The Use of Ultraviolet Light Alone, or in Combination with Cavitational Flow and Ultrasonic Devices, to Inactivate Protozoan Cysts and Oocysts in the Small and Large Scale Treatment of Drinking Water

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by

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EXECUTIVE SUMMARY

Infections by protozoan, specifically *Giardia* and *Cryptosporidium parvum* are now accepted as a common world-wide cause of acute, self-limiting diarrhoeal disease in the human host. The faecal-oral route, transmission of protozoan amongst humans and animals and the consumption of contaminated water are the principal modes of transmission. According to recent statistics on surface water in the USA, respectively some 88 and 98 percent of the sources examined contained *Giardia* cysts and *Cryptosporidium* oocysts. The importance of the observation is the relative inefficiency of conventional water treatment processes to eliminate these organisms. This is emphasised by the fact that respectively 17 and 27 percent of treated water after filtration contain cysts and oocysts of these protozoan (Clancy, 1999; LeChevallier, 1995). Microfiltration and ultrafiltration are barriers to remove these contaminants effectively (Jacangelo, 1997). However, a comprehensive treatment regime, inclusive of disinfection is required when relying on conventional unit treatment processes and operations. Indications are that the removal of the cysts and oocysts is enhanced by the successful reduction of concentrations of suspended matter to produce water with a turbidity as low as possible, preferably < 0,1 NTU (Gregory, 1994). Protozoan cysts and oocysts could be up to 80 times more resistant towards chlorine than bacteria and viruses (Wallis, 1988) and one to three orders more resistant to ozone at low temperatures (Wickramanayake, 1984). Very few water treatment plants in South Africa are equipped to ozonate water. Ozonation is expensive to install and operate, and therefore most water treatment plants in South Africa rely on chlorination for the disinfection of drinking water. Not only may facilities not be adequately equipped but the formation of high concentrations of disinfection by-products, such as trihalomethanes (THM's), which may be mutagenic or carcinogenic, should also deserve consideration.

Karanis (1992) tested the efficiency of ultra-violet (UV) light to inactivate *Giardia* and *Cryptosporidium* cysts and oocysts and found the *Giardia lamblia* cysts to be less sensitive than *Escherchia coli* but more sensitive than *Trichomonas vaginals* towards UV radiation at 254nm. He estimated the required UV fluorescence dose to destroy *G lamblia* cysts as 30 mJ/cm^2. Clancy (1999) demonstrated that UV treatment could be very effective in destroying cysts and oocysts present in water. The UV doses needed to achieve a log 3 reduction of these cysts were 19-41mJ/cm^2.

UV treatment presents an ideal method of instantaneous disinfection, although it does not produce a residual concentration to maintain the microbiological water quality after disinfection.

The aims of the project were:

1 To determine the effect of UV light by itself or / and in combination with either flow cavitation and ultrasound treatment on protozoan cysts and oocysts in water.

2 Establish the minimum UV light energy required to inactivate the cysts and oocysts.

3 Propose design and operating guidelines for the use of UV treatment systems, with or without additional treatment, for small and large installations.

4 Determine the wider benefits that could be obtained by the use of UV disinfection in water treatment.

The following conclusions can be made from experiments conducted to determine the effect of ultraviolet light by itself and in combination with either/or flow cavitation and ultrasound treatment on protozoan cysts or oocysts and other micro-organisms, in water. Although it is believed that the following conclusions hold true in general, the values mentioned for the different treatment methods may only be applicable for the equipment used under the experimental conditions.

- Hydrodynamic cavitation alone is not successful in inactivating bacteria and bacteriophages.

- Of the treatment options tested Ultrasound and UV alone or in combination with each other are the most effective methods.

- A retention time of 2.26s and UV dose of 23.93mW.s/cm^2 were the optimum conditions for the inactivation of bacteria in the equipment used and under the prevailing experimental conditions. A retention time of 2.26s and UV dose of 23.93mW.s/cm^2 were the optimum conditions for the inactivation of bacteria in the equipment used and under the prevailing experimental conditions. This optimum UV dose correspond with UV doses needed for the effectively inactivation of chlorine resistant enteroviruses (25 mW.s/cm^2). This dosage is lower than the dosages of 63 mW.s/cm^2 used by Rice and Hoff (1981) to reduced excystation of *Giardia Lamblia* by 90% and dosages of 41 mW.s/cm^2 used by Bukhari *et al* (1999) for >4log inactivation of *Cryptosporidium parvum* and in the same range as the 19 mW.s/cm^2 for a 3.9-log inactivation of *Cryptosporidium* oocysts (Bukhari *et al,* 1999).

- The optimum retention time for ultrasound is 5.52s to deliver a reduction in standard plate counts (SPC).

- A retention time of 2.26s and UV dose of 23.93mW.s/cm^2 were the optimum conditions for the inactivation of bacteria using UV and ultrasound as treatment option.

- Of all the organisms tested *Clostridium* was the most resistant to the experimental treatment procedures tested. This may be due to spore formation. Best reductions were observed after treatment with UV and Ultrasound in combination.

- Further treatment of clarified and filtered water with any of the possible treatment options resulted in higher inactivation of bacteria and bacteriophages than treatment of raw Klip River water with the same treatment options.

- All the treatment regimes that included either UV alone and/or in combination with cavitation/ultrasound showed higher reductions in bacterial counts than the options of cavitation treatment alone or in combination with ultrasound treatment.

- Ultrasound treatment shows promise for high bacterial and bacteriophage inactivation and was comparable with bacterial and bacteriophage inactivation by the application of only UV treatment.

- UV/cavitation treatment as well as UV/cavitation/ultrasound treatment did not achieve as high a bacterial and bacteriophage reduction as UV alone or in combination with ultrasound. In this case the UV dose was much less because of the constraints imposed by the cavitation, which reduced the reaction time and lead to lower UV doses in the treatment unit.

- UV was the most effective treatment option with ultrasound second best.

- *Clostridium*, which is a spore forming bacteria, showed lower reductions than other bacteria and bacteriophages.

- Results obtained with protozoan cysts and oocysts were difficult to interpret because of the characteristics of the stock dose culture. Formalised cysts, containing 70% viable cysts, were used in the stock suspension. The 70% viability leads to an uncertainty in recoveries percentages and the percentage DAPI positive/negative results. Results from experiments with life cysts can be used for interpretation on the effectiveness of treatment options.

- An important observation made was that the shape of the *Giardia* cysts changed when they were treated with ultrasonic and hydrodynamic cavitation.

- After the cysts and oocysts were subjected to cavitation, the whole cyst stained sky blue and instead of only the nucleus in these instances the DAPI staining was very faint.

- On interpretation of the results of the mouse infectivity tests it was found that UV and Ultrasound contribution war the best to inactivate *Cryptosporidium.*

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"THE USE OF ULTRAVIOLET LIGHT ALONE, OR IN COMBINATION WITH CAVITATIONAL FLOW AND ULTRASOUND DEVICES, TO INACTIVATE PROTOZOAN CYSTS AND OOCYSTS IN THE SMALL AND LARGE SCALE TREATMENT OF DRINKING WATER."

The Steering Committee responsible for this project consisted of the following:

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ABBREVIATIONS AND GLOSSARY

UV	Ultraviolet
mg/ℓ	milligrams per litre
$mW.s/cm^2$	Milliwatts second per square centimetre (equivalent to J/s)
J/s	Joule per second
DAPI	4'6 diamidino-2-phenyl indole
FITC	Fluorescein Isothionate
DIC	differential interference contrast
Clarified water	Water collected from the purification plant after the removal of suspended matter by coagulation, flocculation and sedimentation
Filtered water	Water collected from the purification plant after clarification and filtration before disinfection
SPC	Standard plate count (CFU/mℓ)
CFU	Colony forming units
TC	Total coliforms (TC/100mℓ)
FC	Faecal coliforms (FC/100mℓ)
PFU	Plaque forming units

Chapter 1

INTRODUCTION

1.1 MOTIVATION

Infections by protozoa, specifically *Giardia* and *Cryptosporidium parvum* are now accepted as a common world-wide cause of acute, self-limiting diarrhoeal disease in the human host. The faecal-oral route, transmission of protozoan amongst humans and animals and the consumption of contaminated water are the principal modes of transmission. According to recent statistics on surface water in the USA, respectively some 88 and 98 percent of the sources examined contained *Giardia* cysts and *Cryptosporidium* oocysts. The importance of the observation is the relative inefficiency of conventional water treatment processes to eliminate these organisms. This is emphasised by the fact that respectively 17 and 27 percent of treated water after filtration contain cysts and oocysts of these protozoan (Clancy, 1999; LeChevallier, 1995). Microfiltration and ultrafiltration are barriers to remove these contaminants effectively (Jacangelo, 1997). However, a comprehensive treatment regime, inclusive of disinfection is required when relying on conventional unit treatment processes and operations. Indications are that the removal of the cysts and oocysts is enhanced by the successful reduction of concentrations of suspended matter to produce water with a turbidity as low as possible, preferably < 0,1 NTU (Gregory, 1994). Protozoan cysts and oocysts could be up to 80 times more resistant towards chlorine than bacteria and viruses (Wallis, 1988) and one to three orders more resistant to ozone at low temperatures (Wickramanayake, 1984). Very few water treatment plants in South Africa are equipped to ozonate water. Ozonation is expensive to install and operate, and therefore most water treatment plants in South Africa rely on chlorination for the disinfection of drinking water. Not only may facilities not be adequately equipped but the formation of high concentrations of disinfection

by-products, such as trihalomethanes (THM's), which may be mutagenic, should also deserve consideration.

