vaal catchment area and main sources of pollution in this area are agriculture and poorly operated or dysfunctional sewage systems (DWAF-DNWRP, 2009a). The water quality of the Lower Vaal River has decreased over the past 20 years and will probably decrease even more (Du Preez *et al*., 2000 cited by Le Roux *et al*., 2007).

1.5 PHYSICO-CHEMICAL PARAMETERS

Measuring physico-chemical parameters of water can be a good indication of the quality, productivity and sustainability of that water body (Mustapha, 2008). Changes in the physico-chemical properties not only provide valuable information about the water quality, but the impacts of these parameters on the functions and biodiversity of the reservoir can be determined (Mustapha, 2008). Pollutants in the water cause an increase in physico-chemical parameters such as TDS, COD and metal levels which makes the water inappropriate to use or to drink (Tariq *et al.*, 2006). The importance of each of various parameters that are routinely measured is briefly described in the following sections.

1.5.1 Free chlorine

Chlorination is a very popular used method of disinfection which kills bacteria (Hua *et al*., 1999; Schoenen, 2002; Gião *et al.*, 2010) and it remains in the system for a significant period of time (Hua *et al*., 1999). Sometimes chlorine can produce odours, which are easy to recognise. These odours usually cause people to complain, because the water is unpleasant to drink (Hua *et al*., 1999; Dietrich, 2006). It is thus important to find a balance between the levels of chlorine necessary for bacterial control and providing people with water that is pleasant to drink. Chlorine can also react with the pipe material in water distribution systems (Hua *et al*., 1999). Free chlorine is the residual chlorine that remains 30 minutes after disinfection (DWAF, 2001). Free chlorine is important, because it ensures that final water is microbiologically safe as it moves through the distribution system (DWAF, 2005a). If the concentration of free chlorine is reduced, the contact time needs to be increased, because the effectiveness of chlorination is directly linked to the concentration of free chlorine and the contact time (DWAF, 2005a). The SANS 241 (2011) standard for free chlorine in drinking water is ≤5 mg/L (chronic health level). It is recommended that dosage levels of 2 mg/L are maintained to ensure a free chlorine level of 0.2 - 0.5 mg/L at point of delivery (WHO, 2011). Even though it is important that free chlorine is available in the distribution system, high concentrations will cause the water to have an unpleasant taste and smell (DWAF, 2001).

1.5.2 Total dissolved solids (TDS) & Electrical conductivity (EC)

Total dissolved solids (TDS) consists mainly of inorganic salts such as calcium, magnesium, potassium, sodium, bicarbonates, chlorides and sulphates, but contains a small amount of organic matter as well (WHO, 2011; Heydari & Bidgoli, 2012). Natural sources, sewage, urban runoff and industrial wastewater cause TDS in water (WHO, 2011). Electrical conductivity is a measurement of the ability of water to conduct electricity. Water with low salt levels such as distilled water conducts electricity poorly, whereas water with high salt levels such as sea water conducts electricity effectively (DWAF, 1998). Salinity is thus also a measurement of the amount of TDS present in the water (CSIR, 2010).

The EC level of water can be used to estimate the level of TDS in the water. The EC is related to the TDS by an average conversion factor of 6.5 for most waters (DWAF, 1996b). The conversion equation is as follows: EC (mS/m at 25°C) x 6.5 = TDS (mg/L) (DWAF, 1996b). According to the WHO (2011), there is no reliable data on possible health effects associated with the ingestion of reasonable levels of TDS in drinking water. Drinking water with TDS levels above 1000mg/L becomes increasingly unpalatable to consumers and excessive scaling of water pipes has also been noted (WHO, 2011). Electrical conductivity in drinking water causes a disturbance of salt and water balance in infants, heart patients, persons with high blood pressure, and renal disease (Memon *et al.*, 2008). The SANS 241 (2011) standard for EC in drinking water is ≤170 mg/L and for TDS ≤1200 mg/L in drinking water.

1.5.3 pH

pH is a logarithmic expression of the hydrogen concentration in water. It is a reflection of the degree of acidity (pH lower than 7) or alkalinity (pH greater than 7) of the water. The pH of most unpolluted water lies between 6.5 – 8.5. pH is an important operational water quality parameter (WHO, 2011). Water with a low pH level may cause corrosion in galvanised or copper pipes. The direct health effects of low and high pH levels include acid and alkali burns, respectively. These extreme pH levels may also cause irritation of the mucous membranes (DWAF, 1998). The SANS 241 (2011) standard for pH in drinking water is ≥5 to ≤9.7 pH units.

1.5.4 Sulphate & sulphide

Sulphate occurs in various natural minerals and these dissolved minerals contribute to the mineral content of drinking water (WHO, 2004a). Sulphate influences the taste of drinking water. When large amounts of sulphate containing drinking water are consumed, it can have laxative effects (WHO, 2011). When amounts of sulphate exceeding 600 mg/L are ingested by humans, it can cause cathartic effects (WHO, 2004a). It can also cause diarrhoea in people who is not used to drinking water with high sulphate levels (DWAF, 1998). The SANS 241 (2011) standard for sulphate in drinking water is ≤500 mg/L. Sulphate reducing bacteria utilizes oxidised sulphur compounds as electron acceptors to produce sulphide (Gibson, 1990; Lopes *et al.*, 2009). The presence of sulphide and polysulphides in drinking water distribution systems is of concern. It may cause taste and odour problems due to the reaction with metal ions to form insoluble metal sulphides (Kristiana *et al.*, 2010). Sulphate reducing bacteria may cause corrosion of drinking water distribution pipes (Li *et al.*, 2010). There is no SANS 241 (2011) standard for sulphide in drinking water available.

1.5.5 Nitrate & nitrite

Nitrate and nitrite are part of the nitrogen cycle and are therefore naturally occurring ions (WHO, 2007). Wastewaters and agricultural and urban runoff are natural sources contributing to nitrate in water (Chang *et al.*, 2010). The largest contributor to anthropogenic nitrogen is nitrogen fertilizer and is one of the main sources of nitrate in the drinking water in rural areas (Chang *et al.*, 2010). The SANS 241 (2011) standard for nitrate in drinking water is ≤11 mg/L. Nitrate itself is not toxic, but the microbial reduction of nitrate to nitrite in the intestine is toxic (Adam, 1980; WHO, 2007).

When nitrite in the blood combines with hemoglobin it forms methemoglobin (Yang & Cheng, 2007). In infants this reduces the capability of blood to carry oxygen to body parts (Yang & Cheng, 2007) and is thus a health concern (Fan & Steinberg, 1996; WHO, 2007; U.S. EPA, 2010; Balazs *et al.*, 2011). A further concern associated with nitrates in water is enteric infections (Hanukoglu & Danon, 1996; Charamandari *et al.*, 2001; Balazs *et al.*, 2011). It has been shown that nitrite reacts with nitrosatable compounds in the stomach of humans and some of these compounds were carcinogenic when tested on animals (WHO, 2007). Even though these compounds may also be carcinogenic to humans, data from epidemiological studies are not

conclusive (WHO, 2007). *Nitrosomonas* bacteria can form nitrite in galvanized steel distribution pipes during stagnation of nitrogen-containing and oxygen-poor drinking water (WHO, 2011). The SANS 241 (2011) standard for nitrite in drinking water is ≤0.9 mg/L.

1.5.6 Chemical Oxygen Demand (COD)

Chemical Oxygen Demand (COD) is defined as the amount of oxygen in the form of a strong oxidizing agent consumed when organic matter is oxidized (Noguerol-Arias *et al.*, 2012). It is an indirect indicator of organic matter in the water body (Hur *et al.*, 2010). High levels of COD and BOD (Biological Oxygen Demand) is usually an indication of serious water pollution (Kawabe & Kawabe, 1997; Yin *et al.*, 2011). Industrial, agricultural and domestic wastes are the sources of organic matter in aquatic environments (DWAF, 1996a). Organic matter present in dissolved form causes undesirable tastes and odours of the water. Particulate organic matter, on the other hand, contributes to the suspended solids load (DWAF, 1996a). There is no SANS 241 (2011) standard for COD in drinking water available. COD levels of below 75 mg/L are acceptable for environmental water (DPW, 2012).

1.5.7 Phosphorous

Phosphorus (P) in environmental water can be caused by nonpoint pollution such as agricultural runoff due to excess fertilizer (Capece *et al.*, 2007). In Japan and Finland a slight increase of phosphorous in the water increased microbial growth significantly (Lehtola *et al.*, 2002). Phosphorus itself is harmless to humans, but the toxic algal blooms that grows due to excessive available P, are toxic to humans (Carpenter *et al*., 1998). A study done by Fang *et al.* (2009) indicated that the addition of phosphorous in a drinking water distribution system promotes biofilm formation.

Great concern has been raised about biofilm formation in drinking water distribution systems (Edstrom industries, 1997; Fang *et al.*, 2009). Such biofilms could cause all sorts of operational problems in drinking water distribution systems. This includes microbial induced corrosion of the water distribution pipe (LeChevallier *et al.*, 1993; Zacheus *et al.*, 2000). Although phosphorous have no direct impact on health, there are several indirect effects associated with it. There is no SANS 241 (2011) standard for phosphorous in drinking water available.

1.5.8 Temperature

Temperature is the main factor which affects almost all physico-chemical equilibriums and biological reactions (Delpla *et al.*, 2009). Water temperature can have a direct or indirect effect on physical parameters (LeChevallier *et al*., 1996). It can influence the pH, dissolved oxygen, redox potentials and microbial activity (Park *et al*., 2010). The effect of climate change on drinking water production is described in figure 2. In the figure it can be seen that a rise in temperature causes an increase in the pollution load (chemical and microbiological). For water treatment plants this means that adaption measures (such as complementary treatment steps and process control) must be implemented to treatment processes to ensure that good quality water is produced (Delpla *et al.*, 2009).

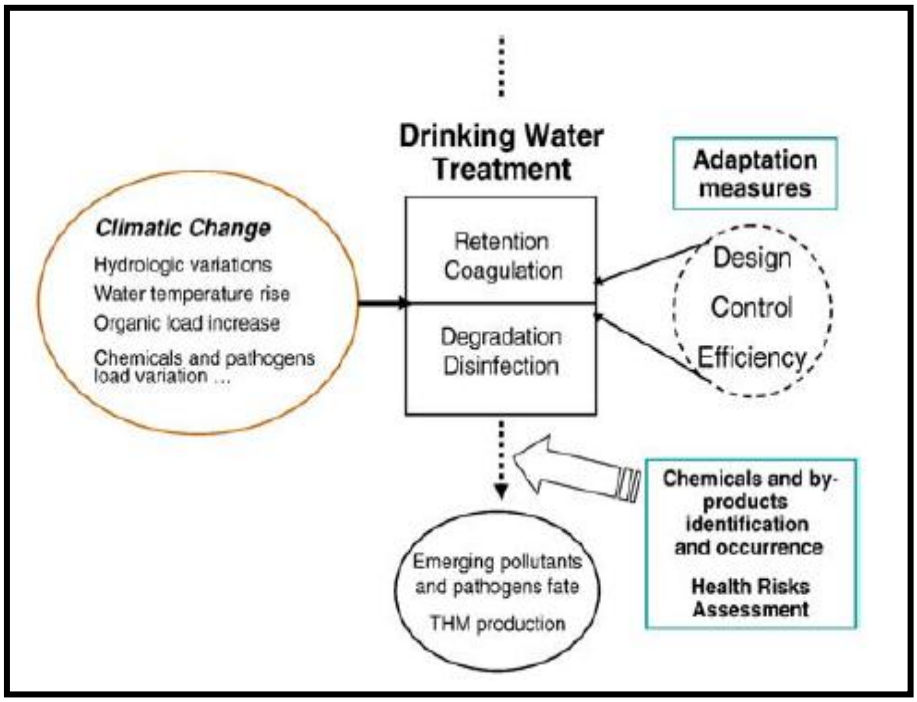

Figure 2: Impacts of climate change on drinking water treatment (Delpla *et al.*, 2009).

1.5.9 Metals

Metals form an integral part of enzymes in all living organisms (Tripathi *et al.*, 1997). These include metals such as iron (Fe), copper (Cu), manganese (Mn) and zinc (Zn) as well as several others (Kavcar *et al.*, 2009). If drinking water with significant high concentrations of metals is consumed over an extended period, it may have chronic toxic effects on consumers (Kavcar *et al.*, 2009). Health effects include shortness of

breath and various types of cancer (Cantor, 1997; Calderon, 2000; Xia & Liu, 2004; Dogan *et al.*, 2005; Kavcar *et al.*, 2009).

pH is an important physical factor affecting the availability of trace metals in drinking water. A low pH can cause corrosion of pipes in distribution systems. This may solubilize the metallic materials and increase the levels of some trace metals in the drinking water (Mora *et al.*, 2009). The SANS 241 (2011) standards for some of the metals include: sodium: ≤200 mg/L; zinc: ≤5 mg/L; aluminium: ≤300 µg/L; arsenic: ≤10 µg/L; copper: ≤2000 µg/L; iron: ≤2000 µg/L; lead: ≤10µg/L; nickel: ≤70 µg/L and mercury: ≤6 µg/L.

1.6 MICROBIOLOGICAL PARAMETERS

The analysis of microbiological quality of water aims to ensure that the consumer is protected from pathogenic organisms such as bacteria, viruses and protozoa (Figueras & Borrego, 2010). Sampling and analysis of microbiological parameters must be done more frequently than physico-chemical parameters, because microbial contamination can have acute health effects on consumers (DWAF, 2005b). Bacteria can be used either as indicators of faecal pollution or to indicate the effectiveness of a water treatment system (Wingender & Flemming, 2011).

1.6.1 Total & faecal coliforms as well as *Escherichia coli*

Coliforms are found naturally in various environments, but drinking water is not considered as a natural environment for them (Rompré *et al.*, 2002). Their presence in drinking water can thus be seen as an indication of possible deteriorating water quality (Rompré *et al.*, 2002). Total coliforms are aerobic and facultative anaerobic, rod-shaped, Gram negative, non-spore forming bacteria which ferment lactose with gas and forms acid within 24h at 35-37°C (WHO, 2011). They develop red colonies with a metallic green sheen within 24h at 35°C on Endo-type media containing lactose (Jain & Pradeep, 2005). The SANS 241 (2011) standard for total coliforms in drinking water is ≤10 CFU/100ml. Total coliforms can be isolated on m-Endo agar using the membrane filtration method. m-Endo agar is selective for coliforms and produce colonies with a metallic sheen (Jain & Pradeep, 2005). m-Endo agar contains various nutrients which promotes the growth of coliforms. It contains lauryl sulphate and deoxychlolate which inhibits the growth of other organisms (Merck, 2012). The reaction of lactose positive

colonies with fuchsin-sulfite releases fuchsin which induces the red colour of the colonies (Merck, 2012). The metallic green sheen of the colonies develops due to the formation of aldehydes during lactose fermentation (Sigma-Aldrich, 2012b). Excessive growth of coliforms on m-Endo agar may inhibit the formation of the distinctive metallic green sheen (Burlingme *et al.*, 1984; Rompré *et al.*, 2002).

Faecal coliforms are used as indicators of sewage in water (Kacar, 2011). The WHO Guidelines for Drinking-water Quality used *Escherichia coli* as faecal indicator of choice (Payment & Robertson, 2004; WHO, 2004b). The presence of *E. coli* in drinking water is an indication of recent or post-treatment faecal contamination. If *E. coli* is present in water samples, it means that the system is contaminated with faecal matter and that pathogenic microorganisms may be present due to failure of the treatment system (Payment & Robertson, 2004). *E. coli* is usually a good indicator of *Salmonella* spp. in the drinking water system (WHO, 2011). Even though *E. coli* forms part of the normal intestinal flora of the human, if present in other parts of the body it can cause serious diseases such as urinary tract infections, bacteraemia and meningitis (WHO, 2011). The SANS 241 (2011) standard for *E. coli* in drinking water is 0 CFU/100ml. m-Fc agar can be used to isolate faecal coliforms using the membrane filtration method. Faecal coliforms produce blue colonies on m-FC agar (Farnleitner *et al.*, 2001). m-Fc agar contains bile salts which inhibit the growth of Gram positive bacteria (Merck, 2012). Peptone and yeast serve as nutrients for the growth of faecal coliforms. The blue colour of the colonies is induced by lactose fermentation at elevated temperatures (44.5°C ± 0.2°C) (Merck, 2012).

Membrane Lactose Glucuronide (MLG) agar can be used to distinguish between total coliforms and *Escherichia coli* (Hallas *et al.*, 2008). Membrane Lactose Glucuronide agar contains lauryl sulphate which inhibits the growth of Gram positive organisms (Oxoid Limited, 2012). The identification of coliforms and *E. coli* on MLG agar is based on two principles: 1) lactose fermentation induces the yellow colour of the colonies when acid is produced; 2) the enzyme glucuronidase cleaves the chromogenic substrate 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (BCIG) and produces a blue chromophore which builds up in the bacterial cells (Oxoid Limited, 2012). Coliforms ferment lactose so colonies will appear yellow on MLG agar, whereas *E. coli* ferments

lactose and possesses the glucuronidase enzyme therefore colonies will appear green (Oxoid Limited, 2012).

If no contamination events took place and indicator organisms are present in the drinking water, it may be an indication of biofilm formation due to regrowth of the indicator organisms in the distribution system (LeChevallier *et al.*, 1987; Wingender & Flemming, 2011).

1.6.2 Heterotrophic plate count bacteria

Heterotrophic plate count (HPC) bacterial levels can be a very useful parameter when assessing water quality. There is a wide variety of heterotrophic plate count bacteria present in water (Allen *et al.*, 2004). Some of the HPC genera commonly found in drinking water include: *Acinetobacter*, *Bacillus*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Nitrosomonas*, *Pseudomonas*, *Staphylococcus* and *Streptococcus* (Allen *et al.*, 2004). An increase in the HPC bacterial level in the final drinking water when this is compared to the raw water may be an indication of the following: post-treatment contamination, growth within the conveyed water and biofilms that are present in the distribution system (Payment & Robertson, 2004). This bacterial parameter is thus a good indicator of the effectiveness of the water treatment process and cleanliness of the distribution system (WHO, 2011).

Heterotrophic plate count bacteria may be used as an indicator of underlying causes of aesthetic problems (Bartram *et al.*, 2003). The number of HPC bacteria in drinking water depends on variables such as source water quality, treatment methods, disinfection type and concentration, age and condition of the distribution system, temperature of the raw and drinking water, isolation methods and incubation conditions (Allen *et al.*, 2004). The sensitivity of coliform bacteria detection in drinking water is reduced when HPC bacteria levels greater than 500 CFU/ml are present (Allen *et al.*, 2004). Therefore it is important that HPC bacterial analysis is carried out together with coliform or *E. coli* analysis (Allen *et al.*, 2004). Heterotrophic plate count bacteria can be isolated on R_2A agar by the spread plate method. Various pigmented and non-pigmented colonies can grow on R_2A agar (Bartram *et al.*, 2003). Pigmented colonies can be useful indicators for changes in the microbiological quality of drinking water (Carter *et al*., 2000). The SANS 241 (2011) standard for heterotrophic bacteria in drinking water is ≤1000 CFU/ml.

1.6.3 Faecal streptococci

Faecal streptococci is a group of facultative anaerobic, Gram positive, catalase negative, non-spore forming bacteria which is found in the gastrointestinal tracts of humans and animals (Mara & Horan, 2003). Like faecal and total coliforms, faecal streptococci are also good indicators of faecal pollution (Kistemann *et al.*, 2002; Baghel *et al.*, 2005; Kacar, 2011). Earlier studies by Sinton *et al.* (1993) stated that the numbers of faecal streptococci are higher in animal faeces than human faeces, whereas faecal coliforms are higher in human faeces than animal faeces. More recent studies contradict this statement, because the ratio differs with exposure to natural environments (Mara & Horan, 2003).

Faecal streptococci are good indicators of faecal pollution, because they tend to survive longer in environmental water than faecal coliforms and are more resistant to chlorine (WHO, 2011). Environmental waters and soils are not natural habitats for faecal streptococci, thus their presence in water is a good indication of faecal contamination (Mara & Horan, 2003). There is no SANS 241 (2011) standard for faecal streptococci in drinking water available. Faecal streptococci can be isolated on KF-Strep agar using the membrane filtration method. Colonies that developed a pink to red colour can be recorded as faecal streptococci (Furukawa *et al*., 2010). KF-Strep agar contains the fermentable carbohydrates, lactose and maltose, which is used as energy sources by the bacteria (Sigma-Aldrich, 2012a). Growth of Gram negative bacteria on KF-Strep agar are inhibited by sodium azide. The reduction of triphenyl tetrazolium chloride to insoluble fomazan by the cells induces the pink or red colonies on the agar (Hayes, 1995; Sigma-Aldrich, 2012a).

1.6.4 Anaerobic bacteria (*Clostridia* & *Bacteroidetes*)

Obligate anaerobic bacteria cannot tolerate oxygen and die in the presence of oxygen (Willey *et al.*, 2008). *Bacteroides, Fusobacterium, Clostridia* and *Methanococcus* are examples of obligate anaerobes (Willey *et al.*, 2008). *Clostridia* are anaerobic, Gram positive, sulphite reducing bacteria. They can resist unfavourable conditions in the environment such as temperature and pH extremities and chlorination by producing spores (WHO, 2011). It is also a useful indicator of faecal contamination in the water (Field & Samadpour, 2007). The detection of *Clostridium perfringens* in drinking water is an indication of possible failure in the filtration process of the water treatment plant

(WHO, 2011). *Clostridium perfringens* can have serious health impacts on humans including gangrene and gastrointestinal disease (Petit *et al.*, 1999).

The genus *Bacteroides* are non-spore forming, obligate anaerobes which are present in human faeces and are also indicators of faecal pollution in water (Field *et al.*, 2003). They indicate recent faecal pollution, because they are strict anaerobes and don't usually survive for extended periods in water (Avelar *et al.*, 1998; Kreader, 1998; Field *et al.*, 2003). There is no SANS 241 (2011) standard for anaerobic bacteria in drinking water. *Clostridia* can be isolated under anaerobic conditions in Reinforced Clostridial broth medium (Ganner *et al*., 2010). This is a present (turbid growth)/absent (no growth) test. Total anaerobic bacteria can be isolated under anaerobic conditions on anaerobic agar. Anaerobic agar contains reducing agents such as thioglycollate, formaldehydesulfoxylate and cysteine to ensure anaerobiosis (Merck, 2012). The decolouration of the redox indictor, methylene blue, indicates anaerobiosis (Merck, 2012).

1.6.5 *Pseudomonas aeruginosa*

Some heterotrophic bacteria are opportunistic pathogens such as *Pseudomonas* (WHO, 2011). *Pseudomonas* species inhabit various environments such as soil, water and vegetation (Shrivastava *et al.*, 2004). *Pseudomonas aeruginosa* may occur in drinking water systems where it originates from source water or due to established biofilms (Bressler *et al.*, 2009). Even though *P. aeruginosa* is sensitive to disinfection (WHO, 2011), their presence in established biofilms makes it more difficult to remove from the drinking water systems (Bressler *et al.*, 2009). Various studies have indicated the antibiotic resistance of *P. aeruginosa* in water. A study by Shrivastava *et al.* (2004) observed that strains of *Pseudomonas aeruginosa* that survived chlorination were resistant to almost all the antibiotics tested for.

No gastrointestinal diseases in humans have been associated with *Pseudomonas* through ingestion with drinking water (WHO, 2011), but *Pseudomonas aeruginosa* is a waterborne opportunistic pathogen which may have health impacts on humans, especially in immunocompromised populations (Wang *et al.*, 2012). High numbers of *Pseudomonas aeruginosa* in drinking water may also cause taste, odour and turbidity problems (WHO, 2011). There is no SANS 241 (2011) standard for *Pseudomonas* sp. in

drinking water available. Cetrimide agar can be used for the isolation of *Pseudomonas aeruginosa* (Rogues *et al*., 2007) by using the membrane filtration method. Cetrimide agar contains the compound cetyltrimethylammonium bromide which inhibits the growth of accompanying microbes (Merck, 2012). It acts as a quaternary ammonium compound as well as cationic detergent which cause bacterial cells other than *Pseudomonas aeruginosa* to release phosphorous and nitrogen (Sigma-Aldrich, 2012c).

1.6.6 Fungi

There are various groups of fungi. These include: filamentous fungi (moulds), mushrooms and the yeasts. Some fungi will naturally be found in water, because they are primarily adapted to aquatic environments (Hageskal *et al*., 2009). Fungi have been found in various water sources, including raw and polluted water as well as purified drinking water (Hageskal *et al*., 2009; WHO, 2011). Fungi can also grow on rubber parts of water distribution systems (WHO, 2011). Filamentous fungi produce many of the taste and odour compounds that bacteria also produce. In addition they produce their own unique off-odours and tastes (Paterson *et al.*, 2009). Investigations for fungi in drinking water started worldwide when several cases of health problems were reported from Finland and Sweden in the 1980's and 1990's (Muittari *et al*., 1980; Åslund, 1984 cited by Hageskal *et al*., 2009). Fungi also produce secondary metabolites called mycotoxins of which some are extremely harmful (Paterson *et al.*, 2009). Food and drink which are contaminated with mycotoxins may have severe health effects on humans and animals (Paterson *et al.*, 2009). Mycotoxins may damage the kidney, liver, and lungs as well as the nervous, endocrine and immune system (Paterson *et al.*, 2009). There is no SANS 241 (2011) standard for fungi in drinking water available. Fungi can be isolated on Sabaraud Dextrose agar (Hageskal *et al.*, 2009) by using the spread plate method.

1.7 METHODS USED FOR PHYSICO-CHEMICAL AND MICROBIOLOGICAL ANALYSIS

In this section the principles and some applications of methods to determine levels of various physico-chemical and microbiological parameters are discussed.

1.7.1 Physico-chemical methods

There are various methods available to measure physico-chemical parameters. The temperature, TDS, EC, pH and salinity could be measured onsite using mobile multiprobe meters. There are several commercially available ones, each with its own advantages and disadvantages. The advantages of one such multiprobe, the PCS Testr 35 (Eutech Instruments, 2005) is provided here: 1) It measures five parameters without having to change sensors. 2) Full pH range can be measured up to 2-decimal places. 3) Low, medium and high TDS/EC ranges can be measured. 4) Multi-ranged salinity measurements of up to 10.00 ppt or 1% can be made. 5) It is waterproof and easy to use.

The Hach Lange DR2800 portable spectrophotometer can be used for more than 240 analytical methods (Hach Company, 2012). The instrument is easy to use and being a portable spectrophotometer means measurements for physical and chemical parameters can be made onsite. Some of the chemical analysis that can be measured with the Hach Lange DR2800 spectrophotometer include: free chlorine, sulphate, sulphide, nitrate, nitrite, chemical oxygen demand and phosphorous.

a. Free chlorine (DPD Free Chlorine Reagent Powder Pillow method)

   The free chlorine in the sample immediately reacts with N,N-diethyl-p-phenylenediamine (DPD) indicator when the powder pillow is added to form a pink colour. The intensity of the pink colour is proportional to the chlorine concentration in the water sample (Hach Company, 2007).

b. Sulphate (SulfaVer 4 Powder Pillow method)

   When the powder pillow is added sulphate ions in the water sample react with barium to form barium sulphate. The amount of turbidity formed is proportional to the sulphate concentration. (Hach Company, 2007).

c. Sulphide (Methylene blue method)

   Sulphide is measured by the reaction of hydrogen sulphide and acid-soluble metal sulphides with N,N-diethyl-p-phenylenediamine sulphate to form methylene blue. The intensity of the blue colour is proportional to the sulphide concentration in the water sample (Hach Company, 2007).

d. Nitrates (NitraVer 5 Nitrate Reagent Powder Pillow method)

   Nitrates in the water sample are reduced by cadmium metal to nitrite. In an acidic medium the nitrite ion reacts with sulfanilic acid to form diazonium salt. An amber coloured solution is formed when the diazonium salt couples with gentisic acid (Hach Company, 2007).

e. Nitrite (NitriVer 2 Nitrite Reagent Powder Pillow method)

   Ferrous sulphate reduces nitrite to nitrous oxide in an acidic medium. Nitrous oxide combines with ferrous ions to form a greenish-brown complex. This is direct proportional to the nitrite present in the water sample (Hach Company, 2007).

f. Chemical oxygen demand (Reactor Digestion Method)

   The sample is heated for two hours with an oxidizing agent, potassium dichromate. The dichromate ion ($Cr_2O_7^{2-}$) is reduced to green chromic ion (Cr^{3+}) when the oxidizable organic compounds begin to react. With the 3-150 mg/L colorimetric method, the amount of Cr^{6+} is determined (Hach Company, 2007).

g. Phosphorous (Amino Acid Method)

   In a highly acidic solution, molybdophosphoric acid is formed when ammonium molybdate reacts with orthophosphate. The amino acid reagent then reduces this complex to form an intensely coloured molybdenum blue complex (Hach Company, 2007).

Inductively Coupled Plasma Mass Spectrophotometry (ICP-MS) is widely used for the detection of trace metals. The ICP-MS process, described by Thomas (2001), consists of the liquid sample being pumped into the introduction system which is made up of the spray chamber and nebulizer. In the form of an aerosol it travels to the base of the plasma and through different heating zones of the plasma torch. During this process the aerosol sample is dried, vaporized, atomized and ionized. It is transformed into solid particles then into a gas. When it reaches the analytical zone of the plasma it occurs as atoms and ions which represent the elemental composition of the sample. Measurement of the sample is then determined. The Agilent 7500ce is the ICP-MS of choice for easy, ppt-level quantification in challenging sample matrices (Agilent Technologies, 2004). One of the advantages of the Agilent 7500ce is its ability and design to handle high

matrix samples such as wastewater, soils, food, biomedical, petrochemical, and geological (Agilent Technologies, 2004).

1.7.2 Culture based methods

Culture based techniques, for example the membrane filtration technique, is used for the isolation of bacteria in water (Venter, 2000). Even though culture based methods are time consuming, taking 18 to 96 hours from sampling to results, it has been widely used, because it is cost-effective and easily implemented (Converse *et al.*, 2012).