Karanis (1992) tested the efficiency of ultra-violet (UV) light to inactivate *Giardia* and *Cryptosporidium* cysts and oocysts and found the *Giardia lamblia* cysts to be less sensitive than *Escherchia coli* (*E.coli*) but more sensitive than *Trichomonas vaginals* towards UV radiation at 254nm. He estimated the required UV fluorescence dose to destroy *G lamblia* cysts as 30 mJ/cm^2. Clancy (1999) demonstrated that UV treatment could be very effective in destroying cysts and oocysts present in water. The UV doses needed to achieve a log three reduction of these cysts were 19-41mJ/cm^2.

UV treatment presents an ideal method of instantaneous disinfection, although it does not produce a residual concentration, to maintain the microbiological water quality after disinfection.

UV treatment:

- Has been proven to be to be effective in inactivating protozoan cysts and oocysts
- Does not contribute to the formation of disinfection by-products
- Can be retrofitted on existing treatment plant
- Enjoys low maintenance and running costs
- Can be used in combination with disinfectants such as chlorine that produces long lasting residuals

UV disinfection can be applied effectively in rural areas for small applications as very little power is consumed and little operating or maintenance input is required.

Further research is required to investigate the principle of UV disinfection and to determine design and operating criteria for UV disinfection systems to inactivate protozoan cysts and oocysts.

1.2 PROJECT AIMS

5 To determine the effect of UV light by itself or / and in combination with either flow cavitation and ultrasonic treatment on protozoan cysts and oocysts, in water.

6 Establish the minimum UV light energy required to inactivate the cysts and oocysts.

7 Propose design and operating guidelines for the use of UV treatment systems, with or without additional treatment, for small and large installations.

8 Determine the wider benefits that could be obtained by the use of UV disinfection in water treatment.

Chapter 2

LITERATURE REVIEW

2.1 INTRODUCTION

The quality of drinking water must be to such that it does not give rise to any health hazards. Such hazards may be caused either by chemical or by microbial contamination. Disinfection of water and wastewater with UV radiation appears to be a potential alternative to the use of chemicals such as chlorine as disinfectant. With the use of chlorine as a disinfectant a possibility exists that by-products may form during disinfection, which could potentially be toxic, mutagenic or carcinogenic (Li JW, *et al*, 1996; Oppenheimer, *et al*, 1997). These concerns and the fact that germicidal UV radiation does not produce undesirable by-products and is effective in inactivating a variety of micro-organisms (Chang, *et al*, 1985) have contributed to the interest in UV light as a disinfectant for drinking and waste water (Cairns, *et al*, 1995).

A number of pathogens particularly spore-forming bacteria and micro-organisms with encapsulating structures such as *Cryptosporidium and Giardia spp.*, are far more resistant towards chlorine disinfection than the indicator organisms used traditionally (Ransome, *et al*, 1995). Filtration has been considered as the only effective means of removing *Cryptosporidium* organisms from drinking water supplies due to the resistance of oocysts to chlorine-based disinfectants. Ozone and UV are now considered excellent alternatives for oocyst inactivation. Although relatively high UV doses are required to inactivate *Giardia* and *Cryptosporidium spp.* (Cairns, 1995; Clancy, 1999) it is more effective than most chemical disinfectants at maximum permissible concentrations.

Advanced oxidation processes are disinfection methods, which could be used for primary disinfection. These processes involve the generation of highly

reactive free radical intermediates such as the hydroxyl radicals. Advanced oxidation processes are potentially beneficial as pre-treatment steps and compliment other treatment processes.

2.2 DISINFECTION METHODS AND MICROBIOLOGY

2.2.1 Theory of disinfection

The chemical nature of the aquatic environment plays a major role in establishing and supporting the aquatic population. The role of the water purification industry is to change the aquatic environment so that it is fit for human consumption. In most disinfection operations, the destruction of micro-organisms is a gradual process that involves a series of physical, chemical and biochemical steps. Various disinfection models have been proposed based on laboratory data and verified with field data in an effort to predict the outcome of the disinfection (Montgomery; 1985).

Although a great deal of work has recently been done on modelling disinfection processes, the main theory of chlorination used today is still based on the Chick or Chick-Watson disinfection model. Chick's law (Trussel and Chao, 1977, Haas and Karra, 1984) expresses the rate of micro-organism destruction in terms of a first-order chemical reaction:

$$\ln\left(\frac{N_t}{N_0}\right) = -k^a t \qquad (2.7)$$

where:

N_t = number of organism present at time t

N_0 = number of organism present at time 0

k^a = rate constant characteristic of the type of disinfectant

t = time

Chick-Watson model:

$$\ln\left(\frac{N_t}{N_0}\right) = -k^b C_d^n t \qquad (2.8)$$

where:

k^b = rate constant characteristic of the type of disinfectant

C_d = residual disinfection concentration

n = reaction order

Unfortunately, the Chick-Watson model is of limited use in most practical disinfection processes. The rate of micro-organism destruction generally does not remain constant. It rather increases or decreases with time depending on the type of micro-organism, the varying concentration or the form of disinfectant used, as well as other operating conditions (Montgomery, 1985).

Disinfection methods can be divided into three groups:

- Chemical methods - which use strong oxidative chemicals (such as HOCl, O_3, etc.) for the destruction of micro-organisms,
- Physico/chemical methods – which includes methods such as heat treatment, cavitation and ultraviolet radiation, and
- Physical methods - which includes methods such as filtration.

2.2.1.1 Chemical disinfection methods

Strong oxidants are used in water treatment as disinfectants because of their ability to cause destruction of the cell wall and interfering with enzyme reactions. The microbiocidal effect, which is the ability of a disinfectant to kill micro-organisms, corresponds approximately to the relative oxidation power of the compound. The order of oxidants used as disinfectants, arranged in order of their bacteriocidal efficiency is: $HOCl<ClO_2<O_3$. The main disadvantages of these oxidants are potential THM formation, taste and

odours in the case of HOCl and the absence of persistent residual of O_3 after dosing.

According to S Winship (1999) when advanced oxidation processes are used in water disinfection organic pollutants can be completely mineralised to carbon dioxide, water and small amounts of acids if the oxidant concentrations are high enough. However if the reaction time is extensive complete mineralisation may not be achieved.

2.2.1.2 Physico/chemical disinfection methods

Physico/chemical methods such as heat treatment is well known methods for the sterilisation of surgical instruments or disinfection of water by boiling. This method is however not effective for the use of disinfecting water of large purification works. In recent years research has been done on other physical methods such as the use of ultraviolet light and cavitation, which include hydrodynamic cavitation and acoustic cavitation for the use as disinfectants.

This project's main focus is on the effective use of the physico/chemical methods alone or in combination for the destruction of micro-organisms, bacteria, bacteriophages and protozoan cysts and oocysts. The physical methods tested are ultraviolet light by itself and in combination with either/or flow cavitation and ultrasound treatment.

2.2.1.3 Physical disinfection methods

Filtration with micro and ultra filtration is acceptable methods for the treatment of water and wastewater. This method is used in various forms for removal of suspended matter and micro-organisms from water and wastewater.

Physical methods are not included in the project objectives and therefore will not be discussed further.

2.3 UV DISINFECTION

2.3.1 Theory, principles and requirements for UV systems

UV radiation forms part of the electromagnetic spectrum and in the wavelengths ranging from 240 to 280 nm is effective for the inactivation of micro-organisms by causing irreparable damage to their nucleic acid (Lykins and Griese, 1986; Wolfe, 1991; Cairns, 1995). The most severe damage to the nucleic acid occurs at a wavelength of 260 nm and the damage incurred is proportional to the UV dose (W.s/cm^2), which is the product of the intensity of the UV radiation (W/cm^2) and the exposure time (seconds).

UV systems can be divided into two general classes:

- Continuous-wave (CW) emission, and
- Pulsed emission systems.

Pulsed systems produce high intensity pulses from xenon-gas flash lamps, while continuous-wave systems produce lower intensity continuous waves from mercury vapour lamps.

The two main types of continuous-wave UV systems that are commercially available are those that use low pressure mercury vapour lamps, and those that use medium pressure mercury vapour lamps (Combs, *et al*, 1989).

The low-pressure lamps produce a narrow band of radiation almost exclusively at a wavelength of 253.7 nm, which is close to the maximum biocidal wavelength of 260nm, however they only convert approximately 40% of the power input as emitted energy at this wavelength (Mofidi *et al*, 2001; Wolfe, 1991). The medium pressure lamps emit a much broader band of UV light, but their overall energy output is significantly higher than that of the low-pressure lamps (Mofidi *et al*, 2001). The low-pressure lamps are however considered by some to be the most efficient source of UV radiation for disinfection purposes (Wolfe, 1991).

The average intensity and the ability to deliver a specific UV dose are affected by the design of the UV system. The selection of an appropriate lamp is only one factor in the total design. Other important considerations are:

a) Optimisation of the hydraulic behaviour around the lamp sleeve during cleaning and while the cleaning mechanism is at rest, (Cairns, 1995),

b) Development of a reliable and effective cleaning mechanism for the UV lamp, (Cairns, 1995),

c) Ensuring compatibility of the selected lamps with available and/or new ballast designs which can provide reliability and process advantages, (Cairns, 1995),

d) Ensuring harmony of lamp configuration with reactor and lamp module designs which provide process advantages and operator convenience (Cairns, 1995; Warne, S, 1986), and

e) Efficiency of energy utilisation (Warne, S, 1986).

Therefore reactor geometries are important with respect to achieving maximum UV light intensity, distribution and hydraulic characteristics, thus specific reactor types must be chosen for a specific application to provide effective disinfection.

Photochemistry is relatively independent of pH, temperature, ionic strength, and therefore variations in these water quality parameters have minimal impact on disinfection of the microbes. Only the UV-absorbing components of dissolved organic matter, colloids and suspended solids will reduce the intensity of light within a well engineered UV system and require an inversely proportional increase in exposure time to compensate for reduced intensity when delivering a given UV dose (Cairns, 1995).