1.7.2.1 The membrane filtration method

Many countries use the membrane filtration method for monitoring drinking water quality (Rompré *et al.*, 2002). The membrane filtration method consists of filtrating the appropriate volume of water sample through a 0.45 µm pore size membrane filter. The filter is then placed onto selective media, incubated at appropriate temperatures and colonies are enumerated on the filter (Rompré *et al.*, 2002). The results are expressed as colony forming units per 100 ml (CFU/100ml) (Edge & Hill, 2007). Various bacteria, such as faecal indicator bacteria (Hijnen *et al.*, 2000) and *Pseudomonas aeruginosa* (Al-Qadiri *et al.*, 2006) can be isolated by means of the membrane filtration method. Advantages of using the membrane filtration method include: 1) increased sensitivity by enabling filtration of large volumes of water; 2) water soluble impurities are separated from the sample which allows no interference with growth of the target organism; 3) an accurate quantitative result of the colonies can be obtained; 4) cost and time effective, because further cultivation steps are not always necessary; 5) the colonies are well separated on the filter making further confirmation easy (Köster *et al.*, 2003).

1.7.2.2 Spread plate method

The spread plate method can also be used for the isolation of bacteria. During this method a dilution of the water sample is made and 100 µl of the diluted sample is placed onto the selective media. The sample is then spread over the surface with a sterile bent-glass rod causing individual cells to separate (Sumbali & Mehrotra, 2009). The plates are incubated at the appropriate temperature and individual colonies are enumerated onto the media (Sumbali & Mehrotra, 2009). The spread plate method can be used to isolate various bacteria, such as heterotrophic bacteria (Jjemba *et al.*, 2010). The advantage of the spread plate method is that colonies can be easily differentiated

(Köster *et al.*, 2003). Quantitative results can also be obtained from the spread plate method. Results are expressed as colony forming units per ml (CFU/ml) (Sumbali & Mehrotra, 2009).

1.7.3 Biochemical confirmation tests for *Escherichia coli*

The identification of the isolated colonies obtained from culture based methods can be done by biochemical tests (Tharannum *et al.*, 2009). Various biochemical tests are available for the identification of *E. coli* namely: activity of the β-D-glucuronidase enzyme using MLG agar, Gram staining (Penney *et al.*, 2002) and the triple sugar iron test.

1.7.3.1 β-D-Glucuronidase

β-D-glucuronidase is an enzyme found in *Escherichia coli* which makes the detection of *E. coli* on media containing substrates of the β-D-glucuronidase enzyme possible (Edberg *et al.*, 1988; Manafi *et al.*, 1991; Eckner, 1998; Fricker *et al.*, 2008). Even though *E. coli* is not the only organism that possesses the β-D-glucuronidase enzyme, media have been formulated to minimise the possibility of false-negatives (Fricker *et al.*, 2008). Membrane Lactose Glucuronide (MLG) agar contains substrates of the β-D-glucuronidase enzyme and can be used to isolate *E. coli* (as discussed in section 2.5.1). Green colonies isolated on the MLG agar can be recorded as *Escherichia coli* (Hallas *et al.*, 2008).

1.7.3.2 Triple Sugar Iron test (TSI)

The TSI test is used to distinguish between members of the family *Enterobacteriaceae*, such as *E. coli*, and other intestinal bacteria (Harley, 2005). *Enterobacteriaceae* have the ability to catabolize the sugars lactose, glucose or sucrose, and to release sulphides from ferrous ammonium sulphate (Harley, 2005). If an organism is not able to catabolize any of these sugars it is an indication that that organism is not part of the *Enterobacteriaceae* group (Winn *et al.*, 2006). Reactions to observe in the test tube are colour changes of the slant and butt, as well as the production of gas and hydrogen sulphide (H_2S). Kligler iron agar or triple sugar iron agar can be used to perform the TSI test (Winn *et al.*, 2006).

Results with TSI agar can be interpreted as follows (Harley, 2005): *yellow butt*: fermentation of glucose takes place and the red colour of the agar turns yellow due to acid production in the butt; *red slant*: there is a limitation of glucose in the medium, because of the fermentation of glucose and therefore no acid production takes place; *Yellow butt and slant*: fermentation of sucrose and/or lactose takes place and causes excessive acid production in the entire medium; *red butt and slant*: none of the sugars were fermented and no gas or H_2S production took place; *H_2S production*: the agar blackens; *gas formation*: splitting of the agar. A yellow slant and butt, gas production and absence of H_2S are indicative of *E. coli* (Munshi *et al.*, 2012).

1.8 MOLECULAR CONFIRMATION OF *ESCHERICHIA COLI*

Culture based methods measure the growth of viable cells whereas molecular methods measure the genetic material of an organism (Converse *et al.*, 2012). Molecular methods, such as the polymerase chain reaction, are more time effective than culture based methods, because the incubation step is eliminated (Converse *et al.*, 2012). Molecular methods can also be used as confirmation tests (Tharannum *et al.*, 2009) of the colonies isolated on selective media.

1.8.1 Polymerase Chain Reaction (PCR)

PCR is a molecular technique and involves the amplification of DNA (Joshi & Deshpande, 2010). PCR consist of several cycles of amplification. Each cycle consist of three stages (Giasuddin, 1995): 1) denaturation of the double stranded DNA whereby the temperature is increased to produce single stranded DNA which serve as templates for the attachment of the synthetic primers; 2) the annealing stage where the temperature is reduced to allow primers to bind to the complimentary single stranded DNA; 3) the final stage is extension of the 3' ends of the annealed primers. This stage is carried out by the enzyme DNA-polymerase (Taq polymerase) which synthesises a complimentary copy of the initial single strands. This technique is widely used, because it is simple as well as cost and time effective (Joshi & Deshpande, 2010).

1.8.1.1 *E. coli* housekeeping genes

Multiplex PCR allows the simultaneous detection of specific genes in a single PCR tube (Kong *et al.*, 2002). A multiplex PCR for the detection of *E. coli* could easily be performed. The two housekeeping genes, *mdh* (Omar *et al.*, 2010) and *lacZ* (Ram &

Shanker, 2005), could be targeted. The *mdh* gene encodes the enzyme malate dehydrogenase (MDH) (Park *et al.*, 1995) and the *lacZ* gene encodes the enzyme β-D-galactosidase (Ram & Shanker, 2005). If the *mdh* and *lacZ* genes are amplified it is an added confirmation that this organism may be *E. coli*. Another housekeeping gene that could be used for the identification of *E. coli* is the *uidA* gene. The *uidA* gene encodes the enzyme β-D-glucuronidase (Martins *et al.*, 1993; Farnleitner *et al.*, 2001).

1.8.1.2 16S rDNA gene

The 16S rDNA gene is universally present in bacteria (Janda & Abbott, 2007). Amplification of the 16S rDNA gene could be used to confirm that the DNA isolated is that from the domain *Bacteria* (Wise *et al.*, 1997). Sequencing of the PCR product is performed for identification of the unknown strain.

1.9 SUMMARY OF LITERATURE REVIEW

One of the most important requirements for domestic water is that the water should be safe to drink (Schutte, 2006; Momba *et al.*, 2009). In the literature review an initial need and context for the study was provided. This was followed by a section that discussed the seven basic steps that water treatment plants carry out to purify raw water to produce drinking water (Obi, 2007; Rand Water, 2012b). The operation and management of the drinking water production plants in the study area was discussed followed by a brief overview of the raw water. In the section that followed the significance of the various physico-chemical and microbiological parameters were presented. This was followed by a section on the methods that could be used to investigate the said parameters.

The literature review started by indicating that many raw water sources are polluted and it is thus very important for water treatment plants to produce water that is fit for domestic use at a reasonable cost (Schutte, 2006). By analysing the physico-chemical and microbiological characteristics of water, the general quality of the water can be determined (Schutte, 2006). Physico-chemical parameters can be a good indication of the quality, productivity and sustainability of that water body (Mustapha, 2008). The main objective of measuring the microbiological quality of water is to protect the consumer from pathogenic organisms (Figueras & Borrego, 2010). Final water produced by the water treatment plant must comply with the South African National

Standard (SANS) 241:2011 for drinking water (DWA, 2012). Faecal coliforms are used as indicators of faecal pollution (Kacar, 2011) and the WHO Guidelines for Drinking-water used *Escherichia coli* as faecal indicator of choice (Payment & Robertson, 2004; WHO, 2004b). The presence of *E. coli* in drinking water indicates that the treatment process is ineffective (Payment & Robertson, 2004).

Domestic water is derived from the Vaal River and purified to provide drinking water to the communities in the Vaalharts region. Vaalharts form part of the Lower Vaal catchment area (DWAF-DNWRP, 2009a) and the quality of the Lower Vaal River has been deteriorating over the past 20 years and will probably deteriorate even more (Du Preez *et al.*, 2000 cited by Le Roux *et al.*, 2007). It is important to have an understanding about the quality of the water in Vaal River, because this will reflect on the quality of water after purification.

CHAPTER 2
MATERIALS & METHODS

2.1 STUDY SITE

The study site consisted of three areas in the Vaalharts irrigation scheme (Hartswater, Pampierstad and Jan Kempdorp) and one site on the border of the irrigation scheme (Warrenton). Vaalharts is located on the border between the Northern Cape- and the North West Province at the confluence of the Harts- and Vaal Rivers and has an area of 29 181 ha (Van Vuuren, 2010). Four samples were taken at each of the areas, giving a total of sixteen sampling points. The sixteen sampling points and their coordinates are given in Table 4. Figure 3 is a map to illustrate the location of the four sampling areas.

Table 4: Sampling points with their coordinates.

Sampling points	Coordinates
HR (Hartswater raw water - at water works)	S 27º 44' 28.5'' E 024º 48' 40.5''
H2 (Hartswater drinking water - at water works)	S 27º 44' 32.7'' E 024º 48' 39.2''
H3 (Hartswater drinking water - middle of distribution)	S 27º 45' 03.8'' E 024º 48' 42.7''
H4 (Hartswater drinking water - furthest point of distribution)	S 27º 43' 56.5'' E 024º 48' 00.1''
PR (Pampierstad raw water - at water works)	S 27º 45' 57.4'' E 024º 42' 11.1''
P2 (Pampierstad drinking water - at water works)	S 27º 45' 57.4'' E 024º 42' 11.1''
P3 (Pampierstad drinking water - middle of distribution)	S 27º 46' 41.6'' E 024º 41' 25.9''
P4 (Pampierstad drinking water - furthest point of distribution)	S 27º 47' 22.9'' E 024º 40' 49.4''
JR (Jan Kempdorp raw water - at water works)	S 27º 54' 55.4'' E 024º 50' 47.8''
J2 (Jan Kempdorp drinking water - at water works)	S 27º 54' 59.1'' E 024º 50' 42.4''
J3 (Jan Kempdorp drinking water - middle of distribution)	S 27º 54' 59.5'' E 024º 49' 40.4''
J4 (Jan Kempdorp drinking water - furthest point of distribution)	S 27º 58' 15.0'' E 024º 46' 06.0''
WR (Warrenton raw water - at water works)	S 28º 05' 58.3'' E 024º 50' 22.3''
W2 (Warrenton drinking water - at water works)	S 28º 06' 01.4'' E 024º 50' 23.9''
W3 (Warrenton drinking water - middle of distribution)	S 28º 06' 36.6'' E 024º 50' 57.1''
W4 (Warrenton drinking water - furthest point of distribution)	S 28º 06' 06.0'' E 024º 52' 11.6''

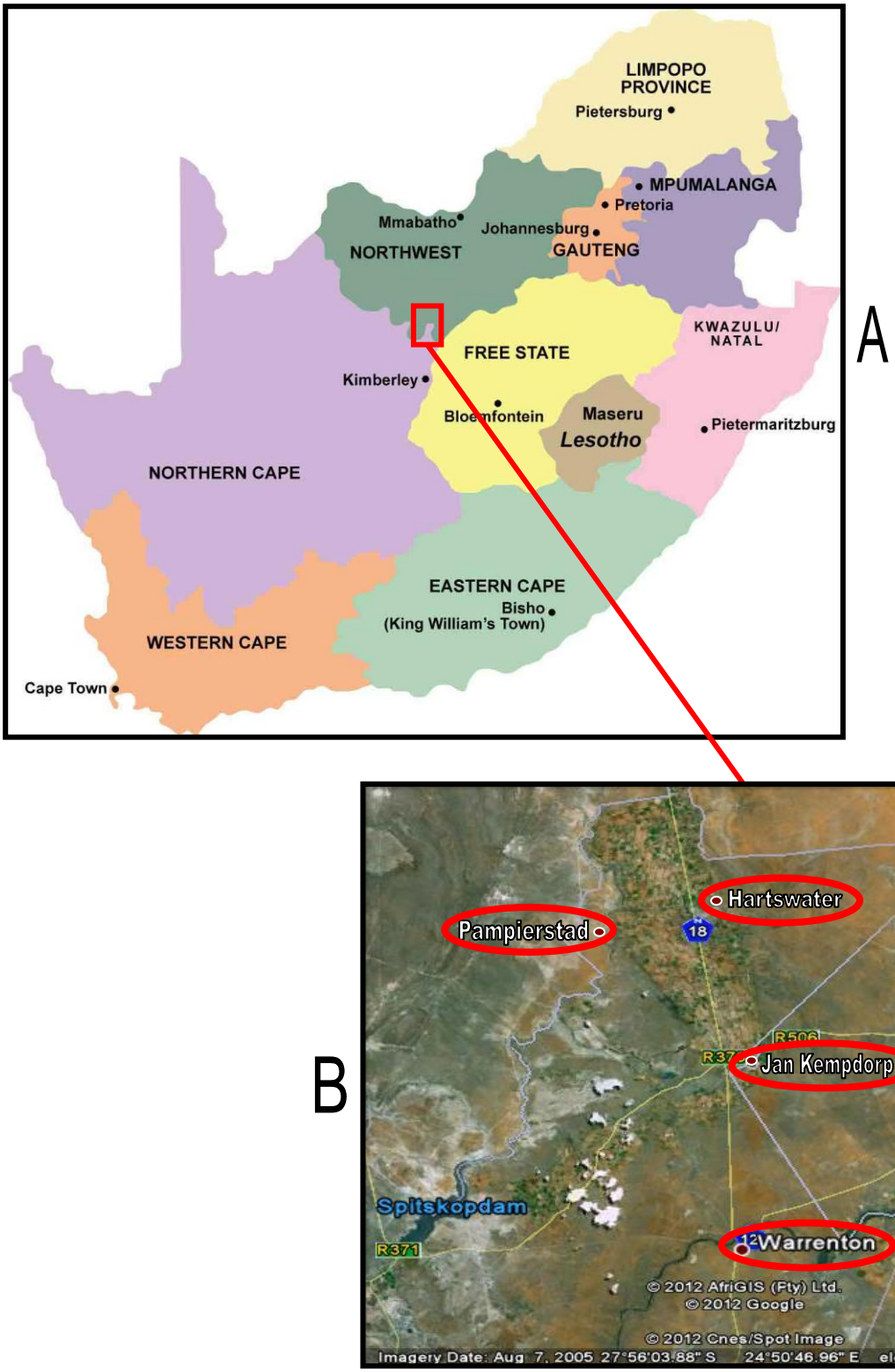

Figure 3: **A**: Map that indicates the location of the Vaalharts irrigation scheme on the border of the North West and Northern Cape Province (red block) (http://towns.bookingsouthafrica.com/). **B**: Map that indicates the Vaalharts region with the different sampling areas (Hartswater, Pampierstad, Jan Kempdorp and Warrenton) (Source: Google Earth).

2.2 SAMPLE COLLECTION

Water samples were collected at the four different residential areas. At each of the areas (Hartswater, Pampierstad, Jan Kempdorp and Warrenton) water samples were collected at the following points:

1. Raw water sample at the water works.
2. Drinking water sample at the water works.
3. Drinking water sample in the middle of the distribution.
4. Drinking water sample at the furthest point of the distribution.

The experimental period was from July 2011 to June 2012. Sampling was done over two seasons during 2011 (July and October) as well as during 2012 (March and June). The water was sampled in sterile glass bottles (Schott Duran, Germany) and transported to the NWU (North-West University) laboratories in cooler boxes with ice. Sampling was done as prescribed by the DWAF Sampling Guide (DWAF, 2000). Analysis of the water samples were done within 48h after sampling.

2.3 PHYSICO- CHEMICAL ANALYSIS

The temperature, pH, salinity, electrical conductivity and TDS were taken onsite with a multi-meter (PCSTestr 35, Eutech Instruments Pte Ltd, Singapore). Before each measurement was taken the measurement beaker was cleaned with distilled water and rinsed with the specific water sample. The probe was also cleaned with distilled water before and after each measurement. The concentrations of nitrate (method 8039), nitrite (method 8153), sulphate (method 8051), sulphide (method 8131), phosphorous (method 8178), free chlorine (method 8021) and COD (method 8000) were measured at the NWU laboratories using the Hach Lange DR 2800 spectrophotometer (Güler & Alpaslan, 2009). Manufacturers' prescription for the various methods was followed (Hach Company, 2007). Total concentrations of 5 major metals (Ca, Mg, K, Na and Zn) and 15 trace metals (Al, Sb, As, Cd, Cr, Co, Cu, Fe, Pb, Mn, Hg, Ni, Se, U and V) were measured at the NWU laboratories with Inductively Coupled Plasma Mass Spectrophotometry (ICP-MS - Agilent 7500CE) (Güler & Alpaslan, 2009). Parameter settings of the ICP-MS were as follows: RF Power: 1500 W; sample depth: 8 mm; carrier gas: 0.99 L/min; makeup gas: 0.15 L/min; nebulizer pump: 0.1 rps; S/C temp: 2°C.

2.4 ENUMERATING BACTERIA

Different volumes of water were filtered through 0.45 µm sterilized membranes (Seperations, UK). Raw water volumes of 10 ml and drinking water volumes of 100 ml were filtered. The membrane filtration technique was used to isolate total coliforms, faecal coliforms (*E.* coli), faecal streptococci and *Pseudomonas aeruginosa*. The membranes were then placed onto various selective media. All the media, except KF-Strep agar and MLG agar, were obtained from Merck (Germany).

Total coliforms were isolated on m-Endo agar and incubated for 48h at 37°C (Potgieter *et al*., 2005; Momba *et al*., 2006). Dark red to purple colonies with a metallic green sheen were recorded as total coliforms (Harley, 2005; Shash *et al.*, 2010). Faecal coliforms (*E. coli*) were isolated on m-FC agar and incubated for 2h at 37°C and then 22h at 45°C (Dufour *et al*., 1981; Alonso *et al.*, 1998; Edge & Hill, 2005; Ishii *et al.*, 2006). Blue colonies were recorded as faecal coliforms (Harley, 2005; Wohlsen, 2011). Faecal streptococci were isolated on KF-Strep agar (Sigma Aldrich, US) and incubated for 48h at 37°C (Sinton *et al*., 1993; Audicana *et al*., 1995; Furukawa *et al*., 2010). Pink to red colonies were recorded as faecal streptococci (Furukawa *et al*., 2010). *Pseudomonas aeruginosa* were isolated on Cetrimide agar and incubated for 48h at 37°C (Rogues *et al*., 2007). All the colonies (all colours) on the membrane were counted.

During July 2011 *Clostridia* were isolated under anaerobic conditions by inoculation in Reinforced Clostridial Medium, incubated for 48h at 37°C (Ganner *et al*., 2010). Results were recorded as present or absent. During October 2011 and during March and June 2012 *Clostridia* were isolated under anaerobic conditions by membrane filtration (to obtain CFU/100ml) and the membrane was placed onto Reinforced Clostridial Agar and incubated for 48h at 37°C. Fungi and yeasts were isolated by means of the spread plate method on Sabouraud Dextrose agar (Hageskal *et al.*, 2009) from a dilution series of 10^{-1} to 10^{-5} and incubated for 5 – 7 days at room temperature. Heterotrophic plate count bacteria were isolated by means of the spread plate method on R_2A agar. A dilution series of 10^{-1} to 10^{-5} was prepared and used. Incubation was for 5 – 7 days at room temperature (Bugno *et al.*, 2010). Total anaerobic bacteria were isolated under anaerobic conditions by means of the spread plate method on Anaerobic agar from a dilution series of 10^{-1} to 10^{-5} and incubated for 5 – 7 days at room temperature.

During July 2011, *Clostridia* and total anaerobic bacteria were isolated under anaerobic conditions using an anaerobic chamber (Forma Scientific Inc., US). The anaerobic chamber was operated according to the manufacturer's prescriptions with nitrogen and an anaerobic gas mixture as auxiliary gasses. Anaerobic gas mix consisted of 5% CO_2, 10% H_2 and 85% N_2. During October 2011 as well as March and June 2012, *Clostridia* and total anaerobic bacteria were isolated using anaerobic jars (Davies Diagnostics, South Africa), which was a more cost effective method. Manufacturers' prescriptions were followed for operating the anaerobic jars.

2.5 BIOCHEMICAL CONFIRMATION TESTS FOR *ESCHERICHIA COLI*

Confirmation of *E. coli* isolated from m-FC agar (blue colonies) was needed. Different biochemical confirmation tests were performed for the identification of *E. coli*.

2.5.1 β-D-Glucuronidase

Selected colonies were streaked out onto MLG (Membrane Lactose Glucuronide) agar (Oxoid, England) and incubated for 24h at 37°C (Hallas *et al*., 2008). Green colonies were recorded as *E. coli* and yellow colonies as total coliforms (Hallas *et al*., 2008).

2.5.2 Gram staining

After the colonies were streaked out onto MLG agar, gram staining was performed on selected colonies to determine if the colonies were pure and gram negative. Except for the fixing step (which was done by heat during the present study) the Gram staining procedure was done as described by Claus (1992). Only pure Gram negative colonies were streak inoculated onto nutrient agar slants and incubated for 24h at 37°C. These pure colonies were used for further confirmation tests.

2.5.3 Triple sugar iron (TSI)

The TSI test can also be used as a confirmation test for *E. coli*. Triple Sugar Iron Agar slants were prepared. The slants were streak- and stab inoculated with a sterile inoculation needle containing the selected colony. The slants were incubated for 24h at 48°C. The colour of the slant and butt was recorded (red or yellow) as well as the production of H_2S (agar blackens) and formation of gas (splitting of the agar) (Harley, 2005).

2.6 MOLECULAR CONFIRMATION OF *ESCHERICHIA COLI*

Molecular tests were performed on the selected colonies. Amplification of the *mdh*, *lacZ*, *uidA* and 16S genes was used for the identification of *E. coli*. An ATCC *E. coli* culture (10536) was used as a positive control for all the molecular tests. A no template control was also included during each set of PCRs.

2.6.1 DNA isolation

DNA isolation was done with overnight cultures which were obtained by inoculating each colony in nutrient broth (Merck, Germany) and incubating it for 24h at 37ºC. Genomic DNA was extracted using a NucleoSpin Tissue kit for bacteria (Macherey-Nagel, Germany). The manufacturer's protocol was followed for the extraction of the DNA. The purity (A_{260nm}:A_{280nm} ratios) and concentrations of the DNA were determined by a NanoDropTM 1000 Spectrophotometer v3.5.2 (Thermo Scientific, US).

2.6.2 DNA amplification

The isolated DNA was amplified using a 1000Cycler (BioRad, UK) thermal cycler. The primer sets were synthesized by Applied Biosystems Inc. (US).

2.6.2.1 Multiplex PCR

The multiplex PCR mix consisted of the *mdh* and *lacZ* primer sets. A 301 bp fragment of the *mdh* gene was amplified using the mdh FW: 5'-GGT ATG GAT CGT TCC GACCT-3' and RV: 5'-GGC AGA ATG GTA ACA CCA GAGT-3' primer pair (Tarr *et al.*, 2002; Omar *et al.*, 2010). A 228 bp fragment of the *lacZ* gene was amplified using the lacZ FW: 5'-CTG GCG TAA TAG CGA AGAGG-3' and RV: 5'-GGA TTG ACC GTA ATG GGA TATG-3' primer pair (Ram & Shanker, 2005). Each reaction tube consisted of 1 x PCR master mix {2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.025 U/µl *Taq*-polymerase} (Fermentas Life Sciences, US), forward and reverse primers (0.8 µM of each) (Applied Biosystems, US) and additional 0.5 mM $MgCl_2$ (Fermentas Life Sciences, US). The total volume of 25 µl was attained by adding nuclease free PCR grade water (Fermentas Life Sciences, US). Eighty nanogram of DNA was used as template in each reaction. The PCR cycling conditions were as follows: initial denaturation at 95°C for 420 seconds, 30 cycles of denaturation at 95°C for 30 seconds, annealing at 57°C for 30 seconds and extension at 72°C for 60 seconds. Final extension was at 72ºC for 420 seconds.

2.6.2.2 Monoplex PCR – *uidA* gene

A 380 bp fragment of the *uidA* gene was amplified using the uid FW: 5'-CCG ATC ACC TGT GTC AATGT-3' and RV: 5'-GTT ACC GCC AAC GCG CAATA-3' primer pair (Bower *et al.*, 2005). Each reaction tube consisted of 1 x PCR master mix {2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.025 U/µl *Taq*-polymerase} (Fermentas Life Sciences, US), forward and reverse primers (0.2 µM of each) (Applied Biosystems, US) and additional 0.5 mM $MgCl_2$ (Fermentas Life Sciences, US). The total volume of 25 µl was attained by adding nuclease free PCR grade water (Fermentas Life Sciences, US). Eighty nanogram of DNA was used as template in each reaction. The PCR cycling conditions were as follows: initial denaturation at 95°C for 300 seconds, 40 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 60 seconds and extension at 72°C for 120 seconds. Final extension was at 72ºC for 600 seconds.

2.6.2.3 Monoplex PCR – 16S rDNA gene

A 1465 bp fragment of the 16S gene was amplified using the 16S FW: 5'-AGA GTT TGA TCM TGG CTCAG-3' and RV: 5'-TAC GGY TAC CTT GTT ACG ACTT-3' primer pair (Lane, 1991). Each reaction tube consisted of 1 x PCR master mix {2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.025 U/µl *Taq*-polymerase} (Fermentas Life Sciences, US), forward and reverse primers (0.6 µM of each) (Applied Biosystems, US) and additional 0.5 mM $MgCl_2$ (Fermentas Life Sciences, US). The total volume of 25 µl was attained by adding nuclease free PCR grade water (Fermentas Life Sciences, US). Eighty nanogram of DNA was used as template in each reaction. The PCR cycling conditions were as follows: initial denaturation at 95°C for 300 seconds, 35 cycles of denaturation at 95°C for 30 seconds, annealing at 54°C for 30 seconds and extension at 72°C for 60 seconds. Final extension was at 72ºC for 300 seconds.

2.6.3 Agarose gel electrophoresis of PCR amplification products

DNA amplifications were confirmed by electrophoresis of the PCR products on a 1.5% (w/v) agarose gel (SeaKem, US) in 1 x TAE buffer [20 mM Acetic acid, 40 mM Tris and 1 mM EDTA at pH 8.0). The agarose gel contained 0.001 mg/ml ethidium bromide (Bio-Rad, UK). A mixture of 5 µl PCR amplification product and 5 µl 6 x Orange loading dye (Fermentas Life Science, US) was loaded into each well of the gel. The fragment sizes of the multiplex (*mdh* & *lacZ*) and *uidA* monoplex PCR amplification products were confirmed respectively by loading a 100 bp molecular marker (O'GeneRuler, Fermentas

Life Science, US) onto the gel. The fragment sizes of the 16S monoplex PCR amplification products were confirmed by loading a 1 Kb molecular marker (O'GeneRuler, Fermentas Life Science, US) onto the gel. Electrophoresis conditions were set at 80 V for 50 minutes for the multiplex and the two monoplex PCR's. Due to the replacement of the imaging system in 2012, some of the gel images were captured by the Gene Bio Imaging System (Syngene, Synoptic, UK) using GeneSnap software (version 6.00.22) and some images were captured by the ChemiDoc MP Imaging System (Bio-Rad, US) using Image Lab software (version 4.0.1). Inverted images of the PCR products were used.

2.7 SEQUENCING OF PCR PRODUCTS

To identify the *mdh, lacZ* and *uidA* genes of the multiplex and monoplex PCR tests, DNA sequence analyses of the PCR amplicons were performed.

2.7.1 First PCR clean-up for sequencing

Using a PCR clean-up kit (NucleoSpin Extract II Kit, Macherey-Nagel, Germany) unwanted products which may have formed during the PCR process were removed. The quality of the product after cleansing was determined by using a NanoDropTM 1000 Spectrophotometer v3.5.2 (Thermo Scientific, US). A 260:280 ratio of 1.7 - 2.1 was considered to be acceptable.

2.7.2 Sequencing PCR

A second round of amplification was performed using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems Inc., US). The reaction mixture consisted of 4µl Ready Reaction Premix (2.5X), 2µl BigDye Sequencing Buffer (5X), 3.2pmol forward primer (Fermentas Life Sciences, US), 10ng Template and the total volume of 20µl was attained by adding nuclease free PCR-grade water (Fermentas Life Sciences, US). Cycling conditions were as follows: initial denaturation at 96°C for 60 seconds, 25 cycles of denaturation at 96°C for 10 seconds, annealing at 50°C for 5 seconds and extension at 60°C for 240 seconds. Amplification was done using a 1000Cycler (BioRad, UK) thermal cycler.

2.7.3 Clean-up of sequencing PCR products

A second clean-up of the sequencing PCR products was performed using a ZR (Zymo Research) Sequencing Clean-up Kit (The Epigenetics Company, US).

Sequences were analysed by Karen Jordaan (North-West University, Potchefstroom Campus, South Africa) using a Genetic Analyzer 3130 (Applied Biosystems Inc., California, US). BLAST (Basic Local Alignment Search Tool) searches were performed (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to compare the obtained sequences to the GenBank database of sequences.