The dose of UV delivered within an UV reactor is the mathematical product of the average intensity (I_{ave}) of light within the reactor multiplied by the retention time (t) of the water passing through the reactor. $D = I_{ave} \times t$. Dose units are

expressed as $mW.s/cm^2$ or $\mu W.s/cm^2$. The actual UV dose received by an organism depends on a number of factors, such as:

- Energy output of the UV lamp (Warne, S, 1986)
- The flow rate of the water through the UV system, that influence the retention time, (Warne, S, 1986)
- The transmission efficiency of the water being treated,
- Number of organisms, and
- The geometric design of the UV radiation chamber.

Sensitivity to UV disinfection of certain species of micro-organisms can vary according to strain, growth medium, life stage of the culture, and influences of the plating medium on the repair of sublethal damage (Chang, *et al*, 1985).

The advantages of UV treatment are:

- An environmentally safe, non-chemical, physical process, (Cairns, *et al*, 1995; Warne, S, 1986),
- A safe and simple system for operator to use, (Cairns, *et al*, 1995; Warne, S, 1986),
- No by-product formation, (Cairns, *et al*, 1995)
- Able to achieve the required disinfection level in a few seconds, (Cairns, *et al*, 1995)
- Able to implement in flow-through channels without the need for contact tanks, thus low space requirements, (Cairns, *et al*, 1995)
- Low energy and maintenance costs, (Cairns, *et al*, 1995)
- Full automatic operation, (Cairns, *et al*, 1995),

- More effective than chlorination on a wide range of organisms including some viruses which may be resistant, especially to chloramines (Cairns, *et al*, 1995),

- UV is less capital intensive than chlorination and dechlorination (Warne, S, 1986)

- UV maintenance costs is very little because of the simplicity in design and low involvement of maintenance personnel (Warne, S, 1986).

Major limitations of UV radiation are the lamp sleeve design, quartz jacket fouling, ageing of the lamp, the concentration of suspended solids and of micro-organisms, the depth of the water column being irradiated and the transmissivity and turbidity of the water.

A disadvantage of using UV lies in the ability of micro-organisms to repair damage caused to their DNA by UV radiation if sub-lethal doses are administered. UV disinfection also leaves no disinfection residual, thus addition of other disinfectants are necessarily to maintain microbiological quality and to prevent aftergrowth of micro-organisms in a distribution system.

Inefficiency of UV systems is mostly due to factors misunderstood. Such as the UV dose required killing different organisms, effect of water quality variations, and the rate of lamp deterioration. Failures are mostly because of the inability to determine the UV dose in a reliable and continuous manner (Warne, S, 1986).

2.3.2 Effect of UV on Micro-organisms

UV disinfection relies on the principle that at 254 nm UV light alters the nitrogenous heterocyclic components of DNA and RNA, causing molecules to form new bonds resulting in dimers, which can prevent the micro-organisms from replicating (Mofidi *et al*, 2001; Parrotta and Bekdash, 1998; Wolfe, 1991).

The sensitivity of many micro-organisms to UV can be influenced by factors such as the growth medium, the life stage of the culture, the strain of micro-

organism and photo reactivation (Harris *et al*, 1987). The majority of bacteria require fairly low UV doses for inactivation, these being in the range of 2 to 6 mW.s/cm^2. Viruses are more resistant toward UV light than bacteria. Parasitic cysts appear to be more resistant to UV radiation than other organisms.

Chlorine resistant enteroviruses are effectively inactivated by UV doses of 25 mW.s/cm^2. Ransome *et al* (1993) found that UV dose of 80 mW.s/cm^2 was needed to reduce excystation of *Cryptosporidium parvum* by 90%. Rice and Hoff (1981) found that an UV dose of 63 mW.s/cm^2 reduced excystation of *Giardia Lamblia* by 90%. Bukhari *et al* (1999) found that dosages as low as 41 mW.s/cm^2 are needed for >4log inactivation of *Cryptosporidium parvum* and dosage as low as 19 mW.s/cm^2 for a 3.9-log inactivation of *Cryptosporidium* oocysts. Mofidi *et al* (2001) found that dosages as low as 11 mW.s/cm^2 are needed for 2 log (99%) inactivation of infectious *Cryptosporidium parvum.* Clancy *et al* (2000) showed that low dosages in the order of 3 mW.s/cm^2 of medium pressure UV light inactivate *Cryptosporidium* oocysts in the order of 3.4-log. It was shown that there is not a significant difference between low and medium pressure UV light when inactivating *Cryptosporidium* oocysts.

Other important dose / effect relationships are reported by Steve Warne (1986) where the D_{10} or the dose required for one log reduction in concentration of the following organisms are:

Streptococcus viridians	2.0 mWs/cm^2
Clostridium tetani	4.9 mWs/cm^2
Salmonella enteritidis	4.0 mWs/cm^2
Staphylococcus aureus	2.2 mWs/cm^2
Polio virus	24.0 mWs/cm^2

These D_{10} values are generally higher than what is required to inactivate *E.coli (*3.0 mWs/cm^2), to the same level.

2.4 CAVITATION

2.4.1 Theory and principles of cavitation

Cavitation is defined as the formation, expansion and implosion of bubbles in a liquid (Lehman *et al*, 1964; Winship, S, 1999). Bubbles are formed due to the reduction of local pressure of the liquid at a specific temperature. The cavitation threshold was theoretically calculated to be at a pressure of 1 013 MPa for the formation of the bubbles. Experimental observations however reports cavitation thresholds of up to 20 260 kPa (Winship, 1999).

It is important to note that the cavities collapse when the local pressure is greater than the vapour pressure of the liquid (Botha, 1993; Lehman *et al*, 1964; Winship, 1999). This leads to the release of high amounts of energy, which is claimed to generate, sufficient localised temperatures and pressures to form hydroxyl and hydrogen radicals from the thermal dissociation of water molecules.

With the implosion of these bubbles noise is generated over a large range of frequencies and the larger the size of the bubble the lower the frequency of noise generated (Botha, 1993).

According to Winship (1999) cavitation can be produced by four different methods in liquids:

- Hydrodynamic cavitation is produced as a result of pressure variations in a flowing liquid due to the geometry of the system
- Acoustic cavitation is produced by sound waves in a liquid, which cause pressure variations
- Optic cavitation is produced by photons of high intensity light (i.e. laser) that rupturing a liquid
- Particle cavitation is a result of any type of elementary particles, i.e. protons, rupturing a liquid.

2.4.1.1 Hydrodynamic cavitation

Cavity formation in flowing liquid occurs at the point of highest velocity and low pressure (Botha, 1993). There are three cases of flow cavitation:

a) Travelling Cavitation: Bubbles formed in the liquid, travels with the liquid as they expand and collapse.

b) Fixed cavitation: The cavity formed in the liquid is attached to a rigid boundary and remains in position in an unsteady state.

c) Vortex cavitation: Cavities formed in the cores of vortices, which form in a region of high shear.

In the Lazur-M3 system that was used in this study, travelling cavitation occurs. The onset of cavitation depends on the flow reduction, the scale of the apparatus and the geometry of the constriction.

2.4.1.2 Ultrasonic cavitation

Ultrasound represents a wide range of frequencies beyond human hearing, these frequencies range from 20 kHz to about 20MHz. Ultrasound is generated from mechanical or electrical energy via an ultrasonic transducer (Botha, 1993).

When ultrasound is applied to water subsequent chemical and physical reactions may result. Thus ultrasound can be used at a range of frequencies and intensities to form and collapse bubbles in the water stream to be treated (Botha, 1993; Mead *et al*, 1976; Neis, 2000; Neppiras *et al*, 1964). This phenomenon is called ultrasonic cavitation. At the collapsing site of the bubbles extreme temperatures (5000K) and high pressures (500bar) exists (Neis, 2000). These extreme temperatures and pressures are then responsible for the physical changes and pronounced chemical reactions (sonochemical reactions) occurring in the water.

Sonochemical reactions are characterised by the formation of radicals ($HO^{\cdot}$, $H^{\cdot}$) and hydrogen peroxide. The lifetime of the radicals is greater than the

lifetime of the bubble (Mead *et al*, 1976) and is therefore available for other reactions in the water. The nature of the sonochemical products is dependant on the:

- Acoustic power (intensity and frequency)(Botha, 1993; Neis, 2000; Mead *et al*, 1976),
- Attenuation of sound (Botha, 1993),
- Design of the isonation cell (Botha, 1993; Mead *et al*, 1976),
- Temperature (Botha, 1993; Neis, 2000; Mead *et al*, 1976),
- External pressure (Botha, 1993; Neis, 2000; Mead *et al*, 1976),
- Solvent characteristics (Botha, 1993), and
- Nature of the dissolved gas (Botha, 1993;Mead *et al*, 1976).

2.4.2 Bactericidal effect of cavitation

2.4.2.1 Hydrodynamic Cavitation

Hydrodynamic cavitation disrupts yeast and bacterial cells (Botha, 1993) and is known to produce the phenomenon of transient cavitation and associated forces. Based on this, the potential for cell destruction akin to that achieved by ultrasonic cavitation exists (Botha, 1993).

2.4.2.2 Ultrasonic Cavitation

The mechanism by which ultrasound inactivates bacteria has not been conclusively established. The following mechanisms have been proposed for cell disruption in a sound field within an aqueous media:

- Forces due to surface resonance of the cell wall, initiated by cavitation, cause mechanical fatigue (Botha, 1993; Neppiras *et al*, 1964).

- Shearing forces occur due to microstreaming of cell fluid (Botha, 1993; Neppiras *et al*, 1964).

- Pressures and pressure gradients result from the collapse of gas bubbles on or near the cell wall. Damage may result from single event or fatigue involving a threshold time (Botha, 1993; Neppiras *et al*, 1964).

- Radial resonance of bubble creates pressure and pressure gradients (Botha, 1993; Neppiras *et al*, 1964).

- Pressure or relative velocity effects resulting from the direct sound beam which are generated from compression and refraction of sound waves (Botha, 1993; Neppiras *et al*, 1964).

- Chemical attack in which a wide range of free radicals, especially $H^{\bullet}$ and $OH^{\bullet}$ radicals are formed in cavitating aqueous liquids. These compounds may attack the cell wall and weaken it to the point of rapture. However, it has been shown that free radical formation and cell rapture occur independently of one another (Botha, 1993; Neppiras *et al*, 1964).

- Combined chemical and mechanical attack (Botha, 1993; Neppiras *et al*, 1964).

2.4.3 CAV-Ox® an advanced combined oxidation process

2.4.2.1 CAV-OX®

The CAV-OX® was tested in a Water Research Commission funded project (Winship, 1995; 1999). This system employs UV radiation, hydrogen peroxide and hydrodynamic cavitation to degrade organic compounds present in water at milligram per litre levels by photolysis and oxidation.

2.5 CONCLUSIONS FROM LITERATURE REVIEW

The efficiency of any UV disinfection system is highly dependant on the water quality. The efficiency depends on the ability to pass ultra violet light through

water and the ability to quantify the energy losses experienced due to impurities in the water. These energy losses are measured as a percentage transmission value. Factors affecting this value are colour, dissolved minerals, turbidity, total hardness, BOD, organic matter and microbiological population.

Disinfection of water and wastewater with UV radiation appears to have the potential to be used in combination with chlorine. Relatively high UV doses (60-80 mW.s/cm^2) are required to inactivate *Giardia* and *Cryptosporidium spp.*, while chlorine resistant enteroviruses are effectively inactivated by rather low UV doses of 25 mW.s/cm^2.

Although UV disinfection leave no disinfectant residual, 5-log reduction in microbial counts can be achieved. Best performance is observed at high UV transmission values that would require shorter retention times.

UV systems perform excellent in disinfection processes specially concerning protozoan, viruses and coliphages and are highly recommended for the disinfection of low turbidity water sources.

Ultrasound and hydrodynamic cavitation employs different techniques to generate cavitation. Little literature is available on the effect of hydrodynamic cavitation on bacterial cell viability or the use of hydrodynamic cavitation for water treatment.

Chapter 3

THE CHEMICAL AND BIOLOGICAL EFFECT OF ULTRAVIOLET LIGHT BY ITSELF OR IN COMBINATION WITH EITHER/OR FLOW CAVITATION AND ULTRASOUND TREATMENT

3.1 METHODS AND EQUIPMENT

3.1.1 Objective

The aim of the investigation was to determine the influence of UV, ultrasound and hydrodynamic cavitation on water quality parameters. The investigation was conducted on two types of water *viz.* on raw water from the Klip River (K19) and on filtered Vaal Dam water. Klip River water was used because it contains high quantities of natural occurring bacteria and bacteriophages. The treated water was taken just after filtration at Vereeniging water purification plant. This water without any further treatment was used as a control in the experiments.

3.1.2 Materials and methods

The Lazur-M3 system (Figure 3.1-3.3) from SVAROG (Russia) was used in this project. This system consists out of three main parts that can be used separately or in combination with each other. The three main parts are a hydrodynamic cavitational unit, a UV lamp and ultrasound generator.

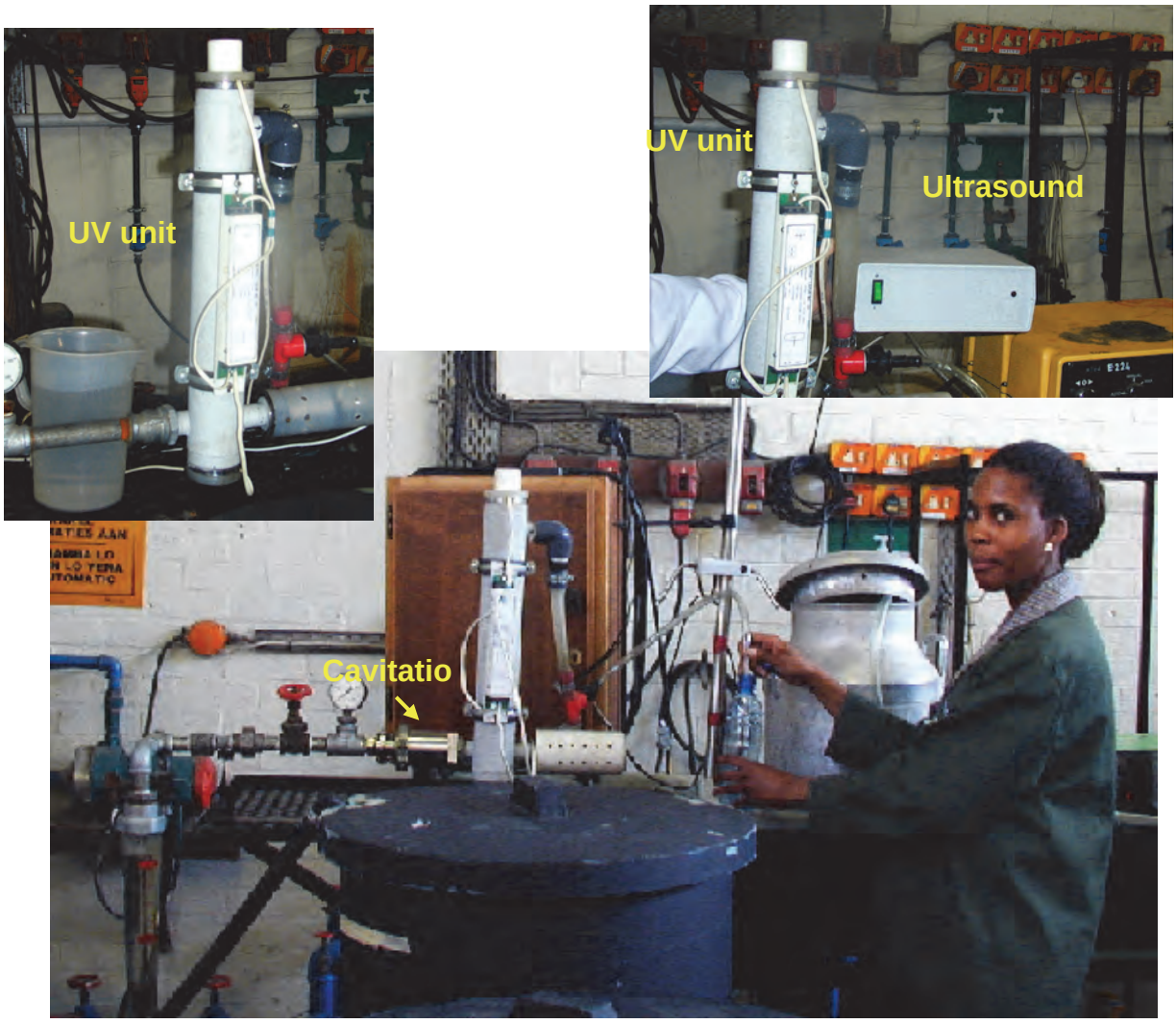

Figure 3.1: Lazur-M3 system

Figure 3.2 is a diagram of the Lazur M3 showing the hydrodynamic cavitation unit, the UV unit and the ultrasonic unit.

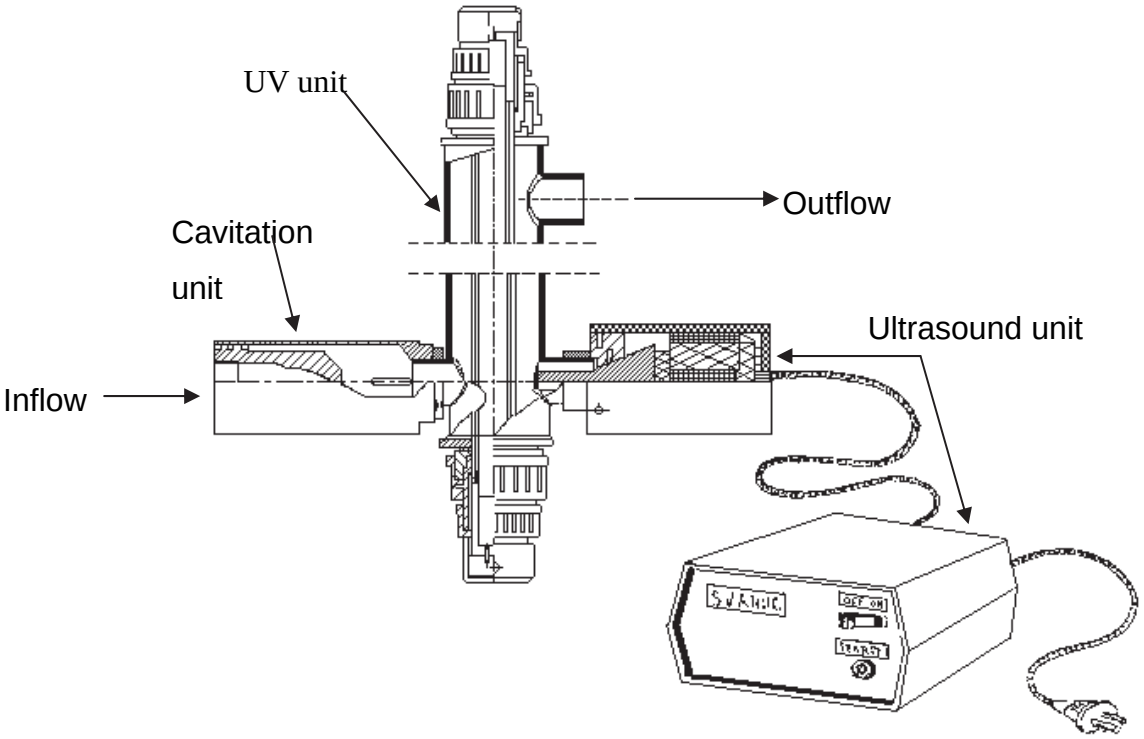

Figure 3.2: Diagram of Lazur M3 system

Figure 3.3 is a flow diagram of the experimental equipment used to exposed water that contained different micro-organisms to hydrodynamic cavitation, ultrasound and UV treatment. Water from the vessel that contained the stock suspension of micro-organisms was pumped through the treatment units. The pump and pipe work was arranged in such a way that the flow could be controlled from 0-4m^3/h by recycling or throttling the flow. The second pump was used to draw water from the secondary collecting vessel that contained the water and cysts that passed through the treatment units, through the Envirocheck cartage in which the (oo)cysts were retained.