2.8 STATISTICAL ANALYSIS

Microsoft Office Excel software was used to calculate the averages and standard errors of the data. To determine the correlation between the physico-chemical parameters and microbiological data during 2011 and 2012 redundancy analysis (RDA), using Canoco software version 4.5 (Ter Braak, 1990), was used. Mean biplots were made of the variables which included: 1) a RDA biplot to indicate the correlation between the microbiological and physico-chemical variables in the raw and drinking water. Average values of the raw and drinking water were used to create the biplot; 2) a RDA biplot to indicate the correlation between the physico-chemical variables and microbiological variables in the drinking water. In this case the measurements at the three points in the distribution system were used to create the biplot. Correlations between the variables were determined by measuring the angle between them on the biplots. The smaller the angle between the variables, the closer the correlation.

CHAPTER 3
INTERPRETATION OF RESULTS

The following chapter contains results obtained from four different water treatment systems in and around the Vaalharts irrigation scheme (Hartswater, Pampierstad, Jan Kempdorp and Warrenton). In section 3.1 the physico-chemical results for 2011 and 2012 are given, followed by microbiological results for 2011 and 2012 in section 3.2. Section 3.3 contains results of the various confirmation tests that were performed for *E. coli* and section 3.4 contains the molecular confirmation results. Section 3.5 contains the sequencing results and section 3.6 contains the statistical analysis of all the physico-chemical and microbiological data for 2011 and 2012. Section 3.7 gives a summary of the results.

3.1 PHYSICO-CHEMICAL RESULTS

Physico-chemical results for 2011 are given in table 5 and table 6 and for 2012 in table 7 and table 8.

3.1.1 Physico-chemical results for 2011

In table 5 it can be seen that Hartswater and Jan Kempdorp had very low free chlorine levels in drinking water during July and October 2011 ranging from 0.03 mg/L to 0.08 mg/L. These levels are very low and it might cause regrowth of microorganisms in the distribution system. Warrenton had the highest free chlorine level in the drinking water during July (0.43 mg/L) and October (0.64 mg/L). Pampierstad also had high levels of free chlorine in the drinking water during July (0.34 mg/L) and October (0.28 mg/L). The free chlorine levels of all four distribution systems complied with the SANS 241 (2011) standard of ≤5 mg/L for drinking water. However, the available chlorine in the distribution systems of Hartswater and Jan Kempdorp were extremely low and not sufficient for disinfection purposes and prevention of regrowth.

Electrical conductivity levels for raw water did not vary much from the levels in the drinking water (table 5). There was, however, an increase in the EC levels from July to October in the raw and drinking water. The highest EC levels in raw and drinking water during July were 40 mS/m and 40.60 mS/m respectively (Warrenton) and during

October 44.80 mS/m (Warrenton) in raw water and 45.50 mS/m (Jan Kempdorp) in drinking water. Electrical conductivity levels of all four distribution systems complied with the SANS 241 (2011) standard of ≤170 mS/m. TDS levels for 2011 are given in Appendix A.

The pH levels measured at all sampling points during 2011 complied with the SANS 241 (2011) standard of ≥5 to ≤9.7 pH units for drinking water. This is also reflected in the pH values that are provided in table 5. A slight decrease in the pH levels from July to October were observed in the raw and drinking water. During July the highest pH level in raw and drinking water was 9.14 and 8.65 respectively (both for Warrenton) and during October 8.32 in raw water (Pampierstad) and 7.64 in drinking water (Jan Kempdorp).

Even though sulphate levels were low during 2011 (table 5), an increase in the levels of the raw and drinking water from July to October was noted. The highest level of sulphate in the raw water during July was 44.50 mg/L (Warrenton) and during October 55.50 mg/L (Jan Kempdorp). In the case of drinking water the highest sulphate measured during July was 47 mg/L (Warrenton) and during October 53.33 mg/L (Hartswater). The sulphate levels of all four distribution systems complied with the SANS 241 (2011) standard of ≤500 mg/L for drinking water. However, a slight increase in sulphate levels after purification was noted. Warrenton had the highest free chlorine, EC, pH and sulphate levels for raw and drinking water during July (table 5).

Nitrate levels were low during July and October 2011 (table 5). Jan Kempdorp had the highest level of nitrate in the raw water (0.70 mg/L) and drinking water (0.40 mg/L) during July. During October Warrenton had the highest nitrate levels for raw water (1.85 mg/L) and drinking water (0.58 mg/L). No nitrate was detected in the raw water of Hartswater and Pampierstad during July, but was detected in the drinking water. This may be due to biofilms forming in the distribution system. Even though the nitrate levels of all four distribution systems complied with the SANS 241 (2011) standard of ≤11 mg/L for drinking water, a slight increase of nitrate levels in drinking water from July to October was noted.

Nitrite levels were considerably high and the levels for all four distribution systems exceeded the SANS 241 (2011) standard of ≤0.9 mg/L for drinking water during July and October. Hartswater had the highest nitrite level in drinking water during July (4.50 mg/L) and Warrenton during October (2.83 mg/L). During July and October no nitrite was detected in the raw water of some of the areas, but detected in the drinking water. This may be due to biofilm formation in the distribution system.

In table 6 it can be seen that COD levels decreased from July to October. During July Hartswater had the highest COD levels in the raw water (42 mg/L) and drinking water (34.75 mg/L). During October Hartswater had again the highest level of COD in the raw water again (20 mg/L) and Jan Kempdorp in the drinking water (15 mg/L).

Sulphide levels were high in the raw water of Hartswater (69 mg/L) and Pampierstad (79 mg/L) during July (table 6). Hartswater had the highest sulphide level in drinking water during July (12.33 mg/L) and October (8.25 mg/L). This indicates a decrease of the sulphate levels in drinking water from July to October. Sulphide levels were higher after purification in some of the distribution systems during July and October. This may be due to possible biofilm formation.

Hartswater had the highest phosphorous level in drinking water during July (0.81 mg/L) and October (0.65 mg/L) (table 6). A marginal increase of phosphorous levels in the raw water of all four areas from July to October was noted. Phosphorous levels were higher after purification for all for distribution systems during July and October. This may be an indication of biofilm formation in the distribution system. During July temperature varied from 10.4°C to 13.1°C and during October from 21.8°C to 25.7°C. The temperature of the raw water correlates with the colder season (July) and warmer season (October).

During July Warrenton had the highest salinity level for raw water (193 ppm) and drinking water (198 ppm). During October Jan Kempdorp had the highest salinity level in raw water (222 ppm) and drinking water (227 ppm). Salinity levels increased in the raw and drinking water for all four distribution systems from July to October. This explains the EC levels that also increased from July to October. A marginal difference between salinity levels of raw and drinking water was noted during 2011. There is no SANS 241 (2011) standard available for COD, sulphide, phosphorous and salinity.

Table 5: Physico-chemical results for July and October 2011.

		Free chlorine (mg/L)		Electrical Conductivity (mS/m)		pH at 25°C (pH units)		Sulphate (mg/L)		Nitrate (mg/L)		Nitrite (mg/L)	
SANS 241:2011		**≤5**		**≤170**		**≥5 to ≤9.7**		**≤500**		**≤11**		**≤0.9**	
Sampling area		**R**	**D**	**R**	**D**	**R**	**D**	**R**	**D**	**R**	**D**	**R**	**D**
Hartswater (July 2011)	**Ave**	**0.04**	**0.03**	**32.60**	**32.96**	**9.06**	**8.34**	**32.50**	**34.33**	**ND**	**0.32**	**4**	**4.50**
	SE	0.01	0.00	0	0.11	0	0.03	2.12	0.54	0	0.09	1.41	0.35
Pampierstad (July 2011)	**Ave**	**0.03**	**0.34**	**33.70**	**34.17**	**8.65**	**7.91**	**32.50**	**35.17**	**ND**	**0.27**	**4.50**	**3**
	SE	0.01	0.23	0	0.11	0	0.06	0.71	0.82	0	0.26	0.71	0.35
Jan Kempdorp (July 2011)	**Ave**	**0.07**	**0.06**	**37.30**	**36.07**	**9.08**	**8.64**	**42.50**	**41.17**	**0.70**	**0.40**	**ND**	**1.17**
	SE	0.01	0.02	0	0.66	0	0.34	0.71	0.41	0	0.04	0	0.20
Warrenton (July 2011)	**Ave**	**0.11**	**0.43**	**40**	**40.60**	**9.14**	**8.65**	**44.50**	**47**	**0.40**	**0.23**	**0.50**	**1.83**
	SE	0.04	0.22	0	0.39	0	0.09	0.71	0.61	0	0.13	0	1.02
Hartswater (Oct. 2011)	**Ave**	**0.05**	**0.04**	**43.60**	**44.10**	**8.24**	**7.77**	**50**	**53.33**	**0.10**	**0.48**	**2.50**	**1.17**
	SE	0.01	0.01	0	0.12	0	0.06	0	2.35	0	0.18	0.71	0.20
Pampierstad (Oct. 2011)	**Ave**	**0.06**	**0.28**	**43.70**	**44.40**	**8.32**	**7.54**	**50.50**	**50.67**	**0.20**	**0.45**	**1**	**1**
	SE	0.01	0.23	0	0.11	0	0.01	0.71	0.54	0	0.04	0	0
Jan Kempdorp (Oct. 2011)	**Ave**	**0.07**	**0.08**	**44.40**	**45.50**	**8.31**	**7.64**	**55.50**	**51.50**	**0.30**	**0.40**	**ND**	**2.66**
	SE	0.01	0.05	0	0.14	0	0.13	0.16	1.22	0.01	0.04	0	0.74
Warrenton (Oct. 2011)	**Ave**	**0.05**	**0.64**	**44.80**	**45.23**	**8.04**	**7.60**	**48**	**51.83**	**1.85**	**0.58**	**ND**	**2.83**
	SE	0.01	0.23	0	0.15	0	0.25	0	0.74	0.07	0.14	0	1.02

R: Raw water; **D**: Drinking water; **Ave**: Average; **SE**: Standard Error; **ND**: Not Detected.

Table 6: Physico-chemical results for July and October 2011 (cont.).

		COD (mg/L)		Sulphide (mg/L)		Phosphorous (mg/L)		Temperature (°C)		Salinity (ppm)	
SANS 241:2011		-		-		-		-		-	
Sampling area		**R**	**D**	**R**	**D**	**R**	**D**	**R**	**D**	**R**	**D**
Hartswater (July 2011)	**Ave**	**42**	**34.75**	**69**	**12.33**	**0.19**	**0.81**	**12.0**	**12.3**	**158**	**160**
	SE	4.42	18.03	4.24	3.05	0.12	0.07	0	0.31	0	0.41
Pampierstad (July 2011)	**Ave**	**33.50**	**28.75**	**79**	**9**	**0.12**	**0.76**	**10.8**	**11.8**	**161**	**164**
	SE	2.12	4.59	5.66	0.35	0.07	0.06	0	0.64	0	0.71
Jan Kempdorp (July 2011)	**Ave**	**33.50**	**23**	**2**	**9.33**	**0.11**	**0.54**	**10.5**	**12.0**	**182**	**173**
	SE	2.12	4.24	1.41	0.82	0.03	0	0	0.86	0	2.67
Warrenton (July 2011)	**Ave**	**33.50**	**14**	**3.50**	**4.83**	**0.14**	**0.31**	**10.4**	**13.1**	**193**	**198**
	SE	2.12	9.19	0.71	0.41	0.03	0.02	0	1.72	0	1.47
Hartswater (Oct. 2011)	**Ave**	**20**	**5.50**	**1.50**	**8.25**	**0.36**	**0.65**	**21.9**	**23.2**	**216**	**219**
	SE	0	3.54	0.71	0.35	0.03	0.07	0	0.25	0	1.47
Pampierstad (Oct. 2011)	**Ave**	**8.50**	**11**	**8**	**5.83**	**0.54**	**0.62**	**21.8**	**23.3**	**218**	**221**
	SE	9.19	0	1.41	0.89	0	0.01	0	0.73	0	0.82
Jan Kempdorp (Oct. 2011)	**Ave**	**11.50**	**15**	**7**	**6.33**	**0.58**	**0.65**	**22.5**	**24.7**	**222**	**227**
	SE	0.71	5.66	1.40	1.43	0.16	0.19	0	1.05	0	0.71
Warrenton (Oct. 2011)	**Ave**	**5.50**	**3**	**ND**	**4.66**	**0.19**	**0.61**	**23.4**	**25.7**	**223**	**226**
	SE	6.36	1.41	0	0.20	0.01	0.08	0	2.13	0	0.41

R: Raw water; **D**: Drinking water; **Ave**: Average; **SE**: Standard Error; **ND**: Not Detected.

3.1.2 Physico-chemical results for 2012

Tables 7 and 8 indicate the physico-chemical results for 2012. In table 7 it can be seen that free chlorine levels were very low for Jan Kempdorp during March (0.04 mg/L) and June (0.06 mg/L). These low levels may cause regrowth of microorganisms in the distribution system. Hartswater had a very high free chlorine level during June (1.39 mg/L). Such high chlorine levels may have health and aesthetic effects. Warrenton and Pampierstad had acceptable free chlorine levels in their drinking water during March and June. The free chlorine levels of all four distribution systems complied with the SANS 241 (2011) standard of ≤5 mg/L for drinking water.

Electrical conductivity levels for raw water did not vary much from the levels in the drinking water during March and June (table 7). Even though EC levels were low, an increase from March to June in the raw and drinking water was noted. During March the highest EC level in raw water was 57 mS/m (Warrenton) and in drinking water 58.03 mS/m (Jan Kempdorp). During June the highest EC level in raw water was 63.40 mS/m (Pampierstad) and in drinking water 64.06 mS/m (Hartswater). The EC levels of all four distribution systems complied with the SANS 241 (2011) standard of ≤170 mS/m during March and June. TDS levels for 2012 are given in Appendix A.

The pH levels measured at all sampling points during 2012 (table 7) complied with the SANS 241 (2011) standard of ≥5 to ≤9.7 pH units for drinking water. This is also reflected in the pH values that are provided in table 7. The pH levels stayed more or less the same during March and June. During 2012 the highest pH level in raw water was 8.50 and in drinking water 7.94.

Sulphate levels increased from March to June (table 7). The highest sulphate level in raw water during March was 70.50 mg/L (Jan Kempdorp) and during June 79.50 mg/L (Hartswater). Hartswater also had the highest sulphate levels in drinking water during March (71.33 mg/L) and June (83.50 mg/L). Even though the sulphate levels were marginally higher after purification for all for distribution systems during March and June (except Pampierstad during March) it complied with the SANS 241 (2011) standard of ≤500 mg/L.

Nitrate levels were low in the raw and drinking water during 2012. During March and June no nitrate were detected in the raw water of Hartswater and Warrenton, but were detected in the drinking water (table 7). This may be an indication of biofilm formation in the distribution system. A slight increase in the nitrate levels from March to June in the drinking water was noted. Hartswater had the highest nitrate level in drinking water during March (0.55 mg/L) and June (0.97 mg/L). Nitrate levels complied with the SANS 241 (2011) standard of ≤11 mg/L for drinking water.

Nitrite levels for all four distribution systems exceeded the SANS 241 (2011) standard of ≤0.9 mg/L for drinking water during March and June, except for Pampierstad during March (0.83 mg/L). No nitrite was detected in the raw water of Hartswater during March and June, but was detected in the drinking water. This may be due to biofilm formation in the distribution system. An increase in nitrate levels in the drinking water from March to June was noted.

COD levels increased from March to June, except for the drinking water of Warrenton (table 8). During March COD levels of Hartswater and Pampierstad were under measuring range. The highest COD level in raw and drinking water during March was 16 mg/L (Warrenton) and during June 28.50 mg/L (Pampierstad). During March 2012, water samples of all four distribution systems were sent to Midvaal Water Company for COD analysis. Midvaal Water Company is a SANAS accredited laboratory. The test reports are given in Appendix C. The COD levels of in the drinking water of all four distribution systems did not vary much with the results from Midvaal. The COD levels in raw water obtained from Midvaal did not vary much from results for Warrenton, but varied for Hartswater, Pampierstad and Jan Kempdorp. The COD levels measured for raw water during our study and by Midvaal respectively were: Hartswater – UMR & 23mg/L; Pampierstad – UMR & 21 mg/L and for Jan Kempdorp – 11 & 21 mg/L. COD results given in table 8 are average results of all three sampling points (for drinking water), whereas the results for Midvaal only consists of a raw water sample and a sample at the furthest point in the distribution of each of the distribution systems.

Sulphide levels did not vary much between March and June (table 8). No sulphide was detected in the raw water of Hartswater during March, but was detected in the drinking water. This may be due to biofilm formation in the distribution system. Pampierstad had

the highest sulphide level in drinking water during March (8.33 mg/L) and June (7.33 mg/L) whereas Jan Kempdorp had the lowest sulphide level in drinking water during March (5.50 mg/L) and June (3.83 mg/L).

Phosphorous levels weren't high during 2012 (table 8). Some of the distribution systems had an increase in phosphorous levels after purification, while other distribution systems had a decrease. The highest phosphorous level in drinking water during March was 1.36 mg/L (Jan Kempdorp) and during June 1.05 mg/L (Hartswater). During March raw and drinking water temperature varied from 21.0°C to 23.7°C and during June from 12.5°C to 16.0°C. The temperature of the raw water corresponds with the warmer season (March) and the colder season (June).

Salinity levels increased from March to June (table 8). This explains the increase in EC levels from March to June. During March the highest level of salinity in raw water was 285 ppm (Warrenton) and in drinking water 290 ppm (Jan Kempdorp). During June the highest level of salinity in raw water was 312 ppm (Pampierstad) and in drinking water 315 ppm (Hartswater and Pampierstad). A marginal increase in salinity levels after purification was noticed.

Table 7: Physico-chemical results for March and June 2012.

		Free chlorine (mg/L)		Electrical Conductivity (mS/m)		pH at 25°C (pH units)		Sulphate (mg/L)		Nitrate (mg/L)		Nitrite (mg/L)	
SANS 241:2011		**≤5**		**≤170**		**≥5 to ≤9.7**		**≤500**		**≤11**		**≤0.9**	
Sampling area		**R**	**D**	**R**	**D**	**R**	**D**	**R**	**D**	**R**	**D**	**R**	**D**
Hartswater (March 2012)	**Ave**	**0.11**	**0.29**	**51.50**	**57.26**	**8.20**	**7.32**	**62**	**71.33**	**ND**	**0.55**	**ND**	**1.83**
	SE	0.04	0.26	0	3.45	0	0.12	2.83	3.17	0	0.14	0	1.33
Pampierstad (March 2012)	**Ave**	**0.04**	**0.23**	**53.80**	**53.96**	**7.98**	**7.56**	**67.50**	**67.50**	**0.30**	**0.53**	**1.50**	**0.83**
	SE	0.01	0.21	0	0.11	0	0	0.70	1.06	0	0.02	0.70	0.41
Jan Kempdorp (March 2012)	**Ave**	**0.07**	**0.04**	**56**	**58.03**	**8.20**	**7.76**	**70.50**	**71**	**0.30**	**0.45**	**1**	**1**
	SE	0.01	0.01	0	1.45	0	0.04	0.70	1.54	0.14	0.04	0	0
Warrenton (March 2012)	**Ave**	**0.04**	**0.47**	**57**	**57.47**	**7.75**	**7.67**	**68.50**	**70.83**	**ND**	**0.48**	**1**	**1**
	SE	0.01	0.29	0	0.18	0	0.16	0.70	0.07	0	0.18	0	0
Hartswater (June 2012)	**Ave**	**0.13**	**1.39**	**62.70**	**64.06**	**8.50**	**7.66**	**79.50**	**83.50**	**ND**	**0.97**	**ND**	**1.83**
	SE	0.01	1.51	0	0.86	0	0.21	0.71	0.35	0	0.27	0	0.41
Pampierstad (June 2012)	**Ave**	**0.07**	**0.32**	**63.40**	**63.90**	**8.46**	**7.85**	**78.50**	**79.17**	**0.45**	**0.62**	**1**	**1.33**
	SE	0.01	0.09	0	0.19	0	0.08	0.71	1.14	0.35	0.02	1.40	0.20
Jan Kempdorp (June 2012)	**Ave**	**0.09**	**0.06**	**62.80**	**63.33**	**8.44**	**7.94**	**77**	**80.83**	**0.35**	**0.73**	**4**	**3**
	SE	0	0	0	0.16	0	0.08	1.41	1.95	0.07	0.15	0	0.94
Warrenton (June 2012)	**Ave**	**0.09**	**0.51**	**63**	**63.20**	**8.08**	**7.90**	**76**	**81.17**	**ND**	**0.66**	**2**	**1.83**
	SE	0.01	0.49	0	0.37	0	0.18	1.41	2.27	0	0.20	1.41	1.34

R: Raw water; **D**: Drinking water; **Ave**: Average; **SE**: Standard Error; **ND**: Not Detected; **UMR**: Under Measuring Range.

Table 8: Physico-chemical results for March and June 2012 (cont.).

		COD (mg/L)		Sulphide (mg/L)		Phosphorous (mg/L)		Temperature (°C)		Salinity (ppm)	
SANS 241:2011		-		-		-		-		-	
Sampling area		**R**	**D**	**R**	**D**	**R**	**D**	**R**	**D**	**R**	**D**
Hartswater (March 2012)	**Ave**	**UMR**	**UMR**	**ND**	**6.17**	**0.87**	**0.93**	**22.5**	**23.7**	**257**	**287**
	SE	0	0	0	0.41	0.04	0.22	0	1.03	0	17.38
Pampierstad (March 2012)	**Ave**	**UMR**	**UMR**	**4.50**	**8.33**	**0.70**	**0.66**	**22.8**	**23.1**	**270**	**270**
	SE	0	0	0.71	1.02	0.04	0.09	0	0.39	0	0
Jan Kempdorp (March 2012)	**Ave**	**11**	**5.50**	**6**	**5.50**	**1.54**	**1.36**	**21.7**	**21.0**	**280**	**290**
	SE	5.70	6.36	1.41	1.23	1.19	0.61	0	0.55	0	6.94
Warrenton (March 2012)	**Ave**	**15.50**	**16**	**UMR**	**5.83**	**0.40**	**0.77**	**19.9**	**21.0**	**285**	**288**
	SE	0.70	0	0	0.54	0.12	0.07	0	1.2	0	2.27
Hartswater (June 2012)	**Ave**	**25.50**	**20.25**	**7**	**5.50**	**2.85**	**1.05**	**13.1**	**15.4**	**308**	**315**
	SE	2.12	6.01	2.83	0.61	1.93	0.35	0	0.65	0	5.31
Pampierstad (June 2012)	**Ave**	**28.50**	**28.25**	**5.50**	**7.33**	**0.46**	**0.70**	**12.5**	**14.1**	**312**	**315**
	SE	3.54	2.75	3.54	0.82	0.05	0.13	0	0.98	0	0.82
Jan Kempdorp (June 2012)	**Ave**	**27**	**20**	**3.50**	**3.83**	**0.85**	**0.84**	**13.2**	**14.7**	**310**	**313**
	SE	4.24	4.95	3.50	0.54	0.40	0.23	0	0.31	0	0.41
Warrenton (June 2012)	**Ave**	**21**	**13.75**	**2**	**6.25**	**0.65**	**0.56**	**13.4**	**16.0**	**308**	**312**
	SE	4.24	7.42	2.82	1.25	0.18	0.11	0	1.66	0	0.82

R: Raw water; **D**: Drinking water; **Ave**: Average; **SE**: Standard Error; **ND**: Not Detected; **UMR**: Under Measuring Range.

The results of the 5 major metals (Ca, Mg, K, Na and Zn) and 15 trace metals (Al, Sb, As, Cd, Cr, Co, Cu, Fe, Pb, Mn, Hg, Ni, Se, U and V) did not raise any concern during 2011 and 2012. Metal concentrations were considerably low in the raw and drinking water for all four distribution systems during 2011 and 2012. Metals such as Zn, U, Pb, Mg, Cd, Se, As, Cu, Ni, Co and Cr were not even detected in most of the samples. The metal levels in the drinking water of all four distribution systems complied with the SANS 241 (2011) standards during 2011 and 2012. The only concern was the mercury levels in the raw and drinking water of Hartswater and Pampierstad during July 2011. The highest level of Mercury in the drinking water of Hartswater was 53.33 µg/L and Pampierstad 18.50 µg/L. These levels exceeded the SANS 241 (2011) standard of ≤6 µg/L for mercury in drinking water.

3.2 MICROBIOLOGICAL RESULTS

Microbiological results for 2011 and 2012 are given in table 9 and table 10 respectively.

3.2.1 Microbiological results for 2011

In table 9 it can be seen that the total coliform levels were very high in the raw water of some of the areas. An increase in total coliform levels from July to October in the raw water was also noted. The highest level of total coliforms in the raw water during July was 677 CFU/100ml and during October 1833 CFU/100ml. Total coliform levels were lower after purification for all four distribution systems during July and October. This is an indication that the purification process effectively removed total coliforms from the raw water. Total coliforms were detected in the drinking water of Hartswater during July (14 CFU/100ml). This did not comply with the SANS 241 (2011) standard of ≤10 CFU/100ml.

Faecal coliform levels (table 9) were low in the raw water during July and October (10 CFU/100ml to 17 CFU/100ml), except for Warrenton during July (100 CFU/ 100ml). No faecal coliforms were detected in the drinking water of all four distribution systems during July and October and thus complied with the SANS 241 (2011) standard of 0 CFU/100ml. This indicates that the purification process effectively removed all the faecal coliforms from the raw water.

Heterotrophic plate count (HPC) bacteria levels were considerably high in the raw water and drinking water of all four distribution systems during July and October (table 9). Heterotrophic plate count bacteria in the raw water of some areas increased from July to October whereas other decreased. Jan Kempdorp had the highest level of HPC bacteria during July for raw water (86.14 x 10^3 CFU/ml) and drinking water (27.73 x 10^3 CFU/ml). During October Hartswater had the highest level of HPC bacteria for raw water (155.42 x 10^3 CFU/ml) and drinking water (10.42 x 10^3 CFU/ml). Heterotrophic plate count levels of Hartswater and Jan Kempdorp did not comply with the SANS 241 (2011) standard of ≤1000 CFU/ml for drinking water during July. During October the HPC bacteria levels of Hartswater, Pampierstad and Jan Kempdorp did not comply with the SANS 241 (2011) standard.

Faecal streptococci levels were low in the raw water during July and October, except for Jan Kempdorp during July which had the highest faecal streptococci level of 72 CFU/100ml (table 9). Faecal streptococci levels decreased from July to October. During July Hartswater was the only distribution system with faecal streptococci in their drinking water (2 CFU/100ml) which means the purification process effectively removed faecal streptococci from the raw water of the other distribution systems during July and October.

During July Hartswater was the only area with *Clostridia* in their raw water (table 9). No *Clostridia* were detected in the drinking water of all four distribution systems during July. During October *Clostridia* were present in the raw and drinking water of all four distribution systems. The presence of *Clostridia* in the drinking water is an indication of possible failure in the treatment process.

Pseudomonas aeruginosa levels increased in the drinking water of all four distribution systems from July to October, except Warrenton which had no *P. aeruginosa* in their drinking water during July and October (table 9). A very high level of *P. aeruginosa* was detected in the raw water of Hartswater during October (8817 CFU/100ml). Hartswater also had the highest potential *P. aeruginosa* level for drinking water during July (22 CFU/100ml) and October (246 CFU/100ml).

Fungi levels increased from July to October 2011 in the raw and drinking water of all four distribution systems (table 9). Fungi levels of 100 CFU/ml were detected in the raw water during July and during October the highest level was 19 000 CFU/ml (Hartswater). No fungi were detected in the raw and drinking water of Warrenton during July (table 9). The highest level of fungi in the drinking water during July was 100 CFU/ml (Hartswater) and during October 667 CFU/ml (Pampierstad).

Even though there was a decrease in anaerobic bacteria from July to October, the levels were considerably high in the raw water (table 9). Hartswater was the only distribution system with anaerobic bacteria in the drinking water during July (75.37×10^3 CFU/ml). This is an indication of possible biofilm formation in the distribution system. No anaerobic bacteria were detected in the drinking water of all four distribution systems during October. There is no SANS 241 (2011) standard for faecal streptococci, *Clostridia*, *Pseudomonas aeruginosa*, fungi and anaerobic bacteria.

Table 9: Microbiological results for July and October 2011.

		Total coliforms (CFU/100ml)		Faecal coliforms (CFU/100ml)		HPC bacteria (x 10^3 CFU/ml)		Faecal streptococci (CFU/100ml)		*Clostridia* (CFU/100ml)		*Pseudomonas aeruginosa* (CFU/100ml)		Fungi (CFU/ml)		Anaerobic bacteria (x 10^3 CFU/ml)	
SANS 241-1:2011		**≤10 CFU/100ml**		**0 CFU/100ml**		**≤1000 CFU/ml**		**-**		**-**		**-**		**-**		**-**	
Sampling area		**R**	**D**	**R**	**D**	**R**	**D**	**R**	**D**	**R**	**D**	**R**	**D**	**R**	**D**	**R**	**D**
Hartswater (July 2011)	**Ave**	**677**	**14**	**13**	**0**	**30.43**	**24.38**	**10**	**2**	**Present**	**Not Present**	**433**	**22**	**100**	**100**	**11.35**	**75.37**
	SE	61.41	3.67	4.08	0	6.04	15.78	0	0	0	0	39.16	18.61	0	0	13.65	57.63
Pampierstad (July 2011)	**Ave**	**437.50**	**2**	**17**	**0**	**19.09**	**0.99**	**34**	**0**	**Not Present**	**Not Present**	**751**	**0**	**100**	**0**	**86.83**	**0**
	SE	132.30	0	4.08	0	7.23	0.29	4.47	0	0	0	76.73	0	0	0	35.79	0
Jan Kempdorp (July 2011)	**Ave**	**48**	**0**	**13**	**0**	**86.14**	**27.73**	**72**	**0**	**Not Present**	**Not Present**	**286**	**2**	**100**	**0**	**49.73**	**0**
	SE	28.43	0	2.88	0	57.99	15.58	14.75	0	0	0	24.71	0	0	0	11.05	0
Warrenton (July 2011)	**Ave**	**67**	**0**	**100**	**0**	**21.88**	**0.69**	**10**	**0**	**Not Present**	**Not Present**	**305**	**0**	**0**	**0**	**-**	**-**
	SE	4.87	0	0	0	13.23	0.22	0	0	0	0	20	0	0	0	-	-
Hartswater (Oct. 2011)	**Ave**	**1833**	**7**	**10**	**0**	**155.42**	**10.42**	**0**	**0**	**Present**	**Present**	**8817**	**246**	**19000**	**100**	**0.58**	**0**
	SE	412.50	1.67	0	0	49.10	11.24	0	0	0	0	386.18	15.94	19799	0	0.13	0
Pampierstad (Oct. 2011)	**Ave**	**550**	**0**	**10**	**0**	**48.97**	**4.59**	**0**	**0**	**Present**	**Present**	**TMTC**	**2**	**500**	**667**	**1.20**	**0**
	SE	212.10	0	0	0	16.64	3.2	0	0	0	0	0	0	0	366.50	0.26	0
Jan Kempdorp (Oct. 2011)	**Ave**	**15**	**9**	**12**	**0**	**26.22**	**4.90**	**2**	**0**	**Present**	**Present**	**TMTC**	**66**	**100**	**100**	**0.07**	**0**
	SE	7.07	1.75	1.82	0	7.61	3.73	1.09	0	0	0	0	66.50	0	0	0.07	0
Warrenton (Oct. 2011)	**Ave**	**TMTC**	**0**	**10**	**0**	**9.78**	**0.38**	**1**	**0**	**Present**	**Present**	**TMTC**	**0**	**200**	**0**	**0.18**	**0**
	SE	0	0	0	0	4.78	0.39	0	0	0	0	0	0	0	0	0.09	0

R: Raw water; **D**: Drinking water; **Ave**: Average; **SE**: Standard Error; **TMTC**: To many to count; **CFU**: Colony forming units; **HPC**: Heterotrophic plate count.