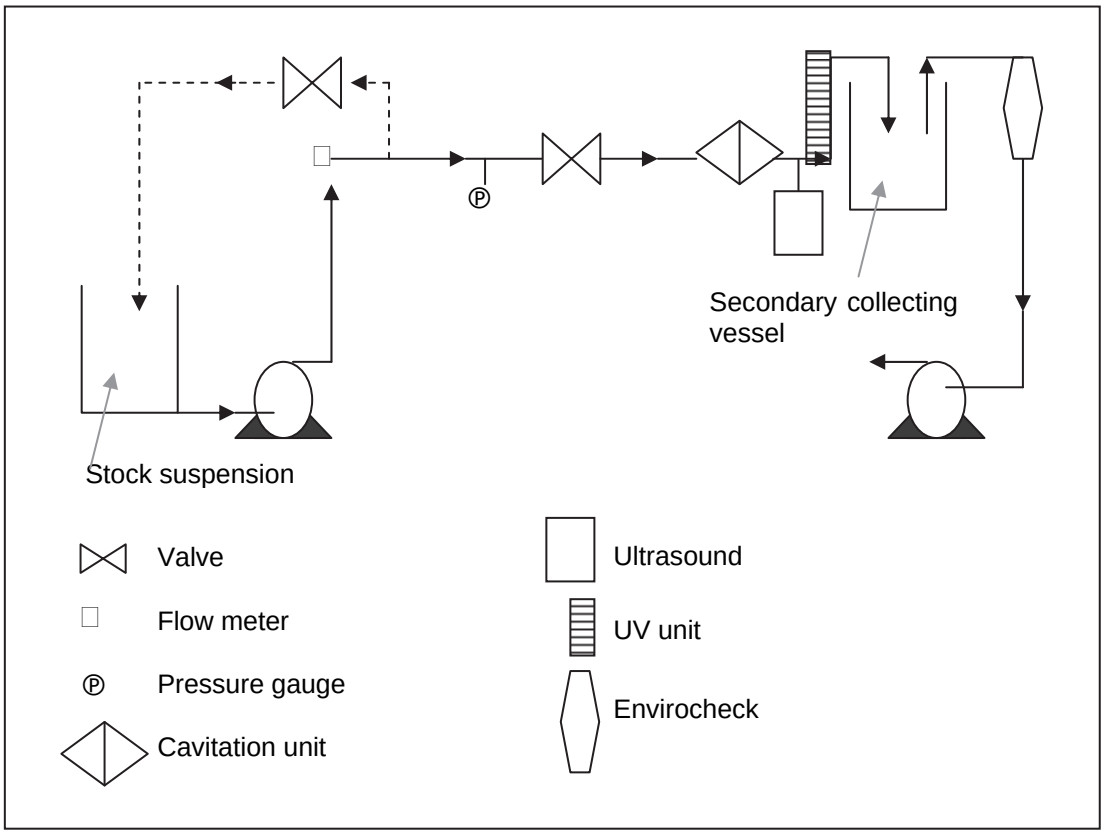

Figure 3.3: Flow diagram of the experimental equipment with Lazur-M3 stainless steel cavitation unit

Any configuration in which the three treatment units could be used is possible by selective switching off or removing one or more of the units as required. Operational conditions used in the experiments are summarised in Table 3.1. All experiments were repeated six times to ensure that reproducible results were obtained. Calibration of the UV was done by measuring the UV light intensity. The UV light intensity was measured when each experiment was conducted using a portable UV flux density meter provided by SVAROG (Russia).

Table 3.1: Operational conditions for the Lazur M3system

Flow rate	$3m^3/h$	$2m^3/h$	$1m^3/h$	$0.5m^3/h$
Pressure	3 bar			
Retention time	0.75s	1.13s	2.26s	5.52s
UV dose	$7.98mW.s/cm^2$	$11.97mW.s/cm^2$	$23.93mW.s/cm^2$	$47.86mW.s/cm^2$

3.1.3 Analysis procedure

Chemical water quality parameters determined were pH, turbidity (NTU), electrical conductivity (mS/m), UV absorbance (%), methyl orange (mo) – alkalinity (mg/l $CaCO_3$), hardness (mg/l $CaCO_3$) and chlorine demand (mg/l Cl_2). Biological water quality parameters tested were Standard plate counts (SPC) at 22 and 37°C (CFU/mℓ), Total coliforms (TC) (TC/100mℓ), Faecal coliforms (FC) (FC/100mℓ), bacteriophages (PFU/10mℓ), *Clostridium* (CFU/100mℓ), *Giardia*, and *Cryptosporidium*

The chemical water quality data collected was treated as follows:

- The average decrease or increase in the water quality parameter was determined
- The following statistical information was obtained for each data set: minimum maximum, average, mean and standard deviation.

The biological water quality data was treated as follows:

- The percentage removal of the biological parameters was determined
- The following statistical information was obtained for each data set: minimum maximum, average, mean and standard deviation.

The results of the processed data of all the determinations for the experiments are tabled in Appendix A.

The first experiments conducted with the Lazur M3 system microbiological determinations that used that could be done relatively cheaply. These included those determinations done on a routine basis to measure potable water quality e.g. SPC, TC, FC, bacteriophages (PFU/10mℓ), and *Clostridium* (CFU/100mℓ). In the experiments conducted on raw Klip River water only natural organisms was used and no laboratory cultures were introduced. This information was used to select the combination of treatment methods that would be more efficient. Further experimentation was then done with the

identified treatment configuration and optimised to improve the destruction of the micro-organisms selected (see Chapter4).

Formalised (oo)cysts was imported from Sterling Parasitology Laboratory, University of Arizona, Department of Veterinary Science and Microbiology by MERCK. The costs for 1 million *Cryptosporidium parvum* was R3200 and for *Giardia lamblia* R3600. Live oocysts were bought from Biotechnology Frontiers (Australia) at cost of US$600. *Cryptosporidium* and *Giardia lamblia* samples was analysed according to USEPA method 1623 (Rand Water accredited method no. 1.2.2.06.1). Stock suspensions of stock doses were prepared according to Rand Water accredited method no. 1.2.2.07.1)

3.2 RESULTS

3.2.1.1 The effect of hydrodynamic cavitation, ultrasound and UV on the chemical quality of water. (See Table A1-A7, A9-A15, A18-A23, A25-A31, A33-A39, A41-A47, A49-A55)

Of the chemical determination done hardness and turbidity are the only two that undergo significant changes when water is treated with hydrodynamic cavitation, hydrodynamic cavitation and ultrasound, hydrodynamic cavitation and UV, hydrodynamic cavitation, UV and ultrasound, ultrasound, and ultrasound and UV.

The increase in turbidity can be attributed to the formation of bubbles and breakdown of particles in water during hydrodynamic and ultrasonic cavitation. This leads to greater light scattering, which is observed as an increase in turbidity and suspended matter.

Reduction in hardness can possibly be attributed to the formation of precipitates or the conversion of carbonate and bicarbonate to carbon dioxide gas, when the $OH^{\bullet}$ radicals form OH^{-} ions that react with bicarbonates to precipitate as carbonates at localised elevated pH values.

3.2.1.2 The effect of hydrodynamic cavitation, UV and ultrasound on the biological quality of water. . (See Table A8, A16, A24, A32, A40,A487, A56)

From the results on the effect of the hydrodynamic cavitation UV and ultrasound on the biological quality of water as summarised in Appendix A we note that all the number of organisms are significantly reduced. The percentage reductions observed varied for the different type of organisms.

The results of the biological analysis are summarised in Table 3.2.1. In this table the average reduction of the various treatment options on different organisms are compared.

Table 3.2.1: Summary of the Percentage reduction of organisms tested for the treatment of Klip River and filtered water with combinations of hydrodynamic cavitation, ultrasound and UV.

Percentage Reduction						
Raw water: Klip River (K19)						
	SPC 22°C	**SPC 37°C**	**TC**	**FC**	**Bacterio -phages**	***Clostri-dium***
Cavitation	43.58	73.66	16.51	10.59	nc[1]	13.66
Cavitation & Ultrasound	23.93	8.36	19.25	28.92	nc	17.50
Cavitation & UV	64.02	69.15	56.03	81.20	nc	41.94
Cavitation, UV & ultrasound	27.48	57.41	77.02	72.95	95.76	30.40
Ultrasound	62.96	57.55	57.81	60.40	66.07	24.23
UV	59.28	64.51	nc	nc	nc	15.15
Treated water: Filtered water						
	SPC 22°C	**SPC 37°C**	**TC**	**FC**	**Bacterio -phages**	***Clostri-dium***
Cavitation	30.32	35.24	44.10	63.83	nc	42.74
Cavitation & Ultrasound	5.96	29.80	72.21	44.27	nc	37.08
Cavitation & UV	91.89	97.30	69.14	67.02	nc	48.69
Cavitation, UV & ultrasound	96.23	97.73	94.64	90.92	99.71	78.73
Ultrasound	99.28	99.03	96.63	89.31	nc	64.54
UV	93.65	87.67	nc	nc	nc	18.11
UV & Ultrasound	95.49	95.60	97.46	97.34	97.75	56.45

From the above results it was concluded that all options that include UV treatment as well as those that used ultrasound alone produce significantly better results that those that used cavitation. An important factor that must be

[1] nc – non conclusive result. Although the experiments was repeated approximately 12 times results varied substantially to such an extent that statistical analysis was not recommended.

taken into account is that the Hydrodynamic cavitation, UV and ultrasound were operated under predetermined experimental conditions (see Table 3.1).

From Table 3.2.1, A8, A16, A24, A32, A40, A487, and A56 it can be conclude that UV, ultrasound and a combination of UV and ultrasound treatment of filtered water reduced the microbiological numbers tester for by the longest margin. It was therefore decided to continue the investigation using only UV, ultrasound and a combination of UV and ultrasound on the treatment of filtered water.

Chapter 4

THE EFFECT OF ULTRAVIOLET LIGHT BY ITSELF AND IN COMBINATION WITH ULTRASOUND TREATMENT ON PROTOZOAN (OO)CYSTS: RESULTS AND DISCUSSION

4.1 INTRODUCTION

4.1.1 Objective

The aim of the investigation was to determine the influence of most successful treatment options (as determined in Chapter 3) on *Cryptosporidium* cysts. The investigation was conducted on formalised and viable cysts suspended in filtered water. This water was collected just after filtration at Vereeniging water purification station. Cysts suspended in filtered water that was not exposed to any of the experimental procedures was used as a control. For each of the treatment options investigated eight observations were made.

The effect of the treatment methods on the following microbiological determinants SPC, TC, FC, coliphages, and *Clostridium* were determined. Formalised oocysts suspended in filtered water were used to optimise the UV and ultrasound dosages to which viable cysts were exposed before performing the mouse infectivity studies. DAPI stain was used to determine the potential viability of the oocysts based on the appearance of the nuclei. The internal morphology by means of Differential Interference Contrast (DIC), size, and nucleus characteristics by means of DAPI staining of the oocysts that was treated with the various treatment methods were compared to the characteristics of the stock suspension to see if there were any changes that could be attributed to the effect of the treatment methods.

Live oocysts were processed through the chosen treatment processes in the experimental system and the concentrates as collected were analysed at Onderstepoort for infectivity using a mouse assay.

4.1.2 Analysis procedure

The accumulated biological water quality data was analysed for:

- The percentage reduction of the biological parameters was determined at various UV and ultrasound dosages and treatment circumstances.
- The percentage infective oocysts

4.2 RESULTS AND DISCUSSION: THE EFFECTIVE ENERGY REQUIREMENTS

4.2.1 UV

Literature (see Chapter 2, section 2.3.2) published before 1999 report that the effective energy requirement for the treatment of water containing protozoa cysts, *Cryptosporidium* was between 60 and 120 mW.s/cm^2. Latest literature indicates that doses of 10 – 20 mW.s/cm^2 are adequate for a 4.4log$_{10}$ inactivation of *Cryptosporidium parvum* (see Chapter 2, section 2.3.2). Based on this the following UV doses were tested: 7.98, 11.97, 23.93 and 47.86 mW.s/cm^2.

The effect of the different UV dosages on different biological determinants is given in Table 4.2.1.

Table 4.2.1: Percentage reduction of microbiological determinants in filtered water exposed to UV treatment.

Percentage Reduction				
	Retention time: 0.75sec **UV dose: 7.98 mW.s/cm^2**	**Retention time: 1.13sec** **UV dose: 11.97 mW.s/cm^2**	**Retention time: 2.26sec** **UV dose: 23.93 mW.s/cm^2**	**Retention time: 5.52sec** **UV dose: 47.86 mW.s/cm^2**
SPC 22^{o}C	99.3	99.95	99.97	99.96
SPC 37^{o}C	99.0	99.94	99.98	99.97
TC	9.6.6	59.3	94.1	86.0
FC	89.3	53.1	96.1	84.4
Bacteriophages	53.0	73.9	61.5	57.1
Clostridium	56.5	86.5	65.9	57.9

From the results in Table 4.2.1 it is noted that the optimum reduction for all the bacterial species occurred at a dose of 23.93 mW.s/cm^2.

4.2.2 Ultrasound

No literature reference on the specific energy or dose requirements for the ultrasound system used in the experiments were found.

The effect of the different ultrasound dosages on different biological determinants is given in Table 4.2.2.

***Table 4.2.2: Percentage reduction of microbiological determinants in filtered water exposed to ultrasound treatment*.**

Percentage Reduction				
	Retention time: 0.75sec	**Retention time: 1.13sec**	**Retention time: 2.26sec**	**Retention time: 5.52sec**
SPC 22^{o}C	93.7	99.92	99.98	99.8
SPC 37^{o}C	87.7	99.7	99.97	99.96
TC	85.2	53.0	81.5	84.0
FC	67.5	68.4	73.9	64.1
Bacteriophages	nc	36.7	35.0	61.5
Clostridium	18.1	nc	72.2	75.9

From the results in Table 4.2.2 it can be seen that the retention time for best removal of most species tested for was in the order of 5.52sec. Longer retention times were not possible due to constraints in the design of the experimental unit.

4.2.3 Ultrasound and UV

The effect of the different UV and ultrasound dosages on different biological determinants is given in Table 4.2.3.

Table 4.2.3: Percentages reduction of microbiological determinants in filtered water exposed to ultrasound and UV treatment.

% Reduction				
	Retention time: 0.75sec **UV dose: 7.98 mW.s/cm^2**	**Retention time: 1.13sec** **UV dose: 11.97 mW.s/cm^2**	**Retention time: 2.26sec** **UV dose: 23.93 mW.s/cm^2**	**Retention time: 5.52sec** **UV dose: 47.86 mW.s/cm^2**
SPC 22^{o}C	95.5	99.91	99.99	99.94
SPC 37^{o}C	95.6	99.90	99.98	99.97
TC	67.3	-	82.9	62.5
FC	68.7	67.7	88.4	65.2
Bacteriophages	53.0	47.3	56.4	54.5
Clostridium	56.5	68.6	68.9	87.9

From the results in Table 4.2.3 it can be seen that the best results were obtained at retention times of 2.26sec.

4.3 RESULTS AND DISCUSSION: *CRYPTOSPORIDIUM* INFECTIVITY

4.3.1 Assessment of oocysts viability using vital dye assay

Experiments were conducted to determine the optimum seed dose to be used. Seed dose recovery of the (oo)cysts were very low with a seed dose of 5000 (oo)cysts/100ℓ of filtered water. The seed dose was increased to

(oo)cysts to 15 000/100ℓ filtered water for the experiments with the live (oo)cysts.

Viability of (oo)cysts was assessed after treatment using vital dye assay, which relay upon morphology and inclusion/exclusion of the following two vital dyes, Fluorescein Isothionate (FITC) and 4'6 diamidino-2-phenyl indole (DAPI). The (oo)cysts showed a viability (using DAPI stain) of approximately 70%, which compared well to the initial stock suspension used.

4.3.2 The effect of different treatment procedures on protozoan cysts and oocysts

The results on experiments conducted on the formalised *Giardia* and *Cryptosporidium* (oo)cysts to evaluate the seed dose concentration and morphological changes is shown in Table 4.3.1, Figure 4.3.1 and 4.3.2.

Table 4.3.1: DAPI and Fluorescence results on the recovered Cryptosporidium and Giardia (oo)cysts ((oo)cysts/10ℓ) from the various treated options

Treated options	Fluorescence		DAPI positive	
	Cryptosporidium	***Giardia***	***Cryptosporidium***	***Giardia***
Hydrodynamic Cavitation	14	31	14	20
Hydrodynamic Cavitation + Ultrasound	34	63	29	37
UV	17	3	12	3
Ultrasound	16	9	14	6
UV + Ultrasound	2	1	0	0

From Table 4.3.1 it is seen that the DAPI positive results are in both cases (with *Giardia* and with *Cryptosporidium*) less than the Fluorescence stained *Giardia* and *Cryptosporidium.*

The (oo)cysts treated with the cavitation showed morphological changes when compared to (oo)cysts before treatment. This can be seen in the images in Figure 4.3.1 and 4.3.2. This change can be attributed to the specific

treatment process and is more prominent when hydrodynamic and ultrasonic cavitation was used.

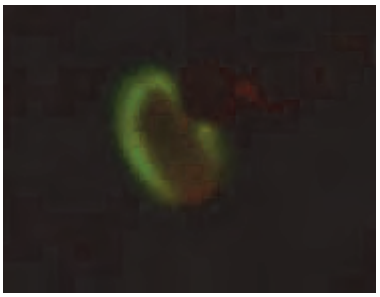

Figure 4.3.1: *Giardia* cysts before treatment

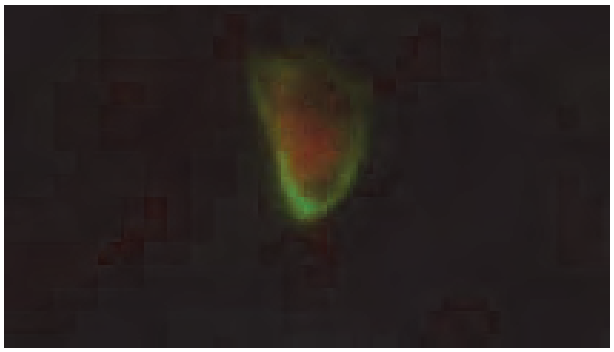

Figure 4.3.2: *Giardia* cysts after treatment

4.3.3 Assessment of oocysts infectivity using mouse assays

The live cysts were exposed to three treatment procedures UV, ultrasound and UV plus ultrasound. The infectivity of the treated oocysts was tested using mouse assays. The mouse assays was conducted under the supervision of Dr JPJ Joubert from the Toxicology Department at Onderstepoort Veterinary Institute. Swiss White mice were used for the mouse assays. The mouse assays were conducted by dosing seven-day-old baby mice with 10µℓ sample. A positive control (stock suspension of live oocysts, see Figure 4.3.5) and negative control (distilled water) was dosed into the mice. Three replicates per procedure were done. The experiment was repeated to confirm the results.