3.2.2 Microbiological results for 2012

Table 10 indicate the microbiological results for March and June 2012. Total coliform levels were high in the raw water of most areas. Warrenton was the only distribution system with total coliforms in the drinking water during 2012 (256 CFU/100ml). This does not comply with the SANS 241 (2011) standard of ≤10 CFU/100ml for drinking water.

Faecal coliform levels in the raw water were low during 2012, except Warrenton during March (TMTC) (table 10). Jan Kempdorp was the only distribution system during 2012 with faecal coliforms in the drinking water (191 CFU/100ml). This does not comply with the SANS 241 (2011) standard of 0 CFU/100ml for drinking water.

Water samples were also sent to Midvaal Water Company during the March sampling period of 2012 for analysis of *E. coli*, faecal coliforms and total coliforms. Test reports are given in Appendix C. Total coliforms obtained in the drinking water of all four samples did not vary much from the samples of Midvaal, except for Warrenton (our study – 256 CFU/ml & Midvaal - 0 MPN/100ml). The results obtained from Midvaal for total coliforms in the raw water of Hartswater, Pampierstad and Warrenton did not vary much to our results, but varied for Jan Kempdorp. The total coliform levels measured for raw water during our study and by Midvaal respectively were: Hartswater - TMTC & >2420 MPN/100ml; Pampierstad - TMTC & 488 MPN/100ml; Jan Kempdorp - 22 CFU/100ml & 866 MPN/100ml and Warrenton - TMTC & 1553 MPN/100ml. This could be due to culture based methods (m-Endo agar) not being as sensitive as the Colilert test, which Midvaal used. The total coliform and faecal coliform results given in table 9 & 10 are average results of all three sampling points (for drinking water), whereas the results for Midvaal only consists of a raw water sample and a sample at the furthest point in the distribution of each of the distribution systems.

Heterotrophic plate count bacteria levels were considerably high in the raw water during 2012 (table 10). Warrenton had the highest HPC bacteria level in raw water during March (183 x 10^3 CFU/ml) and Hartswater during June (119 x 10^3 CFU/ml). The levels of HPC bacteria that were detected in the drinking water during 2012 did not comply with the SANS 241 (2011) standard of ≤1000 CFU/ml. A decrease in HPC bacteria levels from March to June was noted.

During June Hartswater had a high level of faecal streptococci in the raw water (TMTC) (table 10). Jan Kempdorp was the only distribution system with faecal streptococci in the drinking water during March (5 CFU/100ml). No faecal streptococci were detected in the drinking water of all four distribution systems during June. This indicates that the treatment process of the effectively removed the faecal streptococci from the raw water.

Clostridia were detected in the raw water of all four areas during March and June (table 10). No *Clostridia* were present in the drinking water of Pampierstad during March and Warrenton during June. This is an indication that the treatment process effectively removed *Clostridia* from the raw water.

High *Pseudomonas aeruginosa* levels were detected in the raw water of all four areas during March and June (TMTC) (table 10). *P. aeruginosa* levels in drinking water decreased from March to July, except for Hartswater. Jan Kempdorp had the highest *P. aeruginosa* level in drinking water during March (106 CFU/100ml) and Hartswater during June (92 CFU/100ml).

Fungi were only detected in the raw water of Hartswater during March (100 CFU/ml) and Jan Kempdorp during June (100 CFU/ml) (table 10). No fungi were detected in the drinking water of all four distribution systems during March and June. This indicates that the treatment processes of Hartswater and Jan Kempdorp were effective in removing fungi from the raw water.

High levels of anaerobic bacteria were detected in the raw water during 2012, except for Jan Kempdorp which had no anaerobic bacteria in the raw water. The highest level of anaerobic bacteria in raw water during March was 13.75 x 10^3 CFU/ml (Hartswater) and during June 200 x 10^3 CFU/ml (Pampierstad). No anaerobic bacteria were detected in the drinking water of all four distribution systems during March and June. This indicates that the treatment process effectively removed anaerobic bacteria from the raw water.

Table 10: Microbiological results for March and June 2012.

		Total coliforms (CFU/100ml)		Faecal coliforms (CFU/100ml)		HPC bacteria (x 10^3 CFU/ml)		Faecal streptococci (CFU/100ml)		*Clostridia* (CFU/100ml)		*Pseudomonas aeruginosa* (CFU/100ml)		Fungi (CFU/ml)		Anaerobic bacteria (x 10^3 CFU/ml)	
SANS 241-1:2011		**≤10 CFU/100ml**		**0 CFU/100ml**		**≤1000 CFU/ml**		**-**		**-**		**-**		**-**		**-**	
Sampling area		**R**	**D**	**R**	**D**	**R**	**D**	**R**	**D**	**R**	**D**	**R**	**D**	**R**	**D**	**R**	**D**
Hartswater (March 2012)	**Ave**	**TMTC**	**0**	**30**	**0**	**91.20**	**0**	**TMTC**	**0**	**Present**	**Present**	**TMTC**	**6**	**100**	**0**	**13.75**	**0**
	SE	0	0	5.66	0	20.77	0	0	0	0	0	0	1.63	0	0	4.28	0
Pampierstad (March 2012)	**Ave**	**TMTC**	**0**	**12**	**0**	**39.10**	**0**	**20**	**0**	**Present**	**Not Present**	**TMTC**	**0**	**0**	**0**	**1.83**	**0**
	SE	0	0	2.24	0	15.48	0	3.53	0	0	0	0	0	0	0	0.52	0
Jan Kempdorp (March 2012)	**Ave**	**22**	**0**	**47**	**191**	**132.65**	**21.58**	**10**	**5**	**Present**	**Present**	**TMTC**	**106**	**0**	**0**	**0**	**0**
	SE	6.58	0	11.55	13.51	56.45	20.09	0	1.64	0	0	0	61.87	0	0	0	0
Warrenton (March 2012)	**Ave**	**TMTC**	**256**	**TMTC**	**0**	**183**	**8.18**	**35**	**0**	**Present**	**Present**	**TMTC**	**0**	**0**	**0**	**5.45**	**0**
	SE	0	10.04	0	0	68.16	2.25	5.08	0	0	0	0	0	0	0	2.44	0
Hartswater (June 2012)	**Ave**	**30**	**0**	**22**	**0**	**119.66**	**0**	**37**	**0**	**Present**	**Present**	**TMTC**	**92**	**0**	**0**	**5.66**	**0**
	SE	9.35	0	9.12	0	38.41	0	8.33	0	0	0	0	20.26	0	0	2.69	0
Pampierstad (June 2012)	**Ave**	**10**	**0**	**34**	**0**	**20.76**	**0**	**17**	**0**	**Present**	**Present**	**TMTC**	**0**	**0**	**0**	**200**	**0**
	SE	0	0	12.55	0	1.30	0	4.08	0	0	0	0	0	0	0	0	0
Jan Kempdorp (June 2012)	**Ave**	**TMTC**	**0**	**13**	**0**	**17.26**	**2.20**	**40**	**0**	**Present**	**Present**	**TMTC**	**9**	**100**	**0**	**0.63**	**0**
	SE	0	0	4.08	0	3.07	2.93	7.91	0	0	0	0	0.82	0	0	0.25	0
Warrenton (June 2012)	**Ave**	**TMTC**	**0**	**15**	**0**	**10.16**	**0**	**10**	**0**	**Present**	**Not Present**	**TMTC**	**0**	**0**	**0**	**1.75**	**0**
	SE	0	0	7.07	0	2.83	0	0	0	0	0	0	0	0	0	0.52	0

R: Raw water; **D**: Drinking water; **Ave**: Average; **SE**: Standard Error; **TMTC**: To many to count; **CFU**: Colony forming units; **HPC**: Heterotrophic plate count.

3.3 RESULTS OF CONFIRMATION TESTS FOR *ESHERICHIA COLI*

Blue colonies isolated from m-Fc agar were tested to determine if these were possibly *Escherichia coli*. Table 11 and table 12 indicate the results of the various confirmation tests that were performed. Biochemical confirmation tests included β-D-Glucuronidase activity on MLG agar and the TSI test. Molecular confirmation tests included testing for the presence of *mdh*, *lacZ*, *uidA* and 16S rDNA genes. *E. coli* (ATCC 10536) was used as a positive control for all the confirmation tests.

During July 2011 (table 11) confirmation tests were performed on putative *E. coli* obtained from the raw water of Hartswater (HR), Pampierstad (PR), Jan Kempdorp (JR) and Warrenton (WR). The biochemical tests for isolates HR1 and PR2 were all identical and positive for *E. coli*. Except for TSI results, the rest of the biochemical confirmation tests were also positive for PR3. Isolates JR4 and WR5 had the same results for all the confirmation tests and were negative for *E. coli*. The molecular results were all positive for *E. coli*. The isolates from the October 2011 (table 11) sampling period had similar biochemical confirmation results as the July isolates. Except for one isolate (HR7) all the confirmation tests were positive for *E. coli*, but it did not produce gas during the TSI test. All the isolates were positive for *mdh* and *uidA* PCRs. The MLG agar results of three of the seven isolates did not conform to the TSI and PCR results. Of the thirteen isolates from the June 2012 (table 11) sampling period, all were positive for the *uidA* PCR and ten were positive for TSI confirming that these could be *E. coli*. However, the PCR test results of three isolates (HR13, JR19 and JR20) were negative for both *mdh* and *lacZ*. Only six of the thirteen isolates were green on MLG agar.

The isolates from the m-Fc plates collected during March 2012 were lost. However, Colilert sachets of the same water samples that were conducted by the Midvaal Company were available. Representative putative *E. coli* isolates could be tested using the same biochemical and PCR tests. Results for the ten isolates are provided in table 12. All the isolates were positive for the *mdh* and *uidA* PCRs. Nine isolates were positive for the TSI test. Six of the ten isolates were also green on the MLG agar. Three of the isolates (HR27 and PR29) were yellow on the MLG agar, but negative for *lacZ*. Amongst the March isolates there were two representatives (JD*32 and JD*33) from drinking water of Jan Kempdorp (table 12). These were positive for all the *E. coli* confirmatory tests. One of the isolates (WR35) was colourless on MLG agar and had a

red slant during the TSI test. This isolate also tested positive for the PCR markers of *E. coli*. All of the isolates obtained during 2011 and 2012 were Gram negative during the Gram staining test.

The results presented in this part of the study demonstrated that there are shortcomings in the *E. coli* confirmation tests. There were contradictory results for the MLG agar, TSI and PCR markers for some of the samples. The putative *E. coli* isolates obtained from the Colilert test more consistently tested positive with the biochemical and molecular markers.

Table 11: Confirmation tests performed on colonies isolated from m-FC agar during 2011 and 2012.

	Source & colony number	Colour of colony on m-Fc agar	Colour of colony on MLG agar	Triple sugar iron agar results			Molecular confirmation results			
				Slant	Butt	Gas production	***mdh***	***lacZ***	***uidA***	**16S**
	E. coli	Blue	Green	Yellow	Yellow	+	+	+	+	+
July 2011	HR1	Blue	Green	Yellow	Yellow	+	+	+	+	+
	PR2	Blue	Green	Yellow	Yellow	+	+	+	+	+
	PR3	Blue	Green	Red	Yellow	-	+	+	+	+
	JR4	Blue centre	Colourless	Red	Yellow	-	+	+	+	+
	WR5	Blue centre	Colourless	Red	Yellow	-	+	+	+	+
October 2011	HR6	Blue	Yellow	Yellow	Yellow	+	+	+	+	+
	HR7	Blue	Green	Yellow	Yellow	-	+	+	+	+
	PR8	Blue	Green	Yellow	Yellow	+	+	+	+	+
	PR9	Blue centre	Green	Yellow	Yellow	+	+	+	+	+
	PR10	Blue	Yellow	Yellow	Yellow	+	+	+	+	+
	JR11	Blue	Green	Yellow	Yellow	+	+	+	+	+
	JR12	Blue	Yellow	Yellow	Yellow	+	+	-	+	+
June 2012	HR13	Blue centre	Colourless	Yellow	Yellow	+	-	-	+	+
	HR14	Blue	Yellow	Yellow	Yellow	+	+	+	+	+
	HR15	Blue	Green	Yellow	Yellow	+	+	+	+	+
	HR16	Blue	Green	Yellow	Yellow	+	+	+	+	+
	PR17	Blue	Green	Yellow	Yellow	+	+	+	+	+
	PR18	Blue centre	Colourless	Yellow	Yellow	+	+	+	+	+
	JR19	Blue centre	Colourless	Red	Yellow	+	-	-	+	+
	JR20	Blue	Colourless	Red	Yellow	-	-	-	+	+
	JR21	Blue	Green	Yellow	Yellow	+	+	+	+	+
	JR22	Blue	Green	Yellow	Yellow	+	+	+	+	+
	WR23	White	Colourless	Red	Yellow	+	+	+	+	+
	WR24	Blue	Green	Yellow	Yellow	+	+	+	+	+
	WR25	Blue centre	Yellow	Yellow	Yellow	+	+	-	+	+

HR: Hartswater raw water; **PR**: Pampierstad raw water; **JR**: Jan Kempdorp raw water; **WR**: Warrenton raw water.

+: Positive result.

-: Negative result.

Table 12: Confirmation tests performed on colonies isolated from Colilert during March 2012.

	Source & colony number	**Colilert Results** (*E. coli)*	**Colour of colony on MLG agar**	**Triple sugar iron agar results**			**Molecular confirmation results**			
				Slant	Butt	Gas production	***mdh***	***lacZ***	***uidA***	**16S**
March 2012	HR26	Positive	Green	Yellow	Yellow	+	+	+	+	+
	HR27	Negative	Yellow	Yellow	Yellow	+	+	-	+	+
	PR28	Positive	Green	Yellow	Yellow	+	+	+	+	+
	PR29	Negative	Yellow	Yellow	Yellow	+	+	-	+	+
	JR30	Positive	Green	Yellow	Yellow	+	+	+	+	+
	JR31	Negative	Yellow	Yellow	Yellow	+	+	+	+	+
	JD*32	Positive	Green	Yellow	Yellow	+	+	+	+	+
	JD*33	Positive	Green	Yellow	Yellow	+	+	+	+	+
	WR34	Positive	Green	Yellow	Yellow	+	+	+	+	+
	WR35	Negative	Colourless	Red	Yellow	+	+	+	+	+

HR: Hartswater raw water; **PR**: Pampierstad raw water; **JR**: Jan Kempdorp raw water; **WR**: Warrenton raw water.
JD*: Jan Kempdorp drinking water.
+: Positive result.
- : Negative result.

3.4 PCR CONFIRMATION RESULTS OF *ESCHERICHIA COLI*

In this section the PCR results for the confirmation of *E. coli* for 2011 are given. Section 3.4.1 contains the DNA isolation results and section 3.4.2 contains the DNA amplification results. The molecular results for 2012 are given in Appendix B.

3.4.1 DNA isolation results

DNA isolation of the pure colonies was performed as described in section 2.6.1. Figure 5 is a negative image of an ethidium bromide 1.5% (w/v) agarose gel illustrating the quality and quantity of the DNA isolated from the July 2011 isolates. The quantity of isolated DNA was low for some of the samples, as seen in lanes 8 to 12. Samples 5 and 6 had a high quantity of isolated DNA. No fragmentation or RNA was observed. Similar results were obtained for the DNA isolated from the isolates from the rest of the sampling period.

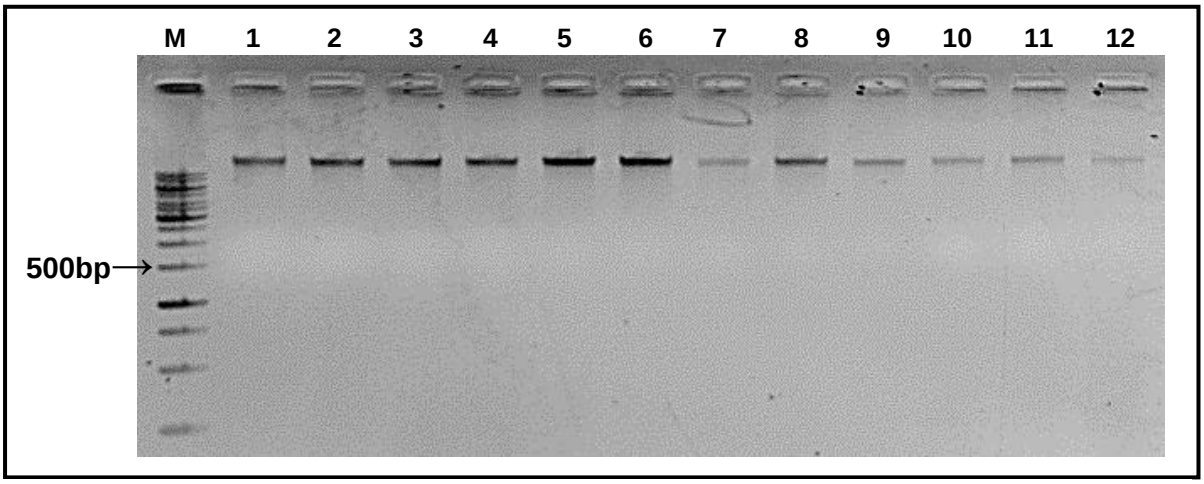

Figure 4: Negative image of the 1.5% (w/v) agarose gel indicating the DNA isolated from the pure colonies. Lane 1 contains the 1 Kb molecular marker (M). Lane 2 to lane 13 indicates the isolated DNA.

These results were confirmed by determining the concentration and purity (A_{260nm}:A_{280nm} ratios) of the isolated DNA by using a NanoDropTM 1000 Spectrophotometer. The quality (A_{260nm}:A_{280nm} ratios) of the DNA isolated during 2011 varied between 1.55 and 2.11 and during 2012 it varied between 1.66 and 2.17. DNA with values between 1.7 and 1.9 are considered to be ideal for PCR. The concentrations of the isolated DNA during 2011 varied between 5.77 ng/µl and 45.59 ng/µl and during 2012 between 15.94 ng/µl and 83.90 ng/µl. The quality and quantity values obtained in the present study

were all suitable for PCR. However, samples with the highest purity and concentrations were used for DNA amplification.

3.4.2 DNA amplification results

Gel electrophoresis was performed with the PCR products for 2011 and 2012. Figure 5 to figure 7 are negative images of the ethidium bromide stained 1.5% (w/v) agarose gels for the multiplex and two monoplex PCR products for 2011. The multiplex PCR amplified fragments of the *mdh* and *lacZ* genes are presented in figure 5. If one or both of these gene fragments are amplified, it indicates the isolate is *E. coli*. The monoplex PCRs amplified fragments of the *uidA* and 16S rDNA genes are presented in figure 6 and 7 respectively. Amplification of the *uidA* gene fragment is also an indication that the isolate is *E. coli*. Amplification of the 16S gene is an indication that the DNA is amplifiable.

In figure 5 lane 2 is the positive control. Bands of the expected fragment sizes for the *mdh* and *lacZ* primer sets were detected in lanes 3 (HR1) to 13 (JR11). Lane 14 (JR12) only amplified the *mdh* fragment. Two fragments, one for *lacZ* (228 bp) and one for *mdh* (301 bp) are visible. All the lanes 3 to 13 were positive for both *E. coli* markers. Very faint bands were detected in lane 5 (PR3), 6 (JR4), 7 (WR5) and 14 (JR12). This could be due to the low concentration of DNA present in the samples and that this yielded a low concentration of amplification products. These were not overflows from adjacent wells and repeat PCRs gave similar results.

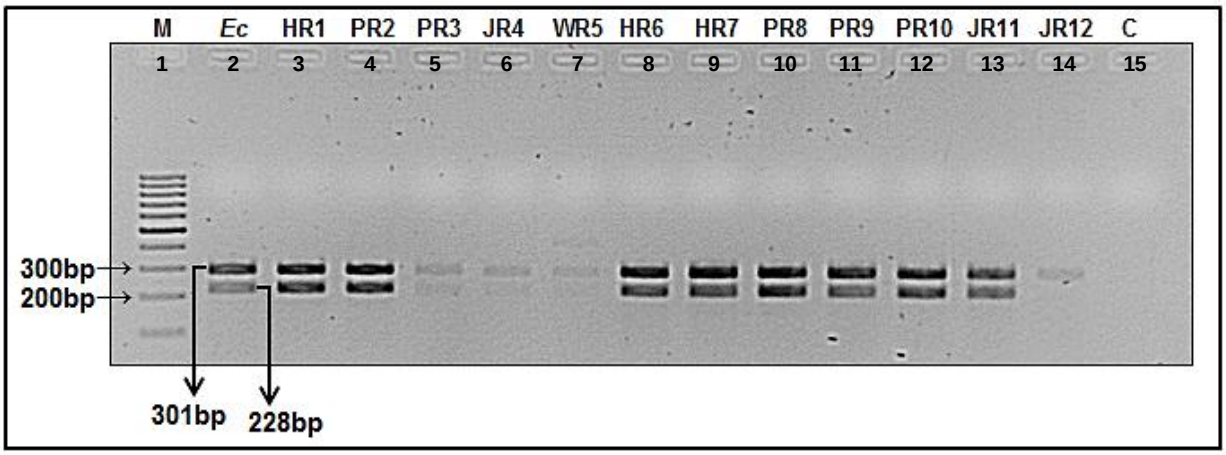

Figure 5: Negative image of the 1.5% (w/v) agarose gel indicating the multiplex PCR results for July and October 2011. The first lane contains the 100 bp molecular marker (M). The second lane contains the positive control (*E. coli* - ATCC 10536). Lane 3 to lane 14 indicates the PCR products of the various isolates. The last lane (15) contains the no template control. The *mdh* gene has a fragment size of 301 bp and the *lacZ* gene 228 bp.

Figure 6 is a negative image of the 1.5% (w/v) agarose gel indicating the *uidA* monoplex PCR results for July and October 2011. Bands of the expected fragment size (380 bp) for the *uidA* primer set were observed for all the isolates. This result indicates that these isolates are all *E. coli*.

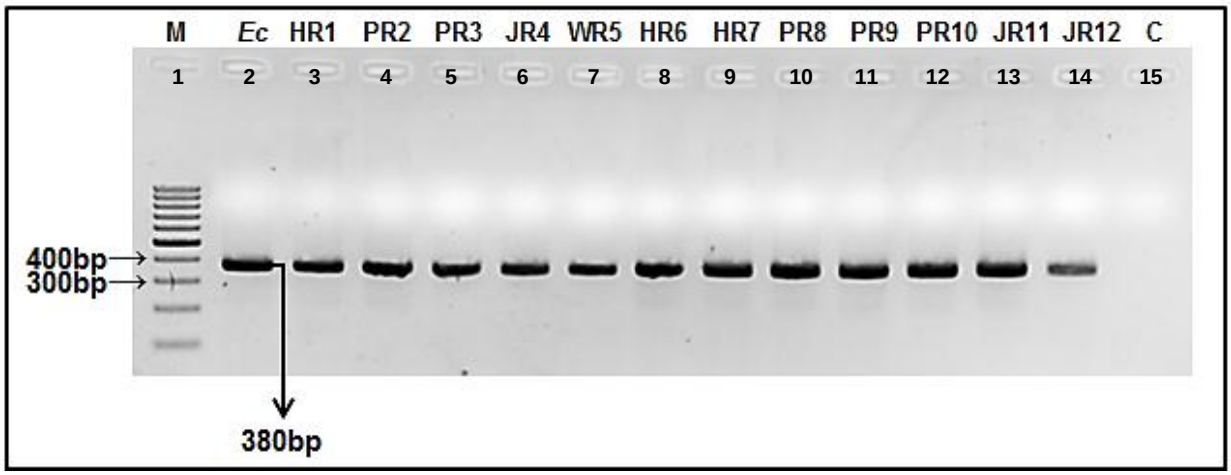

Figure 6: Negative image of the 1.5% (w/v) agarose gel indicating the *uidA* monoplex PCR results for July and October 2011. The first lane contains the 100 bp molecular marker (M). The second lane contains the positive control (*E. coli* - ATCC 10536). Lane 3 to lane 14 indicates the PCR products of the various isolates. The last lane (15) contains the no template control. The *uidA* gene has a fragment size of 380 bp.

Figure 7 is a negative image indicating the 16S rDNA monoplex PCR results for isolates from the July and October 2011 sampling periods. A band of the expected fragment size for the 16S rDNA primer set was detected in all the samples. This PCR was included to demonstrate that the DNA was amplifiable

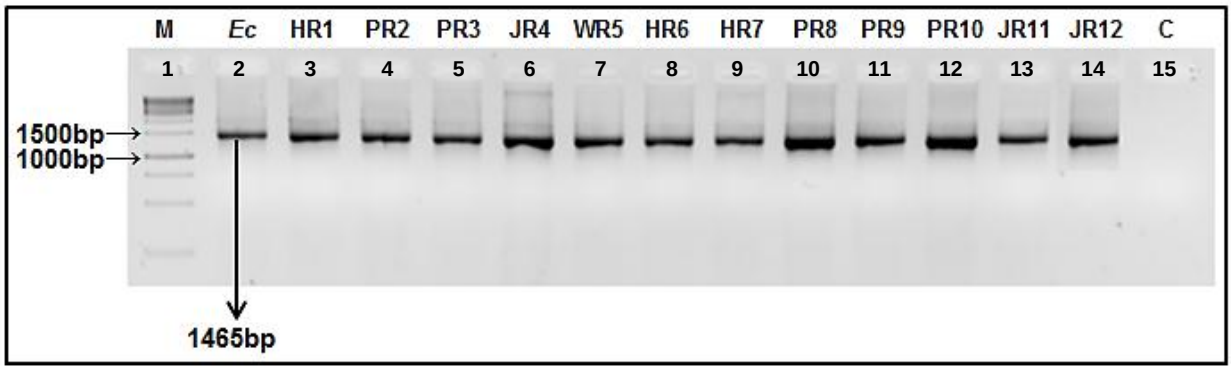

Figure 7: Negative image of the 1.5% (w/v) agarose gel indicating the 16S rDNA monoplex PCR results for July and October 2011. The first lane contains the 1 Kb molecular marker (M). The second lane contains the positive control (*E. coli* - ATCC 10536 Lane 3 to lane 14 indicates the PCR products of the various samples. The last lane (15) contains the no template control. The 16S rDNA gene has a fragment size of 1465 bp.

Similar PCR results were obtained for all the isolates from the 2012 sampling periods. The molecular results are summarized in table 9 and 10. Where the PCR product was formed a positive result was recorded irrespective of band intensity.

3.5 SEQUENCING RESULTS

Only ten samples of the PCR products of the *E. coli* marker bands were selected and sequenced. For sequencing purposes the *mdh* and *lacZ* bands were amplified in monoplex PCRs. Sequences were analysed at the NWU laboratories using a Genetic Analyzer 3130 (Applied Biosystems Inc., California, US). BLAST (Basic Local Alignment Search Tool) searches were performed (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to compare the obtained sequences to the GenBank database of sequences.

Table 13 contains the BLAST results for the selected isolates and the corresponding genes that were sequenced. The sequences of the *mdh* gene fragment of JR12 indicated that this isolate was *Enterobacter cloacae*. This isolate was blue of m-Fc and all the TSI results as well as *uidA* gene PCR results were similar to *E. coli* results. The isolate was negative for *lacZ* PCR and was yellow on MLG agar. All the other sequences matched with *E. coli* sequences. High similarity percentages and very low E-values were obtained for the sequence BLAST searches. This is an indication that the sequencing results are accurate. Isolates HR26, HR1, JR12 and HR13 were identified as *Escherichia coli* O104:H4. This is a pathogenic strain and its presence in the water is cause for concern and should be further explored.

Table 13: Sequence results for confirmation of *Escherichia coli*.

Isolate on electrophoresis gel	GenBank ID	Nucleotides used	E-value	% Similarity
JR12 (*mdh* - figure 5)	*Enterobacter cloacae*	156	2e-70	99%
HR26 (*mdh* - figure 10)	*Escherichia coli* O104:H4	166	2e-78	100%
HR13 (*mdh* - figure 13)	*Escherichia coli*	149	3e-69	100%
HR14 (*mdh* - figure 13)	*Escherichia coli*	201	1e-94	99%
HR13 (*lacZ* - figure 13)	*Escherichia coli*	118	6e-51	99%
HR14 (*lacZ* - figure 13)	*Escherichia coli*	105	1e-45	100%
HR1 (*uidA* - figure 6)	*Escherichia coli* O104:H4	204	5e-99	100%
JR12 (*uidA* - figure 6)	*Escherichia coli* O104:H4	280	1e-139	100%
HR13 (*uidA* - figure 14)	*Escherichia coli* O104:H4	203	2e-98	100%
HR14 (*uidA* - figure 14)	*Escherichia coli*	280	1e-139	100%

3.6 STATISTICAL ANALYSIS RESULTS

Redundancy analysis (RDA) was used to determine the correlation between the physico-chemical and microbiological variables during 2011 and 2012, using Canoco software version 4.5 (Ter Braak, 1990). Mean biplots were constructed of the variables (figure 8 and figure 9) and correlations between the variables were demonstrated by the angle between them on the biplots. The smaller the angle between the variables, the closer the correlation.

Figure 8 is a RDA biplot indicating the correlation between the microbiological and physico-chemical variables in the raw water (red vectors) and the physico-chemical and microbiological variables in the drinking water (blue vectors). Average values of the raw and drinking water were used to create the biplot. The main trend which can be seen in figure 8 is that the quality of the raw water has an impact on the quality of the drinking water. The biplot shows that nitrate in the raw water has a close correlation with nitrate in the drinking water. Sulphate in the raw water correlates with sulphide and anaerobic bacteria in the drinking water. This suggests the possibility of biofilm activity in the distribution system. Heterotrophic plate count bacteria in the raw water correlate with HPC bacteria in the drinking water. The same trend can be seen with faecal streptococci and total coliforms.

Figure 8 also illustrates that nitrite in the drinking water correlates with the anaerobic bacteria in the drinking water. This also suggests the possibility of biofilm activity in the distribution system. Free chlorine in the drinking water does not correlate with fungi, HPC bacteria, anaerobic bacteria, faecal streptococci, faecal coliforms and *P. aeruginosa* in the drinking water, but correlates with total coliforms. Faecal coliforms, faecal streptococci and *P. aeruginosa* in the drinking water correlate with the drinking water temperature.

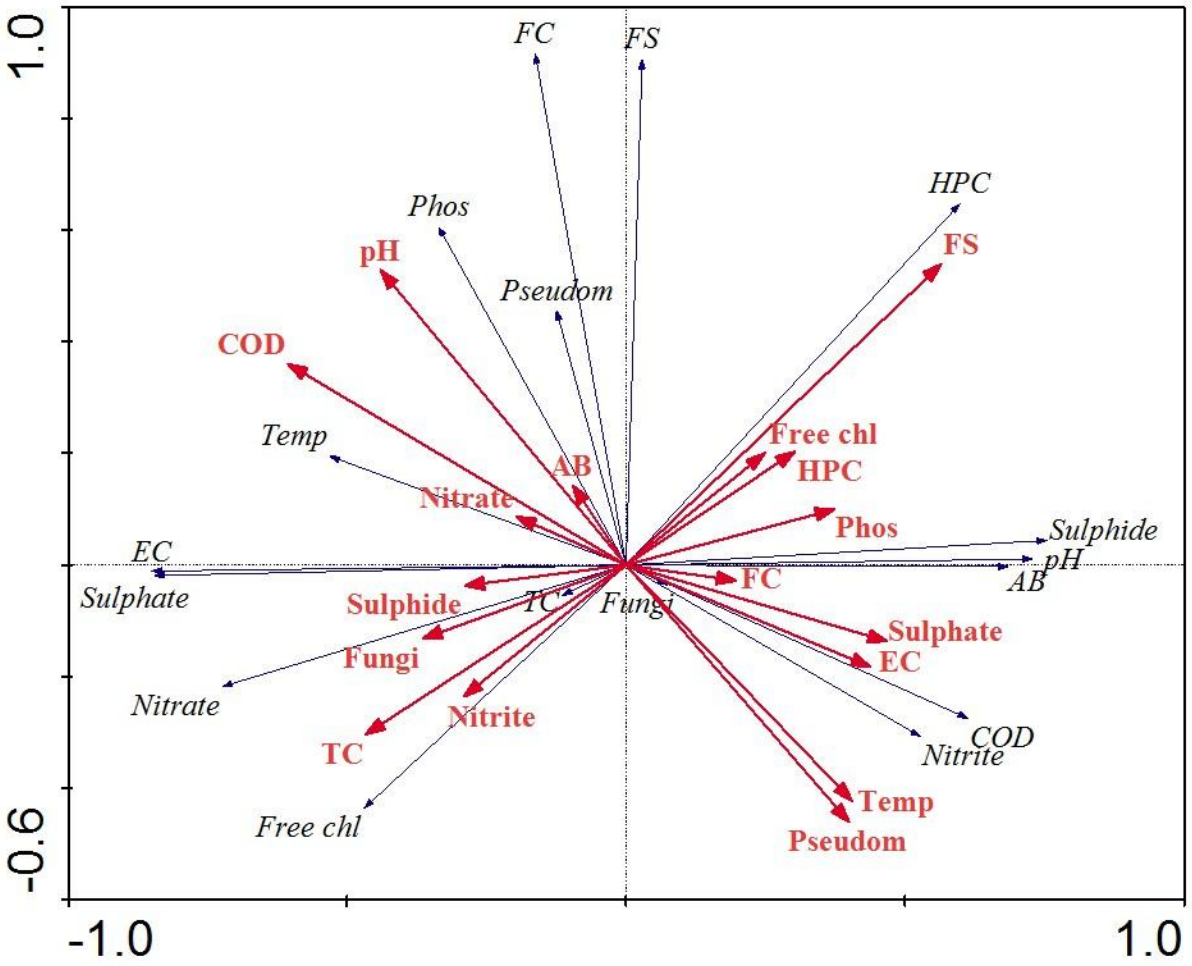

Figure 8: RDA biplot indicating the correlation between the physico-chemical and microbiological variables in the raw water (red vectors) and the physico-chemical and microbiological variables in the drinking water (blue vectors). Temp: temperature; Free chl: free chlorine; EC: electrical conductivity; Phos: Phosphate; COD: chemical oxygen demand; AB: anaerobic bacteria; TC: total coliforms; FC: faecal coliforms; FS: faecal streptococci; HPC: heterotrophic plate count bacteria and Pseudom: *Pseudomonas aeruginosa*.

Figure 9 is a RDA biplot indicating the correlation between the physico-chemical variables (red vectors) and microbiological variables (blue vectors) in the drinking water. In this case the measurements at the three points in the distribution system were used to create the biplot. The biplot shows that faecal coliforms, faecal streptococci and fungi correlate with phosphate. HPC bacteria and anaerobic bacteria correlate with sulphide and nitrite. This further suggests the possibility of biofilm activity in the distribution system. *P. aeruginosa* and total coliforms correlate with sulphate and nitrate. Faecal coliforms, fungi and *P. aeruginosa* showed a correlation with temperature.

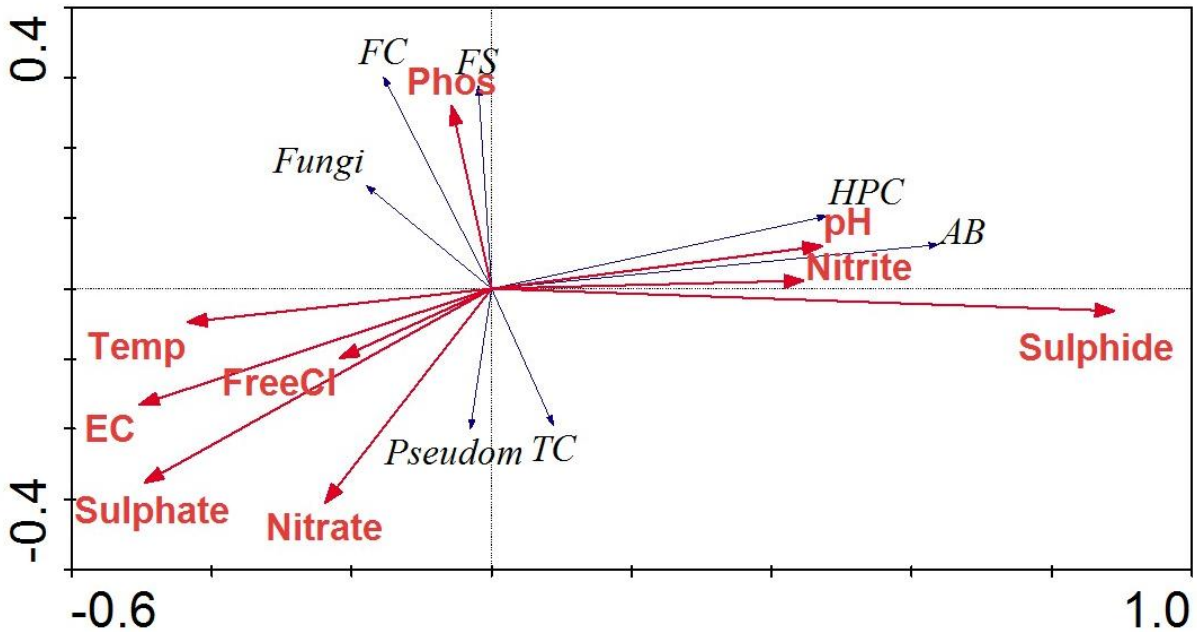

Figure 9: RDA biplot indicating the correlation between the physico-chemical variables (red vectors) and microbiological variables (blue vectors) in the in the drinking water. Temp: temperature; Free chl: free chlorine; EC: electrical conductivity; Phos: Phosphate; COD: chemical oxygen demand; AB: anaerobic bacteria; TC: total coliforms; FC: faecal coliforms; FS: faecal streptococci; HPC: heterotrophic plate count bacteria and Pseudom: *Pseudomonas aeruginosa*.

3.7 SUMMARY OF RESULTS

The physico-chemical results for 2011 and 2012 did not raise any concern, except for the high nitrite levels detected in the drinking water of all four distribution systems during March and October which did not comply with the SANS 241 (2011) standard. It was also noted that the EC, sulphate, nitrate, phosphorous and salinity levels increased gradually from 2011 to 2012. The physico-chemical results of Pampierstad were the most acceptable during 2011 and March 2012 and Warrenton had the most acceptable physico-chemical results during June 2012.

Microbiological results improved from 2011 and 2012, except the *Clostridia* results and the high level of faecal coliforms (191 CFU/100ml) detected in the drinking water of Jan Kempdorp during July 2012. Biochemical and molecular tests were performed on two of the faecal coliform isolates obtained from the drinking water of Jan Kempdorp (JD*32 & JD*33 - table 12) and were identified as *E. coli.* This is concerning, because during 2011 the pathogen *Escherichia coli* O104:H4 was detected in the raw water of Jan

Kempdorp. If the treatment process of Jan Kempdorp does not effectively remove all the faecal coliforms from the raw water, the possibility of this pathogenic strain or other *E. coli* strains leaching into the distribution system increases. Warrenton had the most acceptable microbiological results during 2011 and June 2012 and Pampierstad had the most acceptable microbiological results during March 2012.

Even though the microbiological quality of the drinking water of most of the distribution systems complied with SANS 241 (2011) standard during 2011 and 2012, except for HPC bacteria in some of the distribution systems, the presence of high levels of other bacteria in the drinking water such as *Clostridia*, *P. aeruginosa* and fungi is cause for concern. Statistical analysis indicated that some of the physico-chemical parameters had an impact on the microbiological parameters (figure 9) and that the quality of the raw water does have an impact on the quality of the drinking water (figure 8).

CHAPTER 4
DISCUSSION

Source water is becoming a scarce resource in South Africa and with developments along our important rivers it can be expected to impact water quality negatively (Van der Laan *et al.*, 2012). One of the most important requirements for domestic water is that the water should be safe to drink (Schutte, 2006; Momba *et al.*, 2009). Studies have shown that water purification plants in South Africa may not always produce the quality and quantity of drinking water they are designed for (Momba *et al.*, 2006). Mortality rates due to waterborne pathogens have risen (Exner *et al.*, 2005). To determine if the water provided to communities is safe to drink the physico-chemical and microbiological analysis of water must be performed.

During this study the quality of household water in the Vaalharts irrigation scheme was assessed. The main residential areas where water samples were taken include the distribution systems of Hartswater, Pampierstad, Jan Kempdorp and Warrenton. Sampling was done over two seasons during 2011 as well as during 2012. The water treatment plants of these four towns derive water from the Vaal River, purify the water and distribute it to the communities. The quality of the water in the Vaal River that is channelled into the Vaalharts irrigation scheme has been deteriorating over the past few years (Le Roux *et al*., 2007). One of the objectives of this study was to perform statistical analysis to determine if there is any correlation between the water prior to purification (raw water) and the water after purification (drinking water). Physico-chemical and microbiological analysis of the raw and drinking water of all four areas were performed.

Results of the physico-chemical analysis during 2011 and 2012 are discussed in section 4.1 followed by the discussion of the microbiological results during 2011 and 2012 in section 4.2. In section 4.3 and 4.4 the biochemical and molecular confirmation tests for *E. coli* are discussed. This is followed by section 4.5 which discusses the statistical relevance of the microbiological and physico-chemical results.

4.1 PHYSICO-CHEMICAL PARAMETERS

The physico-chemical results obtained during 2011 and 2012 are discussed in this section. SANS 241:2011 was used as a guide to assess the physico-chemical quality of the drinking water.

4.1.1 Free chlorine

The low free chlorine levels (0.03 mg/L to 0.08 mg/L) in the drinking water of Hartswater and Jan Kempdorp during July and October 2011 raised concern (table 5). The same pattern was observed during March and June 2012 (table 7) for Jan Kempdorp (0.04 mg/L and 0.06 mg/L respectively). Disinfection at these low levels may be compromised with serious risk of infection if the raw water source is contaminated with pathogens (DWAF, 1998). These low levels may also cause regrowth of microorganisms in the distribution system (DiGiano *et al*., 2000).

Hartswater had a very high free chlorine level during June 2012 (1.39 mg/L) (table 7). Free chlorine levels above 0.8 mg/L may cause nausea and mucous membrane irritation as well as unpleasant odour and taste of the drinking water (DWAF, 1998). High chlorine levels may also form disinfection by-products which may cause health problems as well as impact the aesthetic quality of the drinking water (WHO, 2011).

Even though the free chlorine levels of all four areas complied with the SANS 241 (2011) standard of ≤5 mg/L in drinking water during 2011 and 2012, too low and high levels of free chlorine may cause health and aesthetic problems. Sufficient levels of free chlorine for disinfection are between 0.2 - 0.6 mg/L (DWAF, 1998; WHO, 2011). A study done by Tsai (2006) indicated that bulk bacteria levels and biofilm formation decreased in water at free chlorine levels of 0.26 mg/L and 0.57 mg/L. Dosage levels of 2 mg/L ensure a free chlorine level of 0.2 - 0.5 mg/L at point of delivery (WHO, 2011). The free chlorine levels of the Warrenton and Pampierstad distribution systems during 2011 and 2012 were thus sufficient.

4.1.2 Electrical conductivity (EC)

During 2011 (table 5) concern was raised over the increase in EC levels from the first sampling period (July) to the second sampling period (October). The same trend was seen during 2012 where EC levels were higher in June than March (table 7). This could

be due to sampling being done during or after the raining season which causes EC levels to increase due to agricultural runoff upstream from the area that contains ionic substances from fertilizers. Vaalharts is an agricultural region (Van Vuuren, 2009) and the rainy season of the region is from October to March, with little rain during the winter months (Verwey & Vermeulen, 2011). A study done by Phiri *et al.* (2005) indicated that the EC levels were higher during the rainy season due to ground water and surface runoff from surrounding farms.

Not only did the EC levels increase during the two seasons of 2011 and 2012, but an overall increase of EC levels was noted from 2011 to 2012. The highest EC level in raw water during 2011 was 44.80 mS/m and drinking water 45.50 mS/m whereas during 2012 the highest EC in raw water was 63.40 mS/m and drinking water 64.06 mS/m. The SANS 241 (2011) standard for EC in drinking water is ≤170 mS/m. At this level no aesthetic effects can be expected. However, at levels above 150 mS/m corrosion and scaling of the plumbing material and appliances may increase (DWAF, 1996b). The EC levels in the drinking water of all four distribution systems complied with the SANS 241 (2011) standard during 2011 and 2012 and are thus satisfactory.

4.1.3 pH

During 2011 and 2012 the pH levels of all four distribution systems did not raise any concern. The levels stayed neutral to slightly alkaline during 2011 and 2012. The pH of most unpolluted water lies between 6.5 – 8.5 (DWAF, 1998). pH is an important operational water quality parameter (WHO, 2011). To ensure effective disinfection, the pH levels must be controlled when disinfection products are added (WHO, 2011). In section 2.3 the water treatment systems of the four sites are described. From table 3 it is evident that only Hartswater and Jan Kempdorp control the pH after the addition of disinfection materials. This means that the disinfection process of the Pampierstad and Warrenton distribution systems may be compromised. All four distribution systems complied with the SANS 241 (2011) pH level of ≥5 to ≤9.7 pH units for drinking water during 2011 and 2012. The pH levels for 2011 and 2012 were thus satisfactory.

4.1.4 Sulphate

Even though there was an increase in sulphate levels during the two seasons of 2011 and 2012, an increase of about 30 mg/L from 2011 to 2012 was also noted. The highest

sulphate level in raw water during 2011 was 55.50 mg/L and in drinking water 55.33 mg/L. However during October the highest level of sulphate in raw water was 79.50 mg/L and in drinking water 83.50 mg/L. Sulphate contributes to the mineral content of drinking water due to its occurrence in various natural minerals (WHO, 2004a). Treatment may also cause the mean sulphate levels in municipal drinking water supplies to increase (WHO, 2004a). This may explain the increase of ±30 mg/L in sulphate levels for all four distribution systems from 2011 to 2012. If drinking water with sulphate levels exceeding 600mg/L is consumed it may cause health problems (WHO, 2004b). The sulphate levels of all four distribution systems were below 100 mg/L meaning it will not cause any health or aesthetic problems (DWAF, 1998) and complied with the SANS 241 (2011) level of ≤500 mg/L for drinking water.

4.1.5 Sulphide

Sulphide levels varied marginally between the seasons during 2011 and 2012. No sulphide was detected in the raw water of Warrenton during October 2011, but was detected in the drinking water (table 6). The same pattern was seen during March 2012 (table 8) where no sulphide was detected in the raw water of Hartswater, but was detected in the drinking water. This may also be due to biofilm formation in the distribution system. Sulphate reducing bacteria are anaerobic bacteria which utilizes oxidised sulphur compounds as electron acceptors to produce sulphide (Gibson, 1990; Lopes *et al.*, 2009). A study by Kristiana *et al.* (2010) concluded that the sulphide in drinking water distribution systems is associated with sulphate reduction which is an indication of the presence of sulphate reducing bacteria. The presence of sulphide and polysulphides in drinking water distribution systems is of concern. This is because it may cause taste and odour problems that are due to the reaction with metal ions to form insoluble metal sulphides (Kristiana *et al.*, 2010). Even though there is no SANS 241 (2011) level for sulphide in drinking water, detection of sulphide in drinking water distribution systems may indicate the presence of biofilms and sulphate reducing bacteria.

4.1.6 Nitrate

Nitrate levels were low during 2011 and 2012. The highest level of nitrate in the raw water during 2011 was 1.85 mg/L (table 5) and during 2012 0.45 mg/L (table 7). The highest nitrate level in drinking water during 2011 was 0.58 mg/L (table 5) and during

2012 0.97 mg/L (table 7). Chang *et al.* (2010) stated that wastewaters and agricultural and urban runoff are natural sources contributing to nitrate in water.

Nitrates were not detected in the raw water of Hartswater and Pampierstad during July 2011, but were detected in the drinking water (table 5). The same pattern was seen during March and June 2012 (table 7) where no nitrate was detected in the raw water of Hartswater and Warrenton, but was detected in the drinking water. This may be due to biofilms forming in the distribution system containing nitrifying bacteria. Nitrifying bacteria oxidise ammonium via nitrite to nitrate (Lipponen *et al.*, 2004). Unfortunately the ammonium levels in the raw and drinking water were not measured during this study therefore the presence of nitrifying bacteria is only a speculation. A study by Lipponen *et al.* (2004) confirmed the development of nitrifying bacteria in biofilms of drinking water distribution systems. The nitrate levels complied with the SANS 241 (2011) level of ≤11 mg/L for drinking water for all four distribution systems during 2011 and 2012. Nitrate itself is not toxic, but microbial reduction of nitrate to nitrite in the intestine occurs and the nitrite is toxic (Adam, 1980; WHO, 2007). Even though nitrate present in the drinking water may be due to biofilm formation, some of the nitrate in the raw water may have leached through some of the treatment systems and into the distribution networks.

4.1.7 Nitrite

Nitrite levels were very high and the levels for all the distribution systems exceeded the SANS 241 (2011) level of ≤0.9 mg/L for drinking water during 2011 and 2012, except for Pampierstad during March 2012 (0.83 mg/L) (table 7). Alemdar *et al*. (2009) investigated the nitrate and nitrite levels of drinking water in Bitlis Province, Turkey. Results from their study also indicated high levels of nitrite in the tap water with average levels of 1.32±0.09 mg/L.

During 2011 (table 5) no nitrite were detected in the raw water of Jan Kempdorp and Warrenton, but were detected in the drinking water. The same pattern was seen during 2012 (table 7) where no nitrates were detected in the raw water of Hartswater during March and June, but were detected in the drinking water. This may be due to biofilm formation in the distribution system containing *Nitrosomonas* bacteria. *Nitrosomonas* bacteria can form nitrite in galvanized steel distribution pipes during stagnation of nitrogen-containing and oxygen-poor drinking water (WHO, 2007; WHO, 2011). Short

term exposure to nitrite levels of >3 mg/L may cause methaemoglobinaemia in bottle-fed infants (WHO, 2011). Therefore the high level of nitrite in the drinking water of Hartswater during July 2011 (4.50 mg/L) is cause for concern.

4.1.8 Chemical Oxygen Demand (COD)

During 2011 (table 6) COD levels decreased from July to March, but a slight increase in COD levels from October 2011 to June 2012 was noted. Even though there is no SANS 241 (2011) standard for COD in drinking water, the South African Water Quality Guidelines (DWAF, 1996a) indicates that COD levels of 0 – 30 mg/L are acceptable for category 3 industrial processes, which includes water for domestic use. Mamba *et al.* (2009) monitored the natural organic matter and disinfection by-products of two South African water treatment plants, Midvaal in Stilfontein and Sedibeng at Bothaville. Both these plants are upstream from the present study, but they also obtain their water from the Vaal River (Mamba *et al.*, 2009). Midvaal had COD levels of 24.26 mg/L for raw water and 19.04 mg/L in the distribution network. Sedibeng had higher COD levels in the raw water (110.73 mg/L), but lower in their distribution network (13.28 mg/L). High levels of COD and BOD (Biological Oxygen Demand) is usually an indication of serious water pollution (Yin & Tu, 1990; Kawabe & Kawabe, 1997; Yin *et al.*, 2011), but the COD levels in the water of all four distribution system during the present study were acceptable during 2011 and 2012.

4.1.9 Phosphorous

Phosphorus in water can be caused by nonpoint pollution such as agricultural (excess fertilizers) and urban activities (Novotny and Olem, 1994 cited by Carpenter *et al*., 1998) and with Vaalharts being an agricultural region (Van Vuuren, 2009) it is possible for phosphorous to be present in the water. During 2011 (table 6) phosphorous levels were higher after purification for all four distribution systems. During 2012 (table 8) some of the distribution systems had an increase in phosphorous levels after purification, while other distribution systems had a decrease. The highest level of phosphorous in drinking water during 2011 was 0.81 mg/L and during 2012 1.36 mg/L. There is no SANS 241 (2011) standard for phosphorous in drinking water, but a study by Lehtola *et al.* (2002) indicated that a slight increase of phosphorous in the water increased microbial growth. This promotes biofilm formation in water distribution systems (Fang *et al.*, 2009). The

presence of phosphorous in the raw and drinking water of all four distribution systems during 2011 and 2012 is thus cause for concern and may encourage biofilm formation.

4.1.10 Salinity

Not only did salinity levels increase in the raw and drinking water for all four distribution systems from the first to the second sampling trip during 2011 and 2012, but an overall increase in salinity levels from 2011 to 2012 was noted. The same pattern was seen with the electrical conductivity levels (section 4.1.2). During 2011 the highest level of salinity in the raw water was 222 ppm (table 6) and during 2012 it was 312 ppm (table 8). The highest level of salinity in drinking water during 2011 was 227 ppm and during 2012 it was 315 ppm. Salinity is a measurement of the amount of TDS present in the water (CSIR, 2010), thus an increase in salinity would mean an increase in TDS or EC levels. Appendix A indicates the TDS levels for 2011 and 2012. It can be seen that the TDS levels also increased from the first to the second sampling trip during 2011 and 2012. The increase in salinity levels may be due to agricultural runoff during the rainy season (see section 4.1.2). There is no SANS 241 (2011) standard for salinity in drinking water, but with the EC and TDS levels being low and complying with SANS 241 (2011) standards, it could be concluded that the salinity levels are satisfactory.

4.1.11 Temperature

During 2011 the first sampling trip was during the colder season of the year (July) and the second sampling trip was during the warmer season (October). During July temperature varied between 10.4°C and 13.1°C and during October between 21.8°C and 25.7°C (table 6). During 2012 the first sampling trip was during the warmer season of the year (March) and the second sampling trip during the colder season (June). Temperature varied between 21.0°C and 23.7°C during March and during June between 12.5°C and 16.0°C (table 8).

Free chlorine, EC, sulphate, nitrate, nitrite (except Hartswater and Pampierstad), salinity and phosphorous (except Hartswater and Pampierstad) levels in the drinking water increased from the colder season (July) to the warmer season (October) during 2011 (table 5 & 6). A similar trend was seen during 2012 where free chlorine, EC, sulphate, nitrate, nitrite, COD (except Warrenton), salinity and phosphorous (except Jan Kempdorp and Warrenton) levels in drinking water increased from the first to the second

sampling trip. However, during 2012 the parameters increased from the warmer season (March) to the colder season (June) (table 7 & 8). This is an indication that the temperature did not have an impact on these physico-chemical parameters as it gradually increased from July 2011 to June 2012.

During 2011 higher levels of total coliforms, *Clostridia*, *Pseudomonas aeruginosa* and fungi were isolated in October compared to July 2011 (Table 9). During 2012 lower levels of faecal coliforms, total coliforms, heterotrophic bacteria and faecal streptococci were isolated during June compared to March (table 10). This is an indication that the change in temperature had an impact on these microbiological parameters. Temperature is the main factor which affects almost all physico-chemical equilibriums and biological reactions (Delpla *et al.*, 2009) and increases in temperature to more moderate levels will also result in increases in bacterial levels. Temperature influences the efficiency of the treatment plant, microbial growth rate, disinfection efficiency, corrosion rates and water velocity due to water usage during warmer seasons (LeChevallier *et al.*, 1996).

4.1.12 Metals

The metal concentrations were very low in the raw and drinking water for all four distribution systems during 2011 and 2012. Metal levels in the drinking water of all four distribution systems complied with the SANS 241 (2011) standards during 2011 and 2012. The only concern was the mercury levels in the raw and drinking water of Hartswater and Pampierstad during July 2011. Mercury levels in the drinking water of Hartswater (53.33 µg/L) and Pampierstad (18.50 µg/L) exceeded the SANS 241 (2011) level of ≤6 µg/L for drinking water. Some fungicides which are sprayed onto crops may contain copper and mercury and can be easily washed into rivers (Rand Water, 2012a). This may have an impact on the drinking water if not removed during the treatment process. This could be the reason for the high levels of mercury in the raw and drinking water during July 2011.

Overall the physico-chemical parameters that were measured did not raise any concern, except for nitrite levels which did not comply with the SANS 241:2011 level during 2011 and 2012. It was also noted that the EC, sulphate, nitrate, phosphorous and salinity levels increased gradually from 2011 to 2012. Various physico-chemical parameters

also indicated that biofilms were formed within the distribution systems that may affect operational conditions.

4.2 MICROBIOLOGICAL PARAMETERS

The microbiological parameters that were measured during 2011 and 2012 are discussed in this section. These include total and faecal coliforms, heterotrophic plate count bacteria, faecal streptococci, *Clostridia*, *Pseudomonas aeruginosa*, fungi and anaerobic bacteria.

4.2.1 Total coliforms & faecal coliforms

During 2011 and 2012 very high levels of total coliforms were detected in the raw water. The highest level of total coliforms in the raw water during 2011 was 1833 CFU/100ml. During 2012 some of the levels were in the TMTC region (Table 9 & 10). An increase in total coliform levels from July to October 2011 was noted in some of the distribution systems. A study by LeChevallier *et al.* (1996) indicated that an increase in temperature was responsible for an increase in total coliform growth in water. Hartswater had the highest total coliform level in drinking water during 2011 (14 CFU/100ml) (table 9). Although this level was low it did not comply with the SANS 241 (2011) standard of ≤10 CFU/100ml for drinking water. A similar trend was seen during 2012 where great concern was raised over the high level of total coliforms (256 CFU/100/ml) in the drinking water of Warrenton (table 10). Coliforms are found naturally in various environments, but when present in drinking water the levels should be very low (Rompré *et al.*, 2002). Their presence in drinking water can thus be seen as an indication of faecal pollution and possible deteriorating water quality (Rompré *et al.*, 2002). No total coliforms were detected in the drinking water of all four distribution systems during June 2012 (table 10) and thus complied with the SANS 241 (2011) standard. This is an indication that the treatment process was effective in removing total coliforms from the raw water.