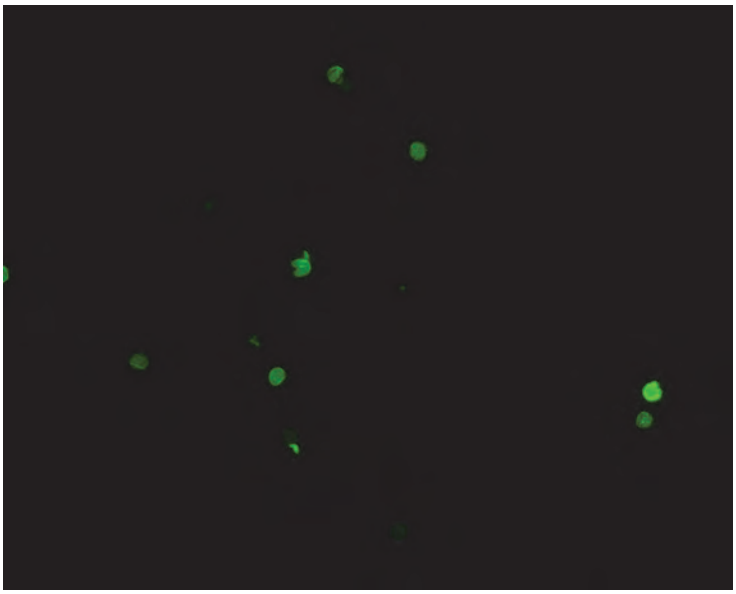

Figure 4.3.5: Stock suspension of LIVE oocysts (FITC)

The mice were kept in single litter cages in the laboratory animal room. They were examined daily and were euthanazed and autopsied after seven days.

Histopathological examination of the ileum of each mouse of each replicate was performed to determine *Cryptosporidium* infestation. Only one of three positive control replicates was positive for *Cryptosporidium* infestation. The ultrasound plus UV treatment resulted in three negative replicates. UV treatment had two positive and one negative infested replicates. The ultrasound treatment had one positive and two negative infested replicates. The negative control showed negative results. *Cryptosporidium* organisms attached to the mucosa and losse in the intestinal lumen of mice can be seen in Figure 4.3.6.

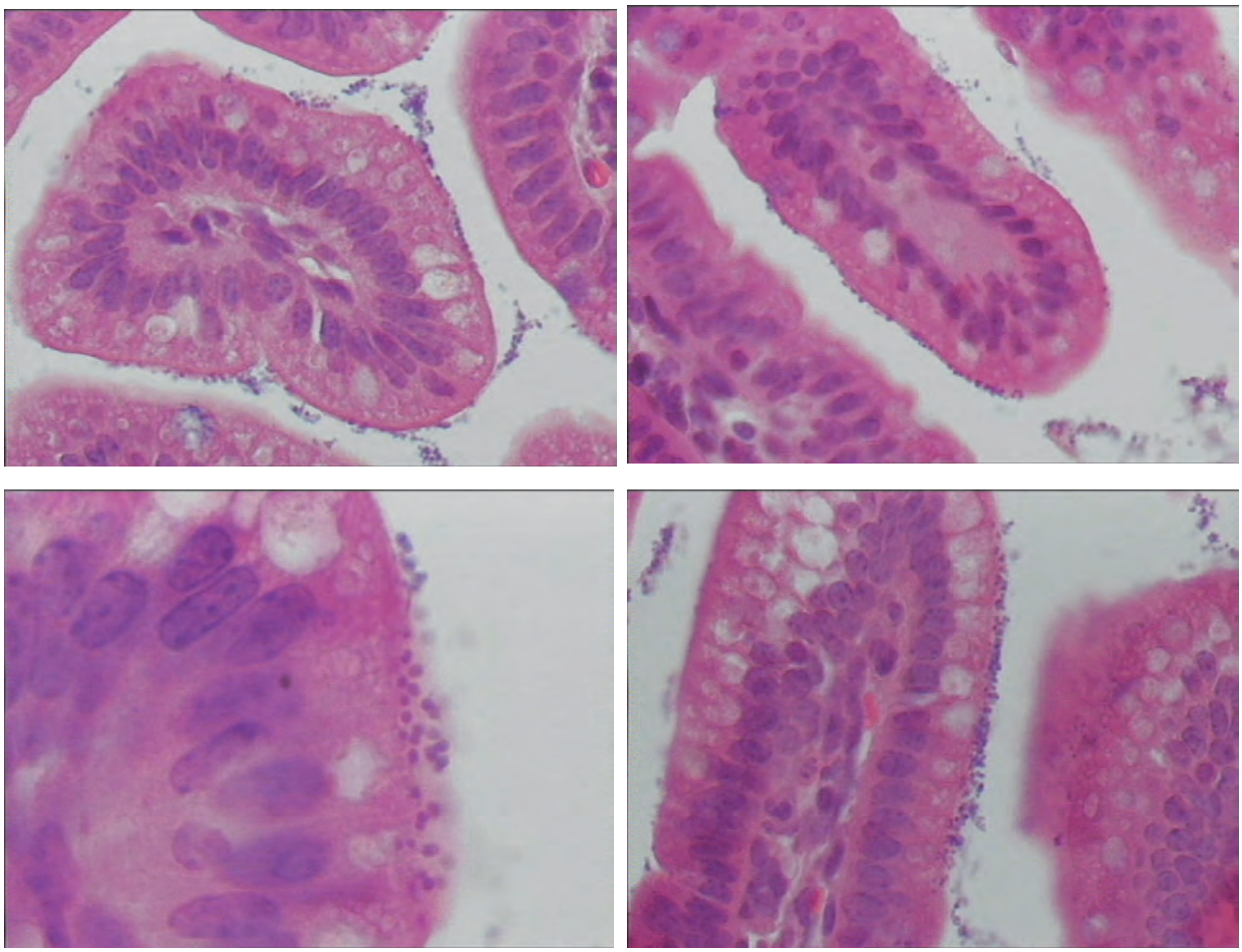

Figure 4.3.6: *Cryptosporidium* organisms attaches to mucosa and losse in intestinal lumen of mice

The total negative results for the faecal samples of all mice may indicated that it was too soon (seven day test period) for the oocysts to have developed. These results may not be as conclusive as it should, but it indicates that the combined treatment may be used.

The experiment was repeated and a summary of the results is as follows:

Thirteen female mice, each with a litter of seven babies, five - Seven days old, were received from the brooding colony and were kept in single litter cages in the laboratory animal room. The 13 litters were fed with the samples and examined daily. The 84 surviving baby mice out of 91 were euthanazed and autopsied after seven days.

Faecal samples were collected from the colon of each baby mouse and preserved in 10% buffered formalin solution in separate containers and were

examined for the presence of *Cryptosporidium* oocysts with the Crypto-Cel Fluorescent Antibody test. All the faecal samples of the 13 groups tested negative. This test may not be sensitive enough, or the seven day period from dosing to sample collection may be too short for oocyst development and excretion.

Histopathological examination of the ileum of each mouse of each replicate was performed to determine *Cryptosporidium* infestation. Only two of the three positive control replicates were positive for Cryptosporidium infestation. This could be as a result that the concentration of the oocyst suspension dosed into the mice was too low.

None of the three replicates, which received water, treated with ultrasound plus UV light had any positive mice. Ultrasound treatment had one out of three replicates with three out of six mice slightly positive. The UV light group also showed one replicate out of three to be positive, but the positive replicate had five out of seven with a moderate infestation. Two out of the six mice in the negative control group showed slight infestation with oocysts. These sections were stained with Giemsa stain to confirm that these organisms were Cryptosporidium and not coccoid bacteria.

The few positive mice in the single negative control group may indicate a few problems, for instance, there may have been a low-grade infestation with C*ryptosporidium* in the mice-breeding colony. The breeding colony used, was not pathogen free. Such an infestation may have caused a measure of immunity in some of the baby mice, explaining the number of negative results in the positive control replicates. Another factor to consider was the possibility that mice at seven days of age may be showing some natural resistance to *Cryptosporidium* and that they need to be dosed at an earlier age. The problem with mice is that the mothers will easily eat their offspring if handled at for instance at one day old, leaving too few to give meaning full results.

Chapter 5

CONCLUSIONS

The following conclusions can be made from experiments conducted to determine the effect of ultraviolet light by itself and in combination with either/or flow cavitation and ultrasound treatment on protozoan cysts or oocysts and other micro-organisms, in water. Although it is believed that the following conclusions hold true in general, the values mentioned for the different treatment methods may only be applicable for the equipment used under the experimental conditions.

- Hydrodynamic cavitation alone is not successful in inactivating bacteria and bacteriophages.

- Of the treatment options tested Ultrasound and UV alone or in combination with each other are the most effective methods.

- A retention time of 2.26s and UV dose of 23.93mW.s/cm^2 were the optimum conditions for the inactivation of bacteria in the equipment used and under the prevailing experimental conditions. A retention time of 2.26s and UV dose of 23.93mW.s/cm^2 were the optimum conditions for the inactivation of bacteria in the equipment used and under the prevailing experimental conditions. This optimum UV dose correspond with UV doses needed for the effectively inactivation of chlorine resistant enteroviruses (25 mW.s/cm^2). This dosage is lower than the dosages of 63 mW.s/cm^2 used by Rice and Hoff (1981) to reduced excystation of *Giardia Lamblia* by 90% and dosages of 41 mW.s/cm^2 used by Bukhari *et al* (1999) for >4log inactivation of *Cryptosporidium parvum* and in the same range as the 19 mW.s/cm^2 for a 3.9-log inactivation of *Cryptosporidium* oocysts (Bukhari *et al,* 1999).