Faecal coliforms are used as indicators of sewage effluent and run-off from farm animals in water (Kacar, 2011). During 2011 and 2012 low levels of faecal coliforms were detected in the raw water, except for Warrenton during July 2011 (100 CFU/100ml) and March 2012 (TMTC). These high levels of faecal coliforms in the raw water of Warrenton may be due to faecal contamination of the source water at that

specific time and close to the sampling point. No faecal coliforms were detected in the drinking water of all four distribution systems during July and October 2011 and thus complied with the SANS 241 (2011) standard of 0 CFU/100ml. This indicates that the purification process effectively removed the faecal coliforms from the raw water. During 2012, however, Jan Kempdorp had a level of 191 CFU/100ml in the drinking water during March (table 10). This does not comply with the SANS 241 (2011) standard. A study done by Pathak and Gopal (2008) indicated the presence of faecal coliforms in the drinking water of residential, commercial and industrial areas during the summer months. The presence of faecal coliforms in drinking water may be due to sewage discharge, insufficient treatment and/or an ineffective distribution system (Pathak & Gopal, 2008). The presence of the faecal coliforms at one sample point in the Jan Kempdorp drinking water distribution system could be due to contamination after treatment. This is possibly due to breakage in the distribution system and contamination because of this. No faecal coliforms were detected in the drinking water of all four distribution systems during June 2012 (table 10). This complies with the SANS 241 (2011) standard.

The faecal coliform results did not vary much to the results obtained from Midvaal, except for the raw water sample of Hartswater (our study - 30 CFU/10ml & Midvaal - 200 CFU/100ml) and Warrenton (our study - TMTC & Midvaal - 8 CFU/100ml). In a report presented by Jackson *et al.* (2002) it was stated that m-Endo and m-Fc media are not as sensitive as the Colilert test for the detection of total coliforms and *E. coli*. Hörman and Hänninen (2006) performed a study to compare different test methods for the detection of coliforms and *E. coli* in water samples. Their study indicated that the Colilert test had significantly higher counts of *E. coli* than m-Endo agar.

4.2.2 Heterotrophic plate count (HPC) bacteria

Heterotrophic plate count bacteria levels were very high in the raw water during 2011 and 2012, but lower in the drinking during 2012. However, all the HPC bacteria detected in the drinking water during 2012 did not comply with the SANS 241 (2011) standard of ≤1000 CFU/ml. Heterotrophic plate count bacteria are good indicators of the effectiveness of the water treatment process and cleanliness of the distribution system (WHO, 2011). Even though the HPC bacteria levels decreased after purification during 2011 and 2012 the high levels detected in the drinking water of some of the distribution

systems is of concern. This is due to the fact that a wide range of primary and secondary pathogens, which may cause health problems in humans are represented by heterotrophic bacteria (Chowdhury, 2012). The heterotrophic bacteria isolated during the study consisted of pigmented (yellow, orange, pink and red) and non-pigmented (white) colonies. Carter *et al.* (2000) isolated pigmented and non-pigmented colonies on R_2A agar from biofilms and bulk water from a distribution system. Reasoner *et al.* (1989) stated that pigmented colonies (especially yellow and orange colonies) in drinking water are an indication of a change in the water quality. They stated furthermore that some of the pigmented colonies are opportunistic pathogens. Tokajian *et al.* (2005) isolated pigmented HPC bacteria from drinking water samples of which some were opportunistic pathogens. The high levels of HPC bacteria in the drinking water during 2011 and 2012 may be due to insufficient levels of free chlorine in the drinking water during that time (section 4.1.1) and is cause for concern.

4.2.3 Faecal streptococci

Like faecal and total coliforms, faecal streptococci are also good indicators of faecal pollution (Kistemann *et al.*, 2002; Baghel *et al.*, 2005; Kacar, 2011). Faecal streptococci levels were low in the raw water during July and October 2011 (table 9) except for Jan Kempdorp during July which had the highest faecal streptococci level of 72 CFU/100ml. During July Hartswater was the only distribution system with faecal streptococci in their drinking water (2 CFU/100ml). The levels were extremely low which means the purification process effectively removed faecal streptococci from the raw water of the other distribution systems during July and October. Faecal streptococci levels were slightly higher in some of the distribution systems during 2012 than 2011. During June 2012 (table 10) Hartswater had a high level of faecal streptococci in the raw water (TMTC). Jan Kempdorp was the only distribution system with faecal streptococci in the drinking water during 2012 (5 CFU/100ml). No faecal streptococci were detected in the drinking water of all four distribution systems during June 2012.

The presence of faecal streptococci in the drinking water of Hartswater during July 2011 and Jan Kempdorp during March 2012 corresponds with total coliforms (July 2011) and faecal coliforms (March 2012) also present in the drinking water of these two distribution systems. Environmental waters and soils are not natural habitats for faecal streptococci, thus their presence in water is a good indication of faecal contamination (Mara & Horan,

2003). There is no SANS 241 (2011) standard for faecal streptococci in drinking water available, but the South African Water Quality Guidelines (DWAF, 1996a) indicated that faecal streptococci counts of >60 CFU/100ml may increase the risk of gastrointestinal effects. The levels of faecal streptococci in the drinking water of Hartswater (2011) and Jan Kempdorp (2012) were low and therefore no cause for concern.

4.2.4 *Clostridia*

Clostridia are useful indicators of faecal contamination in the water (Field & Samadpour, 2007). The detection of *Clostridium perfringens* in drinking water is an indication of possible failure in the filtration process of the water treatment plant (WHO, 2011). During October 2011 *Clostridia* were present in more distribution systems than July 2011 (table 9). During 2012 *Clostridia* were present in all of the raw water samples and most of the drinking water distribution systems (table 10).

A study done by Bisson and Cabelli (1980) indicated that *Clostridium perfringens* was present in drinking water where chlorination was applied. *Clostridium perfringens* can have serious health impacts on humans including gangrene and gastrointestinal disease (Petit *et al.*, 1999). The presence of *Clostridia* in the drinking water of all four distribution systems during October 2011 (table 9) and most of the distribution systems during 2012 (table 10) is an indication of possible failures in the treatment processes and is cause for concern.

4.2.5 *Pseudomonas aeruginosa*

Some heterotrophic bacteria are opportunistic pathogens such as *Pseudomonas aeruginosa* (WHO, 2011). *Pseudomonas* spp. levels increased from July to October 2011 (table 9) in the raw water of all four areas. During 2011 high levels of *P. aeruginosa* were detected in the raw and drinking water of the Hartswater distribution system. High levels of *P. aeruginosa* were detected in the raw water of all four areas during 2012 (TMTC) (table 10). *P. aeruginosa* levels in drinking water decreased from March to July, except for Hartswater. Okeyo *et al.* (2011) assessed the microbiological quality of drinking water of three water treatment plants in the Gauteng Province, South Africa. *Pseudomonas* was isolated in the raw water, after filtration, after chlorination of one of the treatment plants as well as in the distribution system. The water treatment plant had an average chlorine residual of 2.1 mg/L, but it was insufficient for the removal

of *Pseudomonas*. In the present study (section 4.1.1), low levels of free chlorine were measured at some of the distribution systems during 2011 and 2012, especially Hartswater. *P. aeruginosa* may thus be present in the drinking water due to insufficient disinfection.

No gastrointestinal diseases in humans have been associated with *Pseudomonas* through ingestion with drinking water (WHO, 2011). However, *Pseudomonas aeruginosa* is a waterborne opportunistic pathogen which may have impacts on human health, especially in immunocompromised populations (Wang *et al.*, 2012). There is no SANS 241 (2011) standard for *Pseudomonas* in drinking water. High levels of this bacterium in water may cause taste, odour and turbidity problems (WHO, 2011). The increase in *Pseudomonas* levels in the raw water of all four areas from 2011 (table 9) to 2012 (table 10) is cause for concern.

4.2.6 Fungi

Fungi have been found in various types of water, from raw and polluted water to purified water (Hageskal *et al*., 2009; WHO, 2011). An increase in fungi levels in the raw and drinking water from July to October 2011 was noted (table 9). During 2012 fungi levels decreased compared to 2011. Hedayati *et al.* (2011) isolated fungi from tap water in a hospital in Sari city, Iran. Twelve different genera were identified during their study. Higher levels of some genera were isolated during spring and summer time. In the present study higher levels of fungi were isolated in the raw and drinking water during the warmer season (October 2011) (table 9). Fungi produce secondary metabolites called mycotoxins of which some are very harmful (Paterson *et al.*, 2009). Agricultural runoff from contaminated crops may contain mycotoxins and fungi that can subsequently enter the water system (Bucheli *et al.*, 2008; Paterson *et al.*, 2009). Mycotoxins may damage the kidney, liver, and lungs as well as the nervous, endocrine and immune system (Paterson *et al.*, 2009). The presence of fungi in the drinking water during 2011 (table 9) is thus cause for concern.

4.2.7 Anaerobic bacteria

During July 2011 (table 9) anaerobic bacteria levels were very high in the raw water. The highest level of anaerobic bacteria in raw water was 86.83 x 10^3 CFU/ml. Hartswater was the only distribution system with anaerobic bacteria in the drinking

water during 2011 (75.37 x 10^3 CFU/ml). This is an indication of possible biofilm formation in the distribution system (Gomez-Alvarez *et al.*, 2012). During 2012 (table 10) anaerobic bacteria levels were higher in the raw water of some of the areas compared to 2011. The highest level of anaerobic bacteria in raw water during 2012 was 200 x 10^3 CFU/ml (Pampierstad). No anaerobic bacteria were detected in the drinking water of all four distribution systems during 2012. The anaerobic results of October 2011 (table 9) as well as March and June 2012 (table 10) contradicts with the *Clostridia* results. The reason for this is unknown and need to be further investigated.

Overall the microbiological results improved from 2011 to 2012. This may be due to the feedback given to Vaalharts Water during 2011 (O'Reilly & Bezuidenhout, 2011). The impact of the physico-chemical parameters on the microbiological parameters will be discussed with the statistical analysis in section 4.5.

4.3 BIOCHEMICAL AND MOLECULAR CONFIRMATION OF *ESCHERICHIA COLI*

Confirmation tests were performed on selected colonies isolated from m-Fc agar. The housekeeping genes of *E. coli*, *mdh* (Omar *et al.*, 2010) and *lacZ* (Ram & Shanker, 2005), as well as the *uidA gene* encoding the enzyme β-D-Glucuronidase (Martins *et al.*, 1993; Farnleitner *et al.*, 2001) can be used as genetic markers for the molecular identification of *E. coli*. The 16S gene is present all bacteria and is a genetic marker of prokaryotes (Janda & Abbott, 2007).

4.3.1 Biochemical confirmation vs. molecular confirmation

From table 11 it is evident that the biochemical results of some of the colonies, especially the MLG agar results, contradict the molecular results obtained during 2011 and June 2012. Fricker *et al.* (2008) tested the effectiveness of various media for the detection of β-D-glucuronidase activity. The media that were used during their study include: Chromocult, Coliscan, MI agar and MLG agar. Cultures taken from 914 Colilert-18® positive wells as well as 251 *E. coli* isolates grown on m-FC agar were plated onto the different media. The number of false-negatives for the Colilert-18® test was: Chromocult - 4; Coliscan - 6; MI agar - 4 and MLG agar - 114. The number of false-negatives for the *E. coli* isolates grown on m-FC agar was: Chromocult - 0; Colilert-18® - 1; Coliscan - 3; MI agar - 8 and MLG agar – 68. In both tests MLG agar yielded the highest level of false-negatives. Their study concluded that a high number of *E. coli*

strains failed to produce the distinctive green colonies on MLG agar, but instead produced yellow colonies that would be recognised as coliforms.

Table 12 indicates the confirmation results performed on ten colonies isolated from the Colilert test. The MLG agar results correspond to the Colilert results. This indicates that the Colilert method may be more sensitive than membrane filtration for the isolation of *E. coli*. Eckner (1998) studied the efficiency of membrane filtration and multiple-tube fermentation versus the Colilert method of drinking and bathing water samples in Southern Sweden. His study demonstrated that the Colilert test is more sensitive than membrane filtration and multiple-tube fermentation for the isolation of *E. coli* in drinking water. Similar findings were observed by Niemela *et al.* (2003) as well as Al-Turki and El-Ziney (2009).

Results of the present study and previous studies demonstrated that the Colilert test may be more effective for the isolation of total coliforms and faecal coliforms. According to Jackson *et al.* (2002) the laboratories involved in their study found that the Colilert test was also easier to use and less time consuming than other culture based methods. Molecular results of the present study were also more consistent with the Colilert test results. It is thus recommended that the Colilert test, together with molecular tests be used to isolate and identify total coliforms and faecal coliforms in water in future studies.

4.3.2 PCR confirmation results

This section discusses the gel electrophoresis images of the PCR confirmation results given in table 11 and table 12. Section 4.3.2.1 discusses the multiplex PCR results. Section 4.3.2.2 discusses the *uidA* monoplex PCR results followed by section 4.3.2.3 which discusses the 16S rDNA gene PCR results.

4.3.2.1 Multiplex (*mdh* and *lacZ*) PCR results

During 2011 and 2012 one or both of the *E.coli* target gene fragments, *mdh* and *lacZ*, were amplified by the isolates, except for one isolate during June 2012. The agarose gel image of figure 13 (Appendix B) indicated that isolate HR13 did not amplify the *mdh* or *lacZ* gene fragments. This could be due to human error during the loading of the electrophoresis gel, because DNA from this isolate amplified the *uidA* gene (figure 14). Sequence results also indicated that isolate HR13 was *E. coli*.

Faint bands were observed on some of the agarose gel images for some of the PCRs of the isolates. The faint bands could be due to a low concentration of *E. coli* DNA present in the samples and this yielded low concentration of amplification products. During a study by Omar *et al.* (2010) the *mdh* gene was targeted for the identification of *E. coli* in water. Results of the study indicated that 302 bp fragments were successfully amplified which indicates the presence of *E. coli* in the water. Ram and Shanker (2005) performed a study where the *lacZ* gene was targeted in a multiplex PCR for the detection of *E. coli* in water. Their study was based on testing various housekeeping genes of *E. coli* in multiplex PCR's and concluded that the *lacZ* gene can be targeted for the identification of *E. coli*. Therefore the multiplex PCR can be used for the identification of *E.coli*. This approach was successfully used in the present study.

4.3.2.2 *uidA* monoplex PCR results

During 2011 and 2012 the *uidA* gene fragment from all the isolates were amplified. This indicates that all the isolates are *E. coli*. A study by Bower *et al.* (2005) also targeted the *uidA* gene for the identification of *E. coli* in water. Results of their study were similar to the present study where 380 bp *uidA* gene fragments were amplified which is indicative of *E. coli* present in that sample.

4.3.2.3 16S rDNA gene PCR results

During 2011 and 2012 the 16S rDNA gene fragments were amplified by all the isolates. This is an indication that the DNA was amplifiable and that the isolates forms part of the domain *Bacteria* (Wise *et al.*, 1997).

From the molecular data it was demonstrated that the *mdh, lacZ* and *uidA* genes were successfully used for the identification of *E. coli* in raw and drinking water samples. The molecular results also confirm that culture based methods (such as MLG agar) may produce false-negative results for *E. coli*, as seen in table 11 and 12. This agrees with the study performed by Fricker *et al.* (2008). Their study indicated that MLG agar failed to detect glucuronidase activity in 15.6% of the cultures. It is thus important to perform molecular confirmation of isolates obtained from MLG agar to ensure accurate results.

4.4 SEQUENCING RESULTS

Table 13 indicates the results of the ten samples of the PCR products selected for sequencing. Isolate JR12 was the only sample which was not identified as *E. coli*, but as *Enterobacter cloacae*. JR12 sequences, however, were 92% similar to *E. coli* as well. *Enterobacter cloacae and E. coli* forms part of the family *Enterobacteriaceae* which means these two organisms are closely related. All of the other samples were identified as *E. coli*. The sequencing results of isolates HR26, HR1, JR12 and HR13 indicated that these were potentially *Escherichia coli* O104:H4. *Escherichia coli* O104:H4 is a Shiga toxin (Stx)-producing pathogen that can cause diarrhoea, bloody diarrhoea and haemolytic uremic syndrome (HUS) in humans (Mellmann *et al.*, 2011). During May 2011 an outbreak of *E. coli* O104:H4 were reported in Germany (Frank *et al.*, 2011; Mellmann *et al.*, 2011). A total of 3816 cases which included 54 deaths were reported. In the Germany outbreak this strain was associated with agricultural products. The presence of this *E. coli* strain in the water of some of the distribution systems in Vaalharts is thus cause for concern.

4.5 STATISTICAL RESULTS

Figure 8 is a RDA biplot indicating the correlation between the microbiological and physico-chemical variables in the raw water (red vectors) and the physico-chemical and microbiological variables in the drinking water (blue vectors). Average values of the raw and drinking water were used to create the biplots. The biplot shows that nitrate in the raw water has a close correlation with nitrate in the drinking water. This was seen for some of the distribution systems during 2011 and 2012 where nitrate was present in the raw and drinking water of the same distribution system (section 4.1.6). Sulphate in the raw water correlates with sulphide and anaerobic bacteria in the drinking water. This suggests the possibility of biofilm activity in the distribution system. Kristiana *et al.* (2010) confirmed this by detecting sulphide in the drinking water of a distribution system which is associated with sulphate reducing bacteria (section 4.1.5). HPC bacteria in the raw water correlate with HPC bacteria in the drinking water. The same trend can be seen with faecal streptococci and total coliforms. This may be an indication of failure in the treatment processes (WHO, 2011). Figure 8 also indicates that free chlorine in the drinking water does not correlate with heterotrophic bacteria in the drinking water. This confirms the results of low free chlorine levels in the drinking water of some of the distribution systems (discussed in section 4.1.1) and the high levels of heterotrophic

bacteria in the drinking water of the same distribution systems (discussed in section 4.2.2).

The possibility of the presence of biofilms in the distribution systems due to the presence of nitrite in the drinking water were discussed in section 4.1.7. Figure 9 indicates that nitrite in the drinking water correlates with the anaerobic bacteria in the drinking water. This confirms that biofilms may possibly be present in the distribution systems. Faecal streptococci and *Pseudomonas aeruginosa* in the drinking water showed a correlation with the drinking water temperature. This trend was also observed in figure 8 for *Pseudomonas aeruginosa*. It confirms the results given in table 11 and 12 where the levels of these bacteria were higher during the warmer seasons (October and March) and lower during the colder seasons (June and July) of some of the distribution systems. The main trend which can be seen in figure 8 is that the quality of the raw water has an impact on the quality of the drinking water. The trends observed in figure 8 were also observed in figure 9.

Figure 8 and 9 indicate that physico-chemical parameters do have an impact on microbiological parameters. It is also evident that the quality of the raw water does have an impact on the quality of the drinking water (figure 8) and that there are strong indications of biofilm formation. Deterioration in the quality of the raw water will thus have an impact on the quality of the drinking water. Such a scenario may require an adaptation of drinking water production systems. This aspect, however, needs to be further explored. More data points taken over a longer period may be useful to demonstrate such relationships. Tools used in the present study may be useful in such predictive studies.

CHAPTER 5
CONCLUSION AND RECOMMENDATIONS

In this section the conclusions of the study is briefly discussed and recommendations for future research made. Section 5.1 contains the conclusion and section 5.2 the recommendations.

5.1 CONCLUSION

To determine the physico-chemical and microbiological quality of the household water in the Vaalharts irrigation scheme, five objectives were set. A brief discussion of each objective is described below.

i) Survey of the state of drinking water production plants in the Vaalharts Irrigation scheme.

During 2011 a survey of the water treatment plants of the four distribution systems (Hartswater, Pampierstad, Jan Kempdorp and Warrenton) was done (section 2.3). The survey was based on the regulations for the registration of waterworks and process controllers (South Africa, 2006). Information about the design parameters, operating procedures, control processes, advanced processes, microbiological analysis, population size, distribution materials and historical information of each water treatment plant were recorded.

The survey indicated that Pampierstad is the oldest water treatment plant. Pampierstad, Jan Kempdorp and Warrenton use chlorine gas as disinfectant, but Hartswater uses chlorine. None of the water treatment plants perform advanced processes such as reverse osmosis, activated carbon and softening of the water. The pipe material used by the different water treatment plants are as follows: Hartswater: mainly asbestos and PVC; Pampierstad: mainly asbestos; Jan Kempdorp: mostly PVC, but asbestos and galvanised steel in some areas; Warrenton: mainly asbestos. The type of pipe material may influence the proliferation of biofilms attached to the inside surface of the pipe (Zhou *et al.*, 2009). As mentioned in section 3.7 Warrenton had the most acceptable microbiological results during 2011 and June 2012 and Pampierstad had the most acceptable

microbiological results during March 2012. The type of pipe material of these two water treatment plants (asbestos) may have played a role in achieving these acceptable microbiological results. This deduction is supported by findings of Momba and Makala (2004).

ii) Physico-chemical and microbiological quality of the municipal supplied water

The physico-chemical parameters that were measured did not raise any concern, except for nitrite levels in the drinking water of all four distribution systems during 2011 and 2012 (section 4.1.7) which did not comply with the SANS 241:2011 standard. The presence of nitrate, nitrite and sulphide in the drinking water may be due to biofilm formation (as discussed in section 4.1). The EC, sulphate, nitrate, phosphorous and salinity levels increased gradually from 2011 to 2012. Metal results did not raise any concern, except for the mercury levels in the raw and drinking water of Hartswater and Pampierstad during July 2011. The physico-chemical results of Pampierstad were the most acceptable during 2011 and March 2012 and Warrenton had the most acceptable physico-chemical results during June 2012.

Overall the microbiological results improved from 2011 to 2012, except *Clostridia* results. The only concerns were the high levels of HPC bacteria in drinking water of some of the distribution systems during 2011 and 2012 and the high level of faecal coliforms (191 CFU/100ml) detected in the drinking water of Jan Kempdorp during July 2012. The high levels of HPC bacteria in the drinking water of some of the distribution systems during 2011 and 2012 may be due to the considerably low levels of free chlorine in the drinking water of the same distribution systems. The detection of faecal coliforms in the drinking water of Jan Kempdorp during July 2012 is worrisome. DNA sequences that is similar to the pathogen *Escherichia coli* O104:H4 was detected in the raw water of Jan Kempdorp. The sequences of the isolates from the raw water of Hartswater (during 2011 and 2012; table 13) also matched the *Escherichia coli* O104:H4 sequences. If water containing this pathogenic strain or other *E. coli* strains leach into the distribution system, it may have adverse health effects on humans consuming the water.

iii) Compliance of the municipal supplied water with the South African National Standards (SANS) 241:2011

The physico-chemical results complied with the SANS 241 (2011) standards, except nitrite levels of all four distribution systems during 2011 and 2012. Pampierstad was the only distribution system during March 2012 whose nitrite level complied with the SANS 241 (2011) standard of ≤0.9 mg/L. Mercury levels in the drinking water of Hartswater and Pampierstad during July 2011 did not comply with the SANS 241 (2011) standard of ≤6 µg/L. This raised concern. Total coliform levels of all four distribution systems complied with the SANS 241 (2011) standard of ≤10 CFU/100ml during 2011 and 2012, except Hartswater during July 2011 (14 CFU/100ml) and Warrenton during March 2012 (256 CFU/100ml). This may be an indication of deteriorating water quality (Rompré *et al.*, 2002). Faecal coliform levels of all four distribution systems also complied with the SANS 241 (2011) standard of 0 CFU/100ml, except Jan Kempdorp during March 2012 which had a faecal coliform level of 191 CFU/100ml. This is an indication of faecal pollution in the water (Kacar, 2011) and is cause for concern.

The HPC bacteria levels of Warrenton complied with the SANS 241 (2011) standard of ≤1000 CFU/ml during July and October 2011 and Pampierstad during July 2011. The HPC bacteria levels of Hartswater and Pampierstad complied with the SANS 241 (2011) standard during March and June 2012. During June 2012 the heterotrophic bacteria level of Pampierstad also complied with the SANS 241 (2011) standard. Heterotrophic bacteria are good indicators of the effectiveness of the water treatment process and cleanliness of the distribution system (WHO, 2011). The high level of heterotrophic bacteria present in the drinking water of most of the distribution systems during 2011 and 2012 is an indication that the treatment process is not effective.

iv) Impact of seasonal changes on the water quality

The change in seasons did not have an impact on the physico-chemical parameters, but had an impact on some of the microbiological parameters (section 4.1.11). This can be expected, because temperature can influence microbial activity (Park *et al*., 2010).

v) Statistical analysis of the data

Statistical analysis was performed and a positive correlation between the water quality prior to purification and the quality after purification was demonstrated. The biplot results (figure 9) showed that nitrate levels in the raw water has a close correlation with nitrate levels in the drinking water; sulphate levels in the raw water correlates with sulphide levels and anaerobic bacteria levels in the drinking water; heterotrophic bacteria levels in the raw water correlate with heterotrophic bacteria levels in the drinking water and the same trend can be seen with faecal streptococci and total coliforms.

The five objectives that was set for this study was reached. In conclusion the main trends that were observed during the study are: 1) the general drinking water quality of the four distribution systems improved from 2011 to 2012; 2) the physico-chemical parameters had an impact on the microbiological parameters; 3) even though the microbiological results in the drinking water complied with the SANS 241 (2011) levels, the presence of other bacteria, such as *Clostridia*, *Pseudomonas* sp. and fungi is cause for concern; 4) the physico-chemical results also complied with the SANS 241 (2011), but the increase of some of the physico-chemical parameters from 2011 to 2012 is also cause for concern and 5) the quality of the raw water had an impact on the quality of the drinking water.

5.2 RECOMMENDATIONS FOR FUTURE RESEARCH

1) During our study pathogenic strains of *E. coli* were detected in the raw water of some of the areas. This is worrisome as some of these pathogenic *E. coli* may leach into the distribution system. Therefore a detailed study of the *E. coli* strains that are present in the raw water of the Vaalharts region should be conducted.

2) Antibiotic resistance of the *E. coli* isolated in the drinking water can be performed. A study by Chatterjee *et al.* (2012) indicated the antibiotic resistance of *E. coli* in water samples. Emerging antibiotic resistance of bacteria is a major worldwide concern for health officials (Chatterjee *et al.*, 2012). Antibiotic resistance makes the treatment of infections and diseases caused by the antibiotic resistant bacteria more difficult. Therefore, antibiotic analysis of *E. coli* should be performed to establish the effectiveness of certain antibiotics to kill *E. coli*.

3) Molecular based methods such as PCR or real-time PCR can be used for the isolation and identification of *E. coli* instead of culture based methods. During this study molecular tests were more sensitive than culture based methods. The advantage of using Real-time PCR is that it is time effective and quantative results can also be obtained.

4) The Colilert test can also be used for the isolation of *E. coli* as it proved to be more sensitive than culture based methods during this study. The Colilert test is also time effective as it allows for simultaneous detection of total coliforms and *E. coli* within 24h (Eckner, 1998).

5) Water samples before purification, at various points within the treatment plant (for example after coagulation, flocculation, sedimentation and filtration) as well as after purification can be taken. It can't be expected of a single treatment barrier to remove all the various pathogens found in water (Okeyo *et al.*, 2011). Under performance of the chlorination process may take place if water treatment plants contain water with a higher microbial load than what the chlorine delivery process is set to handle (Okeyo *et al.*, 2011). Therefore, the pre-disinfection barriers through which this water passes need to be optimized (Jagals & Jagals, 2004; Okeyo *et al.* 2011). Not only will this ensure that possible pathogens are removed before they reach the final water, it will also identify any failure in the treatment process.

REFERENCE LIST

Adam, J.W.H. 1980. Health aspects of nitrate in drinking-water and possible means of denitrification (literature review). *Water SA*, 6(2):79-84.

Agilent Technologies. 2004. Agilent 7500 Series ICP-MS. http://www.chem.agilent.com/Library/brochures/5989-0774EN_ICP-MS_lo.pdf Date of access: 6 Nov. 2012.

Alemdar, S., Kahraman, T., Agaoglu, S. & Alisarli, M. 2009. Nitrate and nitrite levels of drinking water in Bitlis Province, Turkey. *Journal of Animal and Veterinary Advances*, 8(10):1886-1892.

Allen, M.J., Edberg, S.C. & Reasoner, D.J. 2004. Heterotrophic plate count bacteria-what is their significance in drinking water? *International Journal of Food Microbiology*, 92:265-274.

Alonso, J.L., Soriano, A., Amoros, I. & Ferrus, M.A. 1998. Quantitative determination of *E. coli* and fecal coliforms in water using a chromogenic medium. *Journal of Environmental Science and Health*, A33(6):1229-1428.

Al-Qadiri, H.M., Al-Holy, M.A., Lin, M., Alami, N.I., Cavinato, A.G. & Rasco, B.A. 2006. Rapid detection and identification of *Pseudomonas aeruginosa* and *Escherichia coli* as pure and mixed cultures in bottled drinking water using Fourier transform infrared spectroscopy and multivariate analysis. *Journal of Agricultural and Food Chemistry*, 54:5749-5754.

Al-Turki, A. & El-Ziney, M.G. 2009. Evaluation of commercial Colilert18-Quantitray® method by ISO techniques for enumeration and quantification of total coliforms and *Escherichia coli* in drinking-water of Buraidah, Saudi Arabia. *Journal of Applied Sciences*, 9(18):3357-3363.

Audicana, A., Perales, I. & Borrego, J.J. 1995. Modification of Kanamycin-Esculin-Azide agar to improve selectivity in the enumeration of fecal streptococci from water samples. *Applied and Environmental Microbiology*, 61(12):4178-4183.

Avelar, K.E.S., Moraes, S.R., Pinto, L.J.F., Das Graças Silva E Souza, W., Domingues, R.M.C.P. & De Souza Ferreira, M.C. 1998. Influence of stress conditions on *Bacteroides fragilis* survival and protein profiles. *Zentralblatt für Bakteriologie*, 287:399-409.

Baghel, V.S., Gopal, K., Dwivedi, S. & Tripathi, R.D. 2005. Bacterial indicators of faecal contamination of the Gangetic river system right at its source. *Ecological Indicators*, 5:49–56.

Balazs, C., Morello-Frosch, R., Hubbard, A. & Ray, I. 2011. Social disparities in nitrate-contaminated drinking water in California's San Joaquin Valley. *Environmental Health Perspectives*, 119(9):1272-1278.

Bartram, J., Cotruvo, J., Exner, M., Fricker, C. & Glasmacher, A. 2003. Heterotrophic plate counts and drinking-water safety: The significance of HPCs for water quality and human health. World Health Organization, Geneva, Switzerland.

Bisson, J.W. & Cabelli, V.J. 1980. *Clostridium perfringens* as a water pollution indicator. *Journal of the Water Pollution Control Federation*, 52(2):241-248.

Bower, P.A., Scopel, C.O., Jensen, E.T., Depas, M.M & Mclellan, S.L. 2005. Detection of Genetic Markers of fecal indicator bacteria in Lake Michigan and determination of their relationship to *Escherichia coli* densities using standard microbiological methods. *Applied and Environmental Microbiology*, 71(12):8305-8313.

Braune, E. & Rogers, K.H. 1987. The Vaal River catchment: Problems and research needs. South African national scientific programmes report no. 143. Foundation for Research Development. Pretoria, South Africa.

Bressler, D., Balzer, M., Dannehl, A., Flemming, H.C. & Wingender, J. 2009. Persistence of *Pseudomonas aeruginosa* in drinking-water biofilms on elastomeric material. *Water Science and Technology:Water Supply*, 9(1):81-87.

Bucheli, T., Wettstein, F.E., Hartmann, N., Erbs, M., Vogelgsang, S., Forrer, H. & Schwarzenbach, R. 2008. *Fusarium* mycotoxins: overlooked aquatic micropollutants? *Journal of Agricultural and Food Chemistry*, 56:1029-1034.

Bugno, A., Almodóvar, A.A.B. & Pereira, T.C. 2010. Enumeration of heterotrophic bacteria in water for dialysis: comparison of the efficiency of Reasoner'2 Agar and plate count agar. *Brazilian Journal of Microbiology*, 41:15-18.

Burlingame, G.A., McElhaney, J., Bennett, M. & Pipes, W.O. 1984. Bacterial interference with coliform colony sheen production on membrane filters. *Applied and Environmental Microbiology*, 47(1):56-60.

Calderon, R.L. 2000. The epidemiology of chemical contaminants of drinking water. *Food and Chemical Toxicology*, 38:S13–S20.

Cantor, K.P. 1997. Drinking water and cancer. *Cancer Causes and Control*, 8:292–308.

Capece, J.C., Campbell, K.L., Bohlen, P.J., Graetz, D.A. & Portier, K.M. 2007. Soil Phosphorus, Cattle Stocking Rates, and Water Quality in Subtropical Pastures in Florida, USA. *Rangeland Ecology & Management*, 60(1):19-30.

Carpenter, S.R., Caraco, N.F., Correl, D.L., Howarth, R.W., Sharpley, A.N. & Smith, V.H. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications*, 8(3):559-568.

Carter, J. T., Rice, E. W., Buchberger, S. G. & Lee, Y. 2000. Relationships between levels of heterotrophic bacteria and water quality parameters in a drinking water distribution system. *Water Research*, 34(5):1495-1502.

Chang, C., Chen, C., Wu, D. & Yang, C. 2010. Nitrates in drinking water and the risk of death from rectal cancer: does hardness in drinking water matter? *Journal of Toxicology and Environmental Health Part A*, 73:1337-1347.

Charamandari, E., Meadows, N., Patel, M., Johnston, A. & Benjamin, N. 2001. Plasma nitrate concentrations in children with infectious and noninfectious diarrhea. *Journal of Pediatric Gastroenterology and Nutrition*, 32:423–427.

Chatterjee, R., Sinha, S., Aggarwal, S., Dimri, A.G., Singh, D., Goyal, P., Chauhan, A., Aggarwal, M.L. & Chacko, K.M. 2012. Studies on susceptibility and resistance patterns of various *E. coli* isolated from different water samples against clinically significant antibiotics. *International Journal of Bioassays*, 1(11):156-161.

Chowdhury, S. 2012. Heterotrophic bacteria in drinking water distribution system: a review. *Environmental Monitoring and Assessment*, 184:6087-6137.

Claus, D. 1992. A standardize Gram staining procedure. *World Journal of Microbiology and Biotechnology*, 8:451-452.

Converse, R.R., Griffith, J.F., Noble, R.T., Haugland, R.A., Schiff, K.C. & Weisberg, S.B. 2012. Correlation between quantitative PCR and culture-based methods for measuring *Enterococcus* spp. over various temporal scales at three California marine beaches. *Applied and Environmental Microbiology*, 78(4):1237-1242.

CSIR: Council for Scientific and Industrial Research. 2010. The current status of water quality in South Africa: A review. In: *A CSIR perspective on water in South Africa*. Pretoria, South Africa.

Delpla, I., Jung, A.V., Baures, E., Clement, M. & Thomas, O. 2009. Impacts of climate change on surface water quality in relation to drinking water production. *Environmental International*, 35:1225-1233.

Dietrich, A.M. 2006. Aesthetic issues for drinking water. *Journal of Water and Health*, 4 (supplement 1):11-16.

Digiano, F.A., Francisco, D.E., Zhang, W. & Todd, L. 2000. Bacterial regrowth in drinking water distribution systems: a comparison of Durham and Raleigh. WRRI (Water Resources Research Institute of the University of North Carolina) Project No. 50212.

Dogan, M., Dogan, A.U., Celebi, C. & Baris, Y.I. 2005. Geogenic arsenic and a survey of skin lesions in the Emet region of Kutahya, Turkey. *Indoor and Built Environment*, 14(6):533–536.

DPW: Department of Public Works. 2012. Small waste water treatment works - DPW design guidelines. South Africa. June. http://www.publicworks.gov.za/PDFs/consultants_docs/Design_guidline_for_Small_Waste_Water_Treatment_Works.pdf Date of access: 14 Nov. 2012.

Dufour, A.P., Strickland, E.R. & Cabelli, V.J. 1981. Membrane filter method for enumerating *Escherichia coli*. *Applied and Environmental Microbiology*, 41(5):1152-1158.

DWA: Department of Water Affairs. 2007. Integrated water quality management plan for the Vaal River system: Project Steering Committee Meeting 3.

DWA: Department of Water Affairs. 2012. Bluedrop Handbook: Version 1. http://www.dwa.gov.za/dir_ws/DWQR/Subscr/ViewNewsDoc.asp?FileID=262 Date of access: 23 Oct. 2012.

DWAF: Department of Water Affairs and Forestry. 1996a. South African water quality guidelines. 2nd ed. Volume 3: Industrial use. Pretoria, South Africa: Government Printer.

DWAF: Department of Water Affairs and Forestry. 1996b. South African water quality guidelines. 2nd ed. Volume 1: Domestic use. Pretoria, South Africa: Government Printer.

DWAF: Department of Water Affairs and Forestry. 1998. Quality of domestic water supplies. 2nd ed. Volume 1: Assessment Guide. Pretoria, South Africa: Government Printer.

DWAF: Department of Water Affairs and Forestry. 2000. Quality of domestic water supplies. 1st ed. Volume 2: Sampling Guide. Pretoria, South Africa: Government Printer.

DWAF: Department of Water Affairs and Forestry. 2001. Quality of domestic water supplies. 1st ed. Volume 3: Analysis Guide. Pretoria, South Africa: Government Printer.

DWAF: Department of Water Affairs and Forestry. 2002. Quality of domestic water supplies. 1st ed. Volume 4: Treatment Guide. Pretoria, South Africa: Government Printer.

DWAF: Department of Water Affairs and Forestry. 2005a. Disinfection as a water treatment process pamphlet. Pretoria, South Africa: Government Printer.

DWAF: Department of Water Affairs and Forestry. 2005b. A drinking water quality framework for South Africa. Pretoria, South Africa: Government Printer.

DWAF-DNWRP: Department of Water Affairs and Forestry, directorate national water resource planning (DNWRP). 2009a. Integrated water quality management plan for the Vaal river system: Task 2: Water quality status assessment of the Vaal River system. Report No. P RSA C000/00/2305/1. Pretoria, South Africa.

DWAF-DNWRP: Department of Water Affairs and Forestry, directorate national water resource planning (DNWRP). 2009b. Integrated water quality management plan for the Vaal river system: Task 8: Water quality management strategy. Report No. P RSA C000/00/2305/7. Pretoria, South Africa.

Eckner, K.F. 1998. Comparison of membrane filtration and multiple tube fermentation by the Colilert and Enterolert methods for the detection of waterborne coliform bacteria,

Escherichia coli and enterococci used in drinking and bathing water quality monitoring in Southern Sweden. *Applied and Environmental Microbiology*, 64(8):3079–3083.

Edberg, S.C., Allen, M.J. & Smith, D.B. 1988. National field evaluation of a defined substrate method for the simultaneous enumeration of total coliforms and *Escherichia coli* from drinking water: comparison with the standard multiple tube fermentation method. *Applied and Environmental Microbiology*, 54(6):1595–1601.

Edge, A.E. & Hill, S. 2005. Occurrence of antibiotic resistance in *Escherichia coli* from surface waters and fecal pollution sources near Hamilton, Ontario. *Canadian Journal of Microbiology*, 51:501-505.

Edge, T.A. & Hill, S. 2007. Multiple lines of evidence to identify the sources of fecal pollution at a freshwater beach in Hamilton Harbour, Lake Ontario. *Water Research*, 41:3585-3594.

Edstrom Industries, INC. 1997. Biofilm: The key to understanding and controlling bacterial growth in automated drinking water systems. http://www.edstrom.com /file.aspx?DocumentId=21 Date of access: 23 Oct. 2012.

Eutech Instruments. 2005. PCSTestr 35: Product features. http://www.eutechinst.com /pdt-para-multi-pcstestr35.html Date of access: 30 Oct. 2012.

Exner, M., Kramer, A., Lajoie, L., Gebel, J., Engelhart S. & Hartemann, P. 2005. Prevention and control of health care - associated waterborne infections in health care facilities. *American Journal of Infection Control*, 33(5):S26-S40.

Fan, A.M. & Steinberg, V.E. 1996. health implications of nitrate and nitrite in drinking water: an update on methemoglobinemia occurrence and reproductive and developmental toxicity. *Regulatory Toxicology and Pharmacology*, 23(0006):35-43.

Fang, W., Hu, J.Y. & Ong, S.L. 2009. Influence of phosphorus on biofilm formation in model drinking water distribution systems. *Journal of Applied Microbiology*, 106:1328-1335.

Farnleitner, A.H., Hocke, L., Beiwl, C., Kavka, G.G., Zechmeister, T., Kirschner, A.K.T. & Mach, R.L. 2001. Rapid enzymatic detection of *Escherichia coli* contamination in polluted river water. *Letters in Applied Microbiology*, 33:246-250.

Field, K.G. & Samadpour, M. 2007. Fecal source tracking, the indicator paradigm, and managing water quality - Review. *Water research*, 41:3517-3538.

Field, K.G., Bernhard, A.E. & Brodeur T.J. 2003. Molecular approaches to microbiological monitoring: fecal source detection. *Environmental Monitoring and Assessment*, 81:313-326.

Figueras, M.J. & Borrego, J.J. 2010. New perspectives in monitoring drinking water microbial quality. *International Journal of Environmental Research and Public Health*, 7:4179-4202.

Frank, C., Askar, M., Bernard, H., Spode, A., Jordan, S., Müller, L., Buchholz, U. & Krause, G. 2011. Epidemic profile of Shiga-toxin-producing *Escherichia coli* O104:H4 outbreak in Germany. *The New England Journal of Medicine*, 365(19):1771-17780.

Fricker, C.R., Desarno, M., Warden, P.S. & Eldred, B.J. 2008. False-negative β-D-glucuronidase reactions in membrane lactose glucuronide agar medium used for the simultaneous detection of coliforms and *Escherichia coli* from water. *Letters in Applied Microbiology*, 47:539-542.

Furukawa, T., Yoshida, T. & Suzuki, Y. 2010. Biota of fecal bacteria concentrated in stable foam formed along water's edge in coastal zones. *Bubble Science, Engineering and Technology*, 2(1):25-30.

Ganner, A., Stoiber, C., Wieder, D. & Schatzmayr, G. 2010. Quantitative *in vitro* assay to evaluate the capability of yeast cell wall fractions from *Trichosporon mycotoxinivorans* to selectively bind gram negative pathogens. *Journal of Microbiological Methods*, 83:168-174.

Gião, M.S., Azevedo, N.F., Wilks, S.A., Vieira, M.J. & Keevil, C.W. 2010. Effect of chlorine on incorporation of *Helicobacter pylori* into drinking water biofilms. *Applied and Environmental Microbiology*, 76(5):1669-1673.

Giasuddin, A.S.M. 1995. Polymerase Chain Reaction technique: fundamental aspects and applications in clinical diagnostics. *Journal of Islamic Academy of Sciences*, 8(1):29-32.

Gibson, G.R. 1990. Physiology and ecology of the sulphate-reducing bacteria – a review. *Journal of Applied Bacteriology*, 69:769-797.

Gomez-Alvarez, V., Revetta, R.P. & Domingo, J.W.S. 2012. Metagenome analyses of corroded concrete wastewater pipe biofilms reveal a complex microbial system. *BMC Microbiology*, 12(122):1-14.

Güler, C. & Alpaslan, M. 2009. Mineral content of 70 bottled water brands sold on the Turkish market: Assessment of their compliance with current regulations. *Journal of Food Composition and Analysis*, 22:728-737.

Guo, H., Wyart, Y., Perot,J., Nauleau, F. & Moulin, P. 2010. Low-pressure membrane integrity tests for drinking water treatment: a review. *Water Research*, 44:41-57.

Hach Company. 2007. DR 2800 spectrophotometer: procedures manual. 2nd ed. Germany.

Hach Company. 2012. DR 2800™ portable spectrophotometer with lithium battery. http://www.hach.com/dr-2800-portable-spectrophotometer-with-lithium-ionbattery/product?id=7640439012 Date of access: 6 Nov. 2012.

Hageskal, G., Lima, N. & Skaar, I. 2009. The study of fungi in drinking water. *Mycological Research*, 113:165 - 172.

Hallas, G., Giglio, S., Capurso, V., Monis, P.T. & Grooby, W.L. 2008. Evaluation of chromogenic technologies for use in Australian potable water. *Journal of Applied Microbiology*, 105:1138-1149.

Hanukoglu, A. & Danon, P. 1996. Endogenous methemoglobinemia associated with diarrheal diseases in infancy. *Journal of Pediatric Gastroenterology and Nutrition*, 23:1-7.

Harley, J.P. 2005. Laboratory exercises in microbiology. 6th ed. McGraw-Hill, New-York.

Hayes, P.R. 1995. Food microbiology and hygiene. 2nd ed. Elsevier Science Publishers Ltd, London.

Hedayati, M.T., Mayahi, S., Movahedi, M. & Shokohi, H. 2011. Study on fungal flora of tap water as a potential reservoir of fungi in hospitals in Sari city, Iran. *Journal de Mycologie Médicale*, 21:10-14.

Heydari, M.M. & Bidgoli, H.N. 2012. Chemical Analysis of drinking water of Kashan District, central Iran. *World Applied Sciences Journal*, 16(6):799-805.

Hijnen, W.A.M., van Veenendaal, D.A., van der Speld, Visser, A., Hoogenboezem, W. & van der Kooij, D. 2000. Enumeration of faecal indicator bacteria in large water volumes using on site membrane filtration to assess water treatment efficiency. *Water Research*, 34(5):1659-1665.

Hörman, A. & Hänninen, M. 2006. Evaluation of the lactose Tergitol-7, m-Endo LES, Colilert 18, Readycult Coliforms 100, Water-Check-100, 3M Petrifilm EC and DryCult Coliform test methods for detection of total coliforms and *Escherichia coli* in water samples. *Water Research*, 40:3249-3256.

Hua, F., West, J.R., Barker, R.A. & Forster, C.F. 1999. Modelling of chlorine decay in municipal water supplies. *Water Research*, 33(12):2735 - 2746.

Hur, J., Lee, B., Lee, T. & Park, D. 2010. Estimation of Biological Oxygen Demand and Chemical Oxygen Demand for combined sewer systems using synchronous fluorescence spectra. *Sensors*, 10:2460 - 2471.

Ishii, S., Ksoll, W.B., Hicks, R.E. & Sadowsky, M.J. 2006. Presence and growth of naturalized *Escherichia coli* in template soils from Lake Superior watersheds. *Applied and Environmental Microbiology*, 72(1):612-621.

Jackson, S.A.F., Bailey, I., Britz, R., De Wet, C., Fortune, A., Johnston-Robertson, A.J., Naidoo, K.M. & Sundram, A. 2002. Report on the South African Colilert™ ring trial. Paper presented at the Biennial Conference of the Water Institute of Southern Africa (WISA). 19-23 May 2002, Durban, South Africa. http://www.ewisa.co.za/literature/files /115%20Paper.pdf Date of access: 6 Nov. 2012.

Jagals, C. & Jagals, P. 2004. Managing microbiological hazards at water treatment facilities through the application of HACCP principles. Proceedings of the 2004 Water Institute of Southern Africa (WISA) Biennial Conference. 2-6 May 2004, Cape Town, South Africa. http://www.ewisa.co.za/literature/files/118.pdf Date of access: 6 Nov. 2012.

Jain, P. & Pradeep, T. 2005. Potential of silver nanoparticle-coated polyurethane foam as an antibacterial water filter. *Biotechnology and Bioengineering*, 90(1):59-63.

Janda, J.M. & Abbott, S.L. 2007. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls – Minireview. *Journal of Clinical Microbiology*, 45(9):2761-2764.

Jjemba, P.K., Weinrich, L.A., Cheng, W., Giraldo, E. & LeChevallier, M.W. 2010. Regrowth of potential opportunistic pathogens and algae in reclaimed-water distribution systems. *Applied and Environmental Microbiology*, 76(13):4169-4178.

Joshi, M. & Deshpande, J.D. 2010. Polymerase chain reaction: methods, principles and application. *International Journal of Biomedical Research*, 1(5):81-97.

Kacar, A. 2011. Analysis of spatial and temporal variation in the levels of microbial fecal indicators in the major rivers flowing into the Aegean Sea, Turkey. *Ecological Indicators*, 11:1360–1365.

Kavcar, P., Sofuoglu, A., & Sofuoglu, S.C. 2009. A health risk assessment for exposure to trace metals via drinking water ingestion pathway. *International Journal of Hygiene and Environmental Health*, 212:216-227.

Kawabe, M. & Kawabe, M. 1997. Temporal and spatial characteristics of chemical oxygen demand in Tokyo Bay. *Journal of Oceanography*, 53:19–26.

Kistemann, T., Claben, T., Koch, C., Dangendorf, F., Fischeder, R., Gebel, J., Vacata, V. & Exner, M. 2002. Microbial load of drinking water reservoir tributaries during extreme rainfall and runoff. *Applied and Environmental Microbiology*, 68(5):2188–2197.

Kommineni, S.N., Bryck, J., Stringer, C., Stevens, C., Meyers, N., Karnik, B., Hoffman, R. & Sullivan, L. 2010. Evaluation of an emerging water treatment technology: ceramic membranes. *Water Science and Technology: Water Supply*, 10(5):765-770.

Kong, R.Y.C., Lee, S.K.Y., Law, T.W.F., Law, S.H.W. & Wu, R.S.S. 2002. Rapid detection of six types of bacterial pathogens in marine waters by multiplex PCR. *Water Research*, 36:2802-2812.

Köster, W., Egli, T., Ashbolt, N., Botzenhart, K., Burlion, N., Endo, T., Grimont, P., Guillot, E, Mabilat, C., Newport L., Niemi, M., Payment, P., Prescott, A., Renaud, P. & Rust, A. 2003. Analytical methods for microbiological water quality testing. In: *Assessing microbial safety of drinking water: improving approaches and methods* (A. Dufour, M. Snozzi, W. Koster, J. Bartram, E. Ronchi and L. Fewtrell, eds.). World Health Organization & Organisation for Economic Co-operation and Development. IWA Publishing, London, pp. 237-282.

Kreader, C.A. 1998. Persistence of PCR-detectable *Bacteroides distasonis* from human feces in river water. *Applied and Environmental Microbiology*: 64(10):4103-4105.

Kristiana, I., Heitz, A., Joll, C. & Sathasivan, A. 2010. Analysis of polysulfides in drinking water distribution systems using headspace solid-phase microextraction and gas chromatography–mass spectrometry. *Journal of Chromatography A*, 1217:5995–6001.

Lane, D.J. 1991. 16S/23S rRNA sequencing. In: *Nucleic Acid Techniques in Bacterial Systematics* (E. Stackebrandt and M. Goodfellow, eds.). John Wiley & Sons, New York, pp. 115-175.

Langenbach, K., Kuschk, P., Horn, H. & Kästner, M. 2010. Modeling of slow sand filtration for disinfection of secondary clarifier effluent. *Water Research*, 44:159-166.

Le Roux, P.A.L., Du Preez, C.C., Strydom, M.G., Van Rensburg, L.D. & Bennie, A.T.P. 2007. Effect of irrigation on soil salinity profiles along the Lower Vaal River, South Africa. *Water SA*, 33(4):473-478.

LeChevallier, M.W., Babcock, T.M. & Lee, R.G. 1987. Examination and characterization of distribution system biofilms. *Applied and Environmental Microbiology*, 53(12):2714–2724.

LeChevallier, M.W., Lowry, C.D., Lee, R.G. & Gibbon, D.L. 1993. Examining the relationship between iron corrosion and the disinfection of biofilm bacteria. *Journal of the American Water Works Association*, 85(7):111-123.

LeChevallier, M.W., Welch, N.J. & Smith, D.B. 1996. Full-scale studies of factors related to coliform regrowth in drinking water. *Applied and Environmental Microbiology,* 62(7):2201-2211.

Lehtola, M., Miettinen, I.K., Vartiainen, T. & Martikainen, P.J. 2002. Changes in content of microbially available phosphorus, assimilable organic carbon and microbial growth potential during drinking water treatment processes. *Water Research*, 36:3681-3690.

Li, D., Li, Z., Yu, J., Cao, N., Liu, R. & Yang, M. 2010. Characterization of bacterial community structure in a drinking water distribution system during an occurrence of red water. *Applied and Environmental Microbiology*, 76(21):7171-7180.

Lipponen, M.T.T., Martikainen, P.J., Vasara, R.E., Servomaa, K., Zacheus, O. & Kontro, M.H. 2004. Occurrence of nitrifiers and diversity of ammonia-oxidizing bacteria in developing drinking water biofilms. *Water Research*, 38:4424-4434.

Lopes, F.A., Morin, P., Oliveira, R. & Melo, L.F. 2009. Impact of biofilms in simulated drinking water and urban heat supply systems. *International Journal of Environmental Engineering*, 1(3):276-294.

Mamba, B.B., Krause, R.W., Matsebula, B. & Haarhoff, J. 2009. Monitoring natural organic matter and disinfection by-products at different stages in two South African water treatment plants. *Water SA*, 35(1):121-128.

Manafi, M., Kneifel, W. & Bascomb, S. 1991. Fluorogenic and chromogenic substrates used in bacterial diagnosis. *Microbiological Reviews*, 55(3):335–348.

Mara, D. & Horan, N. 2003. The handbook of water and wastewater microbiology. Academic Press, London.

Martins, M.T., Rivera, I.G., Clark, D.L., Stewart, M.H., Wolfe, R.L. & Olson, B.H. 1993. Distribution of *uidA* gene sequences in *Escherichia coli* isolates in water sources and comparison with the expression of β-glucuronidase activity in 4-methylumbelliferyl-3-β-D-glucuronide media. *Applied and Environmental Microbiology*, 59(7):2271-2276.

Mellmann, A., Harmsen, D., Cummings, C.A., Zentz, E.B., Leopold, S.R., Rico, A., Prior, K., Szczepanowski, R., Ji, Y., Zhang, W., Mclaughlin, S.F., Henkhaus, J.K., Leopold, B., Bielaszewska, M., Prager, R., Brzoska, P.M., Moore, R.L., Guenther, S., Rothberg, J.M. & Karch, H. 2011. Prospective genomic characterization of the German enterohemorrhagic *Escherichia coli* O104:H4 outbreak by rapid next generation sequencing technology. *Plos One*, 6(7):1-9.

Memon, N.A., Unar, M.A., Ansari, A.K., Khaskheli, G.B. & Memon, B.A. 2008. Prediction of parametric value of drinking water of Hyderabad City by artificial network modelling. *WSEAS Transactions on Environment and Development*, 4(8):707-716.

Merck. 2012. Merck microbiology manual. 12th ed. http://www.scribd.com/doc /94454909/ Merck-Microbiology-Manual-12th Date of access: 15 Nov. 2012.

Momba, M.N.B. & Makala, N. 2004. Comparing the effect of various pipe materials on biofilm formation in chlorinated and combined chlorine-chloraminated water systems. *Water SA*, 30(2):175-182.

Momba, M.N.B., Obi, C.L. & Thompsons, P. 2009. Survey of disinfection efficiency of small drinking water treatment plants: challenges facing small water treatment plants in South Africa. *Water SA*, 35(4):485-493.

Momba, M.N.B., Tyafa, Z., Makala, N., Brouckaert, B.M. & Obi, C.L. 2006. Safe drinking water still a dream in rural areas of South Africa. Case study: the Eastern Cape Province. *Water SA*, 32(5):715-720.

Mora, A., Mac-Quhae, C., Calzadilla, M. & Sánchez, L. 2009. Survey of trace metals in drinking water supplied to rural populations in the eastern Llanos of Venezuela. *Journal of Environmental Management*, 90:752-759.

Muittari, A., Kuusisto, P., Virtanen, P., Sovijärvi,A., Grönroos, P., Harmoinen, A., Antila, P. & Kellomäki, L. 1980. An epidemic of extrinsic allergic alveolitis caused by tap water. *Clinical Allergy*, 10:77 - 90.

Munshi, S.K., Rahman, M.M. & Noor, R. 2012. Detection of virulence potential of diarrhoeagenic *Escherichia coli* isolated from surface water of rivers surrounding Dhaka City. *Journal of Bangladesh Academy of Sciences,* 36(1):109-121.

Mustapha, M.K. 2008. Assessment of the water quality of Oyun reservoir, Offa, Nigeria, using selected physico-chemical parameters. *Turkish Journal of Fisheries and Aquatic Sciences*, 8:309-319.

Niemela, S.I., Lee, J.V. & Fricker, C.R. 2003. A comparison of the International Standards Organisation reference method for the detection of coliforms and *Escherichia coli* in water with a defined substrate procedure. *Journal of Applied Microbiology*, 95:1285-1292.

Noguerol-Arias, J., Rodríguez-Abalde, A., Romero-Merino, E. & Flotats, X. 2012. Determination of chemical oxygen demand in heterogeneous solid or semisolid samples using a novel method combining solid dilutions as a preparation step followed by optimized closed reflux and colorimetric measurement. *Analytical Chemistry*, 84:5548-5555.

O'Reilly, G. 2010. Assessment of the physico-chemical quality and detection of faecal indicator bacteria in household water of the Vaalharts region in the Northern Cape Province, South Africa. North-West University: Potchefstroom Campus. (Mini-dissertation - BSc.Hons.) 30 p.

O'Reilly, G. & Bezuidenhout, C.C. 2011. A preliminary assessment of the microbiological and physico-chemical quality of household water in the Vaalharts irrigation scheme, South Africa. Document presented to Vaalharts Water - Executive Committee, 17 Feb. 2011. 8 p.

Obi, C.L., Momba, M.N.B., Samie, A., Igumbor, J.O., Green, E. & Musie, E. 2007. Microbiological, physico-chemical and management parameters impinging on the efficiency of small water treatment plants in the Limpopo and Mpumalanga Provinces of South Africa. *Water SA*, 33(2):229-238.

Okeyo, A., Momba, M.N.B. & Coetzee, M. 2011. Application of the HACCP concept for the microbiological monitoring of drinking water quality: a case study of three water treatment plant in the Gauteng Province, South Africa. *Trends in Applied Sciences Research*, 6(3):369-281.

Omar, K.B., George, B.T. & Jagals, P. 2010. Development of a competitive PCR assay for the quantification of total *Escherichia coli* DNA in water. *African Journal of Biotechnology*, 9(4):564-572.

Oxoid Limited. 2012. Oxoid microbiology products. Membrane Lactose Glucuronide Agar (MLGA). http://www.oxoid.com/UK/blue/prod_detail/prod_detail.asp?pr=CM1031&org=71&c=UK&lang Date of access: 15 Nov. 2012.

Park, J., Duan, L., Kim, B., Mitchell, M.J. & Shibata, H. 2010. Potential effects of climate change and variability on watershed biogeochemical processes and water quality in Northeast Asia. *Environmental International*, 36:212-225.

Park, S., Cotter, P.A. & Gunsalus, R.P. 1995. Regulation of malate dehydrogenase (*mdh*) gene expression in *Escherichia coli* in response to oxygen, carbon, and heme availability. *Journal of Bacteriology*, 177(22):6652-6656.

Paterson, R.R.M., Hageskal, G., Skaar, I. & Lima, N. 2009. Occurrence, problems, analysis and removal of filamentous fungi in drinking water. In: *Fungicides: chemistry, environmental impact and health effects* (P. De Costa and P. Bezerra, eds.). Nova Science Publishers, Inc., pp. 1-21.

Pathak, S.P. & Gopal, K. 2008. Prevalence of bacterial contamination with antibiotic resistant and enterotoxigenic fecal coliforms in treated drinking water. *Journal of Toxicology and Environmental Health, Part A*, 71:427-433.

Payment, P. & Robertson, W. 2004. The microbiology of piped distribution systems and public health. World Health Organization. IWA Publishing, London, UK.

Penney, D.P., Powers, J.M., Frank, M., Willis, C. & Churukian, C. 2002. Analysis and testing of biological stains - the biological stain commission procedures. *Biotechnic & Histochemistry*, 77(5&6):237-275.

Petit, L., Gibert, M. & Popoffv, M.R. 1999. *Clostridium perfringens*: toxinotype and genotype. *Trends in Microbiology*, 7(3):104-110.

Phiri, O., Mumba, P., Moyo, B.H.Z. & Kadewa, W. 2005. Assessment of the impact of industrial effluents on water quality of receiving rivers in urban areas of Malawi. *International Journal of Environmental Science and Technology*, 2(3):237-244.

Potgieter, N., Obi, C.L., Bessong, P.O., Igumbor, E.O., Samie, A. & Nengobela, R. 2005. Bacterial contamination of Vhuswa - a local weaning food and stored drinking-water in impoverished households in the Venda region of South Africa. *Journal of Health, Population and Nutrition*, 23(2):150-155.

Ram, S. & Shanker, R. 2005. Computing TaqMan probes for multiplex PCR detection of *E. coli* O157 Serotypes in water. *In Silico Biology*, 5(5-6): 499-504.

Rand Water. 2012a. Water pollution and your health. http://www.randwater.co.za/CorporateResponsibility/WWE/Pages/WaterPollution.aspx Date of access: 5 Nov. 2012.

Rand Water. 2012b. Water purification. http://www.randwater.co.za/WaterAndInfastructureManagement/Pages/WaterPurification.aspx Date of access: 22 Oct. 2012.

Reasoner, D.J., Blannon, J.C., Geldreich, E.E. & Barnick, J. 1989. Nonphotosynthetic pigmented bacteria in a potable water treatment and distribution system. *Applied and Environmental Microbiology*, 55(4):912-921.

Rogues, A.M., Boulestreau, H., Lashéras, A., Boyer, C., Gruson, D., Merle, C., Castaing, Y., Bébear, C.M. & Gachie, J.P. 2007. Contribution of tap water to patient colonisation with *Pseudomonas aeruginosa* in a medical intensive care unit. *Journal of Hospital Infection*, 76:72-78.

Rompré, A., Servais, P., Baudart, J., De-Roubin, M. & Laurent, P. 2002. Detection and enumeration of coliforms in drinking water: current methods and emerging approaches. *Journal of Microbiological Methods*, 49:31-54.

SANS: South African National Standards, 241-1:2011. Drinking water. Part 1:Microbiological, physical, chemical, aesthetic and chemical determinands. SABS Standards Division. Pretoria, South Africa.

Schoenen, D. 2002. Role of disinfection in suppressing the spread of pathogens with drinking water: possibilities and limitations. *Water Research*, 36:3874-3888.

Schutte, F. 2006. Handbook for the operation of water treatment works. South Africa: The Water Research Commission. Pretoria, South Africa.

Shash, S.M., Kamel, M.M., Wasify, R.S. & Samhan, F.A. 2010. Rapid detection and enumeration of coliforms and *Escherichia coli* in River Nile using membrane filtration technique. *Environmental Biotechnology*, 6(1):6-10.

Shrivastava, R., Upreti, R.K., Jain, S.R., Prasad, K.N., Seth, P.K. & Chaturvedi, U.C. 2004. Suboptimal chlorine treatment of drinking water leads to selection of multidrug-resistant *Pseudomonas aeruginosa*, 58:277-283.

Sigma-Aldrich. 2012a. Sigma-Aldrich - product information. http://www.sigmaaldrich.com/etc/medialib/docs/Sigma/Datasheet/2/k2510dat.Par.0001.File.tmp/k2510dat.pdf Date of access: 15 Nov. 2012.

Sigma-Aldrich. 2012b. Sigma-Aldrich - product information. http://www.sigmaaldrich.com/etc/medialib/docs/Fluka/Datasheet/63646dat.Par.0001.File.tmp/63646dat.pdf Date of access: 15 Nov. 2012.

Sigma-Aldrich. 2012c. Sigma-Aldrich - product information. http://www.sigmaaldrich.com/etc/medialib/docs/Fluka/Datasheet/70887dat.Par.0001.File.tmp/70887dat.pdf Date of access: 15 Nov. 2012.

Sinton, L.W., Donnison, A.M. & Hastie, C.M. 1993. Faecal streptococci as faecal pollution indicators: a review. Part 1: taxonomy and enumeration. *New Zealand Journal of Marine and Freshwater Research*, 27:101-115.

South Africa. 2006. Draft regulations for the registration of waterworks and process controllers. (Notice 28557). *Government Gazette*, 181, 24 Feb.

Sumbali, G. & Mehrotra, R.S. 2009. Principles of Microbiology. New Delhi: Tata McGraw Hill.

Tariq, M., Ali, M. & Shah, Z. 2006. Characteristics of industrial effluents and their possible impacts on quality of underground water. *Soil and Environment*, 25(1):64-69.

Tarr, C.L., Large, T.M., Moeller, C.L., Lacher, D.W., Tarr, P.I., Acheson, D.W. & Whittam, T.S. 2002. Molecular characterization of a serotype O121:H19, a distinct Shiga toxin-producing clone of pathogenic *Escherichia coli. Infection and Immunity*, 70(12):6853-6859.

Ter Braak, C.J.F. 1990. Interpreting canonical correlation analysis through biplots of structure correlations and weights. *Psychometrika,* 55: 519–531.

Tharannum, S., Sunitha, S., Nithya, J., Chandini, M., Vanitha, J., Manjula, T.S., Shyam Sundar, C. 2009. Molecular confirmation of the presence of coliforms in drinking water using polymerase chain reaction. *Kathmandu University Journal of Science, Engineering and Technology*, 5(II):130-136.

Thomas, R. 2001. Beginner's guide to ICP-MS - Part 1. *Spectroscopy*, 16(4):38-42.

Tokajian, S.T., Hashwa, F.A., Hancock, I.C. & Zalloua, P.A. 2005. Phylogenetic assessment of heterotrophic bacteria from a water distribution system using 16S rDNA sequencing. *Canadian Journal of Microbiology*, 51:325-335.

Tripathi, R.M., Raghunath, R. & Krishnamoorthy, T.M. 1997. Dietary intake of heavy metals in Bombay city, India. *The Science of the Total Environment*, 208:149-159.

Tsai, Y. 2006. Interaction of chlorine concentration and shear stress on chlorine consumption, biofilm growth rate and particle number. *Bioresource Technology*, 97:1912-1919.

U.S. EPA (Environmental Protection Agency). 2010. Drinking water contaminants. http://www.epa.gov/safewater/contaminants/index.html Date of access: 22 Nov. 2012.

Van Der Laan, M., Van Antwerpen, R. & Bristow. 2012. River water quality in the northern sugarcane-producing regions of South Africa and implications for irrigation: A scoping study. *Water SA*, 38(1): 87-96.

Van Vuuren, L. 2009. Water savings: persistence pays off at Vaalharts. *The Water Wheel*, 12-15.

Van Vuuren, L. 2010. Vaalharts: A garden in the desert. *The Water Wheel*, 20-24.

Venter, S.N. 2000. Rapid microbiological monitoring methods: The status quo. International Water Association: the Blue Pages, IWA, London, UK. http://www.iwahq.org/contentsuite/upload/iwa/Document/RAPID_MICROBIOLOGICAL_MONITORING_METHODS_2000.pdf Date of access: 29 Oct. 2012.

Verwey, P.M.J. & Vermeulen, P.D. 2011. Influence of irrigation on the level, salinity and flow of groundwater at Vaalharts Irrigation Scheme. *Water SA*, 37(2):155-164.

Wang, H., Masters, S., Hong, Y., Stallings, J., Falkinham, J.O., Edwards, M.A. & Pruden, A. 2012. Effect of disinfectant, water age, and pipe material on occurrence and persistence of *Legionella*, *Mycobacteria*, *Pseudomonas aeruginosa*, and two amoebas. *Environmental Science & Technology*, 46(21):11566-11574.

WHO: World Health Organization. 2004a. Sulfate in drinking-water: Background document for development of WHO guidelines for drinking-water quality. Geneva, Switzerland.

WHO: World Health Organization. 2004b. Guidelines for drinking-water quality. 3rd ed. Volume 1. Geneva, Switzerland.

WHO: World Health Organization. 2007. Nitrate and nitrite in drinking-water. Background document for development of WHO guidelines for drinking-water quality. Geneva, Switzerland.

WHO: World Health Organization. 2011. Guidelines for drinking-water. 4th ed. Geneva, Switzerland.

Willey, J.M., Sherwood, L.M. & Woolverton, C.J. 2008. Prescott, Harley, and Klein's microbiology. 7th ed. McGraw-Hill, New-York.

Wingender, J. & Flemming, H. 2011. Biofilms in drinking water and their role as reservoir for pathogens. *International Journal of Hygiene and Environmental Health*, 241:417-423.

Winn, W.C., Koneman, E.W., Allen, S.D., Janda, W.M., Schreckenberger, P.C., Procop, G.W. & Woods, G.L. 2006. Koneman's Color Atlas and Textbook of Diagnostic Microbiology, 6th ed. Baltimore: Lippincott Williams & Wilkins.

Wise, M.G., Mcarthur, J.V. & Shimkets, L.J. 1997. Bacterial diversity of a Carolina bay as determined by 16S rRNA gene analysis: confirmation of novel taxa. *Applied and Environmental Microbiology*, 63(4):1505-1514.

Wohlsen, T.D. 2011. Comparative evaluation of chromogenic agar CM1046 and mFC agar for detection of *E. coli* and thermotolerant coliform bacteria from water samples. *Letters in Applied Microbiology*, ISSN 0266-8254.

Xia, Y. & Liu, J. 2004. An overview of chronic arsenism via drinking water in PR China. *Toxicology*, 198:25–29.

Yang, H. & Cheng, H. 2007. Controlling nitrite level in drinking water by chlorination and chloramination. *Separation and Purification Technology*, 56:392-396.

Yin, Y., Zhang, Y., Liu, X., Zhu, G., Qin, B., Shi, Z. & Feng, L. 2011. Temporal and spatial variations of chemical oxygen demand in Lake Taihu, China, from 2005 to 2009. *Hydrobiologia*, 665:129-141.

Zacheus, O.M., Iivanainen, E.K., Nissinen, T.K., Lehtola, M.J. & Martikainen, P.J. 2000. Bacterial biofilm formation on polyvinyl chloride, polyethylene and stainless steel exposed to ozonated water. *Water Research*, 34(1):63-70.

Zhou, L., Zhang, Y. & Li, G. 2009. Effect of pipe material and low level disinfectants on biofilm development in a simulated drinking water distribution system. *Journal of Zhejiang University SCIENCE A*, 10(5):725-731.

Zularisam, A.W., Ismail, A.F. & Salim, R. 2006. Behaviours of natural organic matter in membrane filtration for surface water treatment - a review. *Desalination*, 194:211-231.

References were done based on instructions to authors of the ***Journal of Water and Health.***

APPENDIX A

Total dissolved solids (TDS) results for 2011 & 2012

Table A1: TDS results for July and October 2011.

		SANS 241-1:2011 ≤ 1200 mg/L	
Sampling area		**Raw water**	**Drinking water**
Hartswater (July 2011)	Ave	230	234
	SE	0	1.08
Pampierstad (July 2011)	Ave	240	243
	SE	0	0.71
Jan Kempdorp (July 2011)	Ave	266	256
	SE	0	4.55
Warrenton (July 2011)	Ave	286	287
	SE	0	2.04
Hartswater (Oct. 2011)	Ave	309	313
	SE	0	1.22
Pampierstad (Oct. 2011)	Ave	310	315
	SE	0	0.71
Jan Kempdorp (Oct. 2011)	Ave	315	323
	SE	0	0.71
Warrenton (Oct. 2011)	Ave	318	320
	SE	0	0.41

Table A2: TDS results for March and June 2012.

		SANS 241-1:2011 ≤ 1200 mg/L	
Sampling area		**Raw water**	**Drinking water**
Hartswater (March 2012)	Ave	366	407
	SE	0	25.13
Pampierstad (March 2012)	Ave	384	383
	SE	0	1.41
Jan Kempdorp (March 2012)	Ave	397	412
	SE	0	10.01
Warrenton (March 2012)	Ave	405	408
	SE	0	2.27
Hartswater (June 2012)	Ave	445	453
	SE	0	5.31
Pampierstad (June 2012)	Ave	451	453
	SE	0	0.71
Jan Kempdorp (June 2012)	Ave	445	441
	SE	0	10.87
Warrenton (June 2012)	Ave	445	448
	SE	0	3.19

APPENDIX B

PCR confirmation results for 2012

March 2012

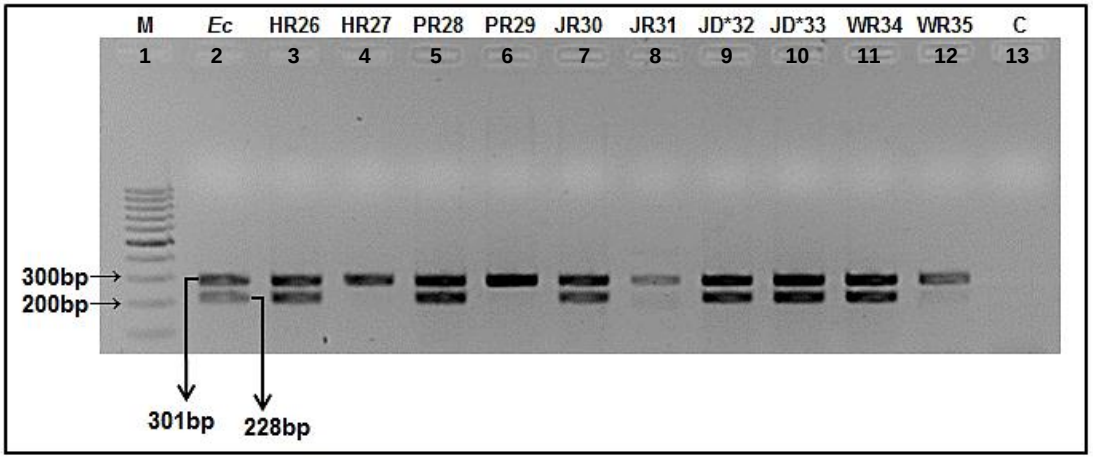

Figure 10: Negative image of the 1.5% (w/v) agarose gel indicating the multiplex PCR results for March 2012. The first lane contains the 100 bp molecular marker (M). The second lane contains the positive control (*E. coli* - ATCC 10536). Lane 3 to lane 12 indicates the PCR products of the various isolates. The last lane (13) contains the no template control. The *mdh* gene has a fragment size of 301 bp and the *lacZ* gene 228 bp.

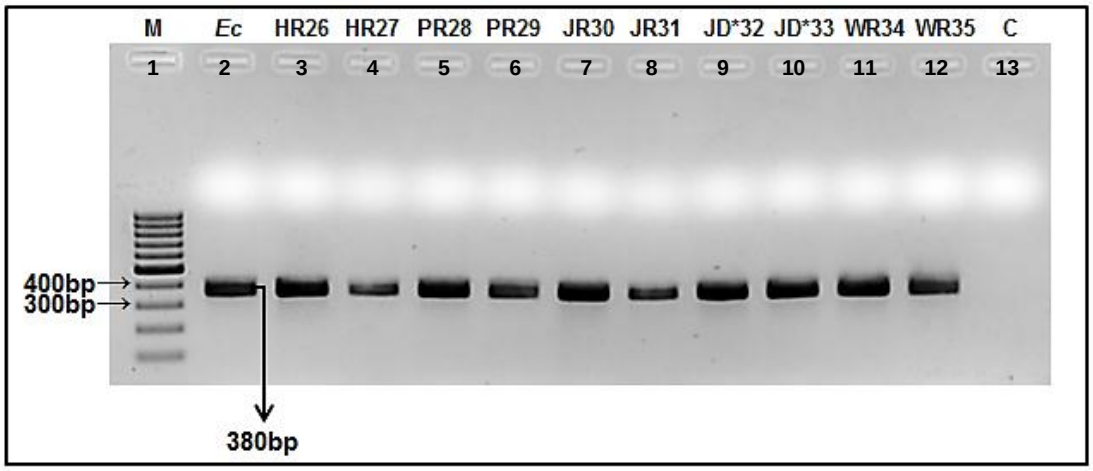

Figure 11: Negative image of the 1.5% (w/v) agarose gel indicating the *uidA* monoplex PCR results for March 2012. The first lane contains the 100 bp molecular marker (M). The second lane contains the positive control (*E. coli* - ATCC 10536). Lane 3 to lane 12 indicates the PCR products of the various isolates. The last lane (13) contains the no template control. The *uidA* gene has a fragment size of 380 bp.

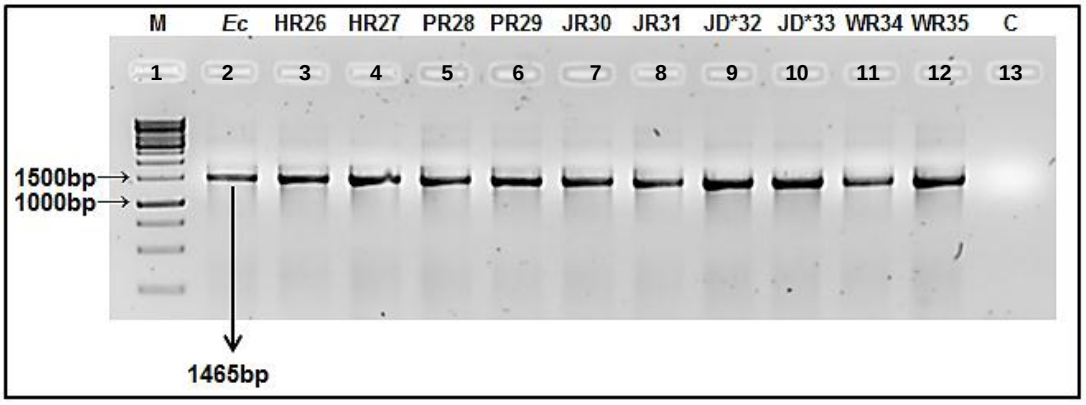

Figure 12: Negative image of the 1.5% (w/v) agarose gel indicating the 16S monoplex PCR results for March 2012. The first lane contains the 1 Kb molecular marker (M). The second lane contains the positive control (*E. coli* - ATCC 10536 Lane 3 to lane 12 indicates the PCR products of the various samples. The last lane (13) contains the no template control. The 16S gene has a fragment size of 1465 bp.

June 2012

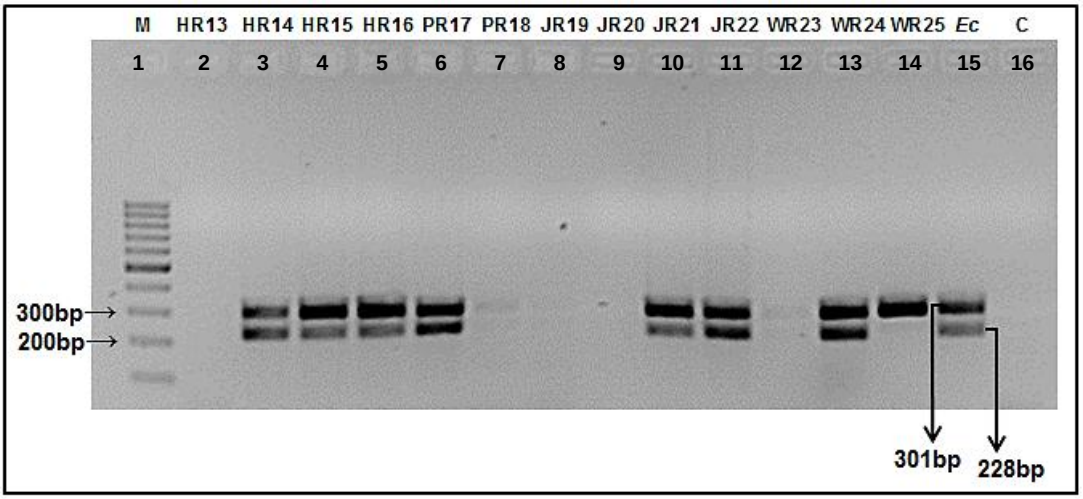

Figure 13: Negative image of the 1.5% (w/v) agarose gel indicating the multiplex PCR results for June 2012. The first lane contains the 100 bp molecular marker (M). Lane 2 to lane 14 indicates the PCR products of the various isolates. Lane 15 contains the positive control (*E. coli* - ATCC 10536). The last lane (16) contains the no template control. The *mdh* gene has a fragment size of 301 bp and the *lacZ* gene 228 bp.

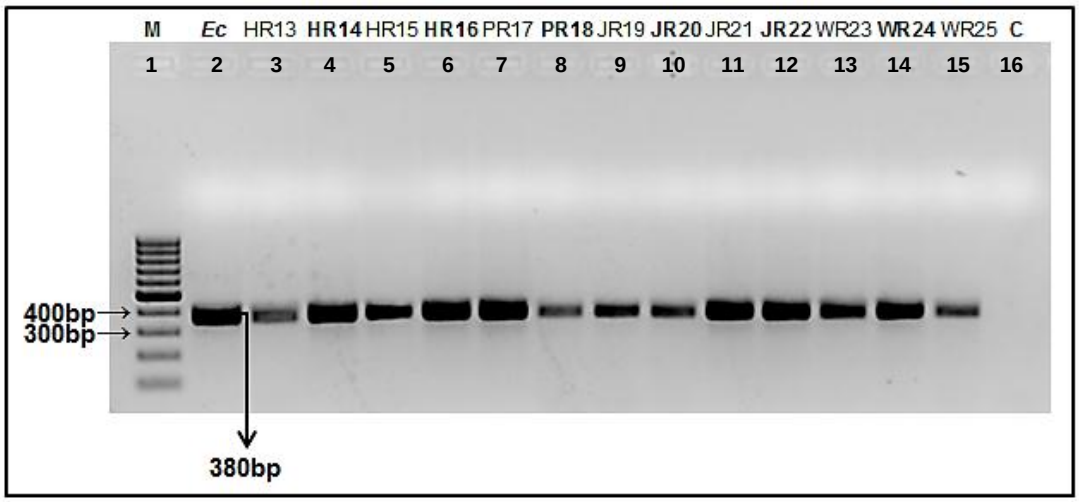

Figure 14: Negative image of the 1.5% (w/v) agarose gel indicating the *uidA* monoplex PCR results for June 2012. The first lane contains the 100 bp molecular marker (M). The second lane contains the positive control (*E. coli* - ATCC 10536). Lane 3 to lane 15 indicates the PCR products of the various isolates. The last lane (16) contains the no template control. The *uidA* gene has a fragment size of 380 bp.

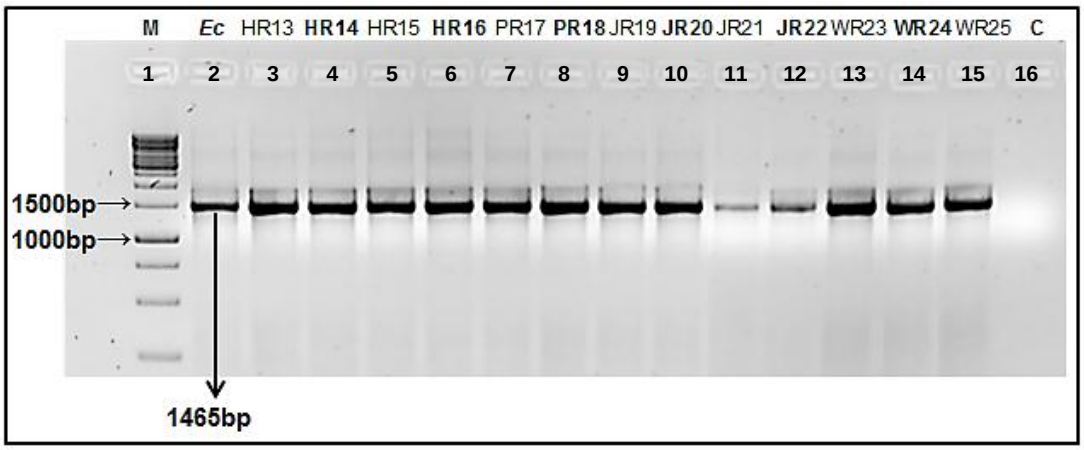

Figure 15: Negative image of the 1.5% (w/v) agarose gel indicating the 16S monoplex PCR results for June 2012. The first lane contains the 1 Kb molecular marker (M). The second lane contains the positive control (*E. coli* - ATCC 10536 Lane 3 to lane 15 indicates the PCR products of the various samples. The last lane (16) contains the no template control. The 16S gene has a fragment size of 1465 bp.

APPENDIX C

Midvaal Company Reports for March 2012

(see next four pages)

*NWU H1: Hartswater raw water sample.

*NWU H4: Hartswater drinking water sample.

*NWU P1: Pampierstad raw water sample.

*NWU P2: Pampierstad drinking water sample.

*W1 - NWU Mikro: Warrenton raw water sample.

*W4 - NWU Mikro: Warrenton drinking water sample.

*J1 - NWU Mikro: Jan Kempdorp raw water sample.

*J4 - NWU Mikro: Jan Kempdorp drinking water sample.

MIDVAAL Water Company

sanas Testing Laboratory T0132

SCIENTIFIC SERVICES

PO Box 31, Stilfontein, 2550 ♦ Tel: 018 482 9500 ♦ Fax: 018 482 1110

lab@midvaalwater.co.za ♦ www.midvaalwater.co.za

TEST REPORT

Submitter: NWU Dept. Mikrobiologie
Private Bag x6001
2520 Potchefstroom

Sample type: Water sample
Date Received: 2012-03-29
Debit Number: NWU002
Certificate Number: 2012-14435

Analyses Results

Sample number			14435	14436	14437	14438	14439
Sample date			2012-03-29	2012-03-29	2012-03-29	2012-03-29	2012-03-29
Identification on container			Gans	War	NWU H1	NWU H4	NWU P1
Determinand	**Unit**	**Method Number**					
Chemical - Organic and Related Determinands							
Chemical Oxygen Demand	mg/L	N9	20	37	23	<20	21
Microbiological Determinands							
Bacteria, coliform, E. coli - Colilert®	MPN/100 ml	BL5	19	1 733	114	Not detected	2
Bacteria, coliform, faecal	cfu/100 ml	BL1	2	1 300	200	Not detected	9
Bacteria, coliform, total - Colilert®	MPN/100 ml	BL5	>2 420	>2 420	>2 420	Not detected	488

If a test is marked ** in this report it is not included in the SANAS Schedule of Accreditation for this laboratory.

Signed:_____

J W D Pietersen (Head: Scientific Services)

Date of issue: 2012-04-04

This test report is only valid if signed.

This report relates only to the samples supplied to Midvaal Water Company Scientific Services.

MIDVAAL Water Company

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SCIENTIFIC SERVICES

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lab@midvaalwater.co.za ♦ www.midvaalwater.co.za

TEST REPORT

Submitter:	NWU Dept. Mikrobiologie Private Bag x6001 2520 Potchefstroom	**Sample type:** **Date Received:** **Debit Number:** **Certificate Number:**	Water sample 2012-03-29 NWU002 2012-14440

Analyses Results

Sample number			14440
Sample date			2012-03-29
Identification on container			NWU P2
Determinand	**Unit**	**Method Number**	
Chemical - Organic and Related Determinands			
Chemical Oxygen Demand	mg/L	N9	<20
Microbiological Determinands			
Bacteria, coliform, E. coli - Colilert®	MPN/100 ml	BL5	Not detected
Bacteria, coliform, faecal	cfu/100 ml	BL1	Not detected
Bacteria, coliform, total - Colilert®	MPN/100 ml	BL5	Not detected

If a test is marked ** in this report it is not included in the SANAS Schedule of Accreditation for this laboratory.

Signed:_____

J W D Pietersen (Head: Scientific Services)

Date of issue: 2012-04-04

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This report relates only to the samples supplied to Midvaal Water Company Scientific Services.

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lab@midvaalwater.co.za ♦ www.midvaalwater.co.za

TEST REPORT

Submitter: NWU Dept. Mikrobiologie
Private Bag x6001
2520 Potchefstroom

Sample type: Water sample
Date Received: 2012-04-25
Debit Number: NWU002
Certificate Number: 2012-15890

Analyses Results

Sample number			15890	15891	15892	15893	15894
Sample date			2012-04-25	2012-04-25	2012-04-25	2012-04-25	2012-04-25
Identification on container			Gans	Warrenton	W1 - NWU Mikro	W4 - NWU Mikro	J1 - NWU Mikro
Determinand	**Unit**	**Method Number**					
Chemical - Organic and Related Determinands							
Chemical Oxygen Demand	mg/L	N9	<20	51	20	26	21
Microbiological Determinands							
Bacteria, coliform, E. coli - Colilert®	MPN/100 ml	BL5	Not detected	291	9	Not detected	13
Bacteria, coliform, faecal	cfu/100 ml	BL1	Not detected	1 100	8	Not detected	10
Bacteria, coliform, total - Colilert®	MPN/100 ml	BL5	>2 420	>2 420	1 553	Not detected	866

If a test is marked ** in this report it is not included in the SANAS Schedule of Accreditation for this laboratory.

Signed:_____

J W D Pietersen (Head: Scientific Services)

Date of issue: 2012-05-08

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This report relates only to the samples supplied to Midvaal Water Company Scientific Services.

MIDVAAL Water Company

sanas Testing Laboratory T0132

SCIENTIFIC SERVICES

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lab@midvaalwater.co.za ♦ www.midvaalwater.co.za

TEST REPORT

Submitter: NWU Dept. Mikrobiologie
Private Bag x6001
2520 Potchefstroom

Sample type: Water sample
Date Received: 2012-04-25
Debit Number: NWU002
Certificate Number: 2012-15895

Analyses Results

Sample number			15895
Sample date			2012-04-25
Identification on container			J4 - NWU Mikro
Determinand	**Unit**	**Method Number**	
Chemical - Organic and Related Determinands			
Chemical Oxygen Demand	mg/L	N9	<20
Microbiological Determinands			
Bacteria, coliform, E. coli - Colilert®	MPN/100 ml	BL5	326
Bacteria, coliform, faecal	cfu/100 ml	BL1	400
Bacteria, coliform, total - Colilert®	MPN/100 ml	BL5	387

If a test is marked ** in this report it is not included in the SANAS Schedule of Accreditation for this laboratory.

Signed:_____

J W D Pietersen (Head: Scientific Services)

Date of issue: 2012-05-08

This test report is only valid if signed.

This report relates only to the samples supplied to Midvaal Water Company Scientific Services.