- The optimum retention time for ultrasound is 5.52s to deliver a reduction in standard plate counts (SPC).

- A retention time of 2.26s and UV dose of 23.93mW.s/cm^2 were the optimum conditions for the inactivation of bacteria using UV and ultrasound as treatment option.

- Of all the organisms tested *Clostridium* was the most resistant to the experimental treatment procedures tested. This may be due to spore formation. Best reductions were observed after treatment with UV and Ultrasound in combination.

- Further treatment of clarified and filtered water with any of the possible treatment options resulted in higher inactivation of bacteria and bacteriophages than treatment of raw Klip River water with the same treatment options.

- All the treatment regimes that included either UV alone and/or in combination with cavitation/ultrasound showed higher reductions in bacterial counts than the options of cavitation treatment alone or in combination with ultrasound treatment.

- Ultrasound treatment that shows promise for high bacterial and bacteriophage inactivation and was comparable with bacterial and bacteriophage inactivation by the application of only UV treatment.

- UV/cavitation treatment as well as UV/cavitation/ultrasound treatment did not achieve as high a bacterial and bacteriophage reduction as UV alone or in combination with ultrasound. In this case the UV dose was much less because of the constraints imposed by the cavitation, which reduced the reaction time and lead to lower UV doses in the treatment unit.

- UV was the most effective treatment option with ultrasound second best.

- *Clostridium*, which is a spore forming bacteria, showed lower reductions than other bacteria and bacteriophages.

- Results obtained with protozoan cysts and oocysts were difficult to interpret because of the characteristics of the stock dose culture. Formalised cysts, containing 70% viable cysts, were used in the stock suspension. The 70% viability leaded to an uncertainty in recovery percentages and the percentage DAPI positive/negative results. Results from experiments with life cysts can be used for the interpretation of the effectiveness of treatment options.

- On interpretation of the results of the mouse infectivity tests it was found that UV and Ultrasound treatment contribution war the best to inactivate *Cryptosporidium.*

- An important observation made was that the morphology of the *Giardia* cysts changed when they were treated with ultrasonic and hydrodynamic cavitation.

- After the cysts and oocysts were subjected to cavitation, the whole cyst stained sky blue and instead of only the nucleus in these instances the DAPI staining was very faint.

The minimum energy input required to inactivate the cysts and oocysts using the Lazur M3 system was 23.93mW.s/cm^2 (J/s).

Proposed design and operating guidelines for the use of ultraviolet treatment systems, with or without additional treatment, for small and large installations are:

- A UV lamp age factor (decrease in UV lamp intensity over specified period of time) of 0.5 is recommended for all lamp systems.

- It is important that reactors, which contain the UV lamps, must promote plug flow.

- UV equipment must be designed and evaluated to operate at the specified approach velocity ranges that occur in the specified unit.

- UV equipment must be set up in such a manner that it is easily accessible for efficient and regular cleaning
- Sleeve cleaning procedures must be effective and efficient and must not impact negatively on operations
- Standby equipment must be available to ensure continuous operation in case of component failure
- Timely maintenance, replacement and calibration of equipment must be ensured at all times
- Reliable and back-up power supply must be available at all times
- Continuous monitoring of operation UV dose is essential to ensure effective disinfection

The wider benefits for the use of ultraviolet light disinfection in water treatment are:

- No significant influence on chemical properties of water.
- System does not need much maintenance and is easy to operate.
- Organisms that are difficult to kill with chlorine are susceptible to UV radiation

Chapter 6

RECOMMENDATIONS FOR FUTURE STUDIES

1. A research project could be undertaken to evaluate the best test method for the determining the viability of *Cryptosporidium* in case of water treatment plant failure. Mouse assays and cell culture are methods, which may be used to detect *Cryptosporidium* infectivity.

2. Improved methods for the recovery of protozoa (oo)cysts from turbid water.

Chapter 7

TECHNOLOGY TRANSFER

The following article, presentation and thesis are based on results from the study:

"The effect of ultraviolet light, cavitational flow and ultrasound on protozoa cysts and oocysts, bacteriophages and Clostridium", E van der Walt, WISA, 2002, Durban

Mrs. Mohohlo used the microbiological data for a project for the partial fulfilment of a B. Tech. (Biotechnology) at the Vaal Triangle Technicon, 2002.

"The effect of ultraviolet light, cavitational flow and ultrasound on protozoa cysts and oocysts, bacteriophages and Clostridium", E van der Walt, Water SA, Special Edition, Gezina, March 2003

"The use of UV in combination with physical unit processes for the treatment of water in small or rural communities", E. van der Walt, The 2nd international congress on ultraviolet technologies, Vienna, Austria July 9 – 11, 2003

"The use of ultraviolet light alone, or in combination with cavitational flow and ultrasonic devices, to inactivate protozoan cysts and oocysts in the small and large scale treatment of drinking water", M. Grundlingh, M. Tech, Vaal Triangle Technicon, 2003

Possible application of the information:

- Design of new water and waste water disinfection treatment facilities
- Optimisation of treatment procedures for protozoan cysts and other bacteria and viruses,
- Test different operational procedures for new and existing disinfection facilities

- Optimisation of treatment of water containing protozoan cysts with minimal use of chlorine or other chemical.

Chapter 8

CAPACITY BUILIDING

CAPACITY BUILDING TOWARDS INDIVIDUALS:

Two persons of previously disadvantage groups was part of the research team:

1) Puseletso Mohohlo

Mrs. Mohohlo was appointed as an Assistant Researcher specifically for this project. She is currently enrolled for a B. Tech. (Biotechnology). Mrs. Mohohlo will be using the biological data for her project and practicum to complete her B. Tech..

Mrs. Mohohlo is partially responsible for the experiments and is fully responsible for the biological analysis.

This project gave Mrs. Mohohlo experience in:

- Analytical techniques
- Microbiological techniques
- Partial project scheduling and planning
- Some project management skills
- Reporting skills
- Communication skills.

2) Aubrey Mohluoa

Mr. Mohluoa is appointed as a laboratory Assistant at Process technology, Rand Water. He is currently enrolled for a Water care diploma.

Mr. Mohluoa is partially responsible for the experiments and is fully responsible for the chemical analysis.

This project gave Mr. Mohluoa experience in:

- Analytical techniques
- Partial project scheduling and planning
- Project management skills
- Reporting skills
- Communication skills

The experience Mr. Mohluoa gained will give him the opportunity to enhance his career to apply for analytical and supervisory posts.

CAPACITY BUILDING INITIATIVES AFFECTING COMMUNITIES:

UV and Ultraviolet are non-chemical ways of disinfection it does not need expensive capital infrastructures. Operators can be easily trained and no needs for highly qualified and skilled personnel.

Both the above-mentioned methods can be used for the disinfection of ground and surface water in small communities. The small communities may benefit in two ways, firstly by acquisition of new skills and responsibility and secondly by receiving better quality water which improve life standards. 5

REFERENCES

1. Botha, CJ, “A study of hydrodynamic cavitation as a method of water treatment”, M.Sc. in Engineering, University of Natal, September, 1993

2. Bukhari, Z, Hargy T M, Bolton, J R, Dussert, B, and Clancy J L, “ Medium pressure UV for oocysts inactivation”, Journal AWWA, Vol. 91, Issue 3, March, 1999, p 86 – 94

3. Cabajl, A, “ The Disinfection of Water with UV radiation and Type-Testing of UV-Disinfection Plants for Drinking Water”, Poster presented at 24th session of CIE, June 24 – 30, 1999

4. Cairns, W L and Jim McKee, “New Advances in Ultraviolet Light Disinfection technology”, Trojan Technologies Inc., London, 1995

5. Cairns, W L, “Comparing Disinfection By Ultraviolet Light and Chlorination of Mechanism for Practice”, Trojan Technologies Inc., London, 1995

6. Cairns, W L, “UV Technology for water supply treatment”, Trojan Technologies Inc., London

7. Cairns, W L, *et al*, “The impacts of wastewater quality on Ultraviolet disinfection of reclaimed wastewater”, Trojan Technologies Inc., London, 1995.

8. Cairns, W L, “Advances In UV Disinfection Technology For Treatment Of Low Quality Wastewater “, For Australian Water & Wastewater Association (AWWA) 16th Federal Convention, Sydney, Australia Apr 2-6, 1995

9. Chang, J C H, *et al*, “UV inactivation of Pathogenic and Indicator Micro-organisms”, Applied and Environmental Microbiology, Vol. 49, No. 6, June, 1985 p 1361 – 1365

10. Clancy J L, Ph.D., ‘Ultraviolet Light – A Solution to the *Cryptosporidium* Threat?”, UV News, Vol.1 No. 1, 1999, p 18 – 22

11. Clancy J L, Bukhari Z, Hargy T M, Bolton JR, Dussert BW, and Marshall MM, “Using UV to inactivate *Cryptosporidium*”, Journal AWWA, Vol. 92, Issue 9, September, 2000, p 97 - 104

12. Combs, R F; and McGuire, P J; Ultrapure Water, Vol. 6, no. 3, 1989, p 62 - 68

13. Eaton, A, “Measuring UV-absorbing organics: A standard method” Journal AWWA, February, 1995, p 86 – 90

14. Harris, GD, Adams, DV, Sorensen, DL, and Curtis, MS, “ Ultraviolet inactivation of selected bacteria and viruses with photoreactivation of the bacteria”, Wat. Res., Vol. 21, No. 6, 1987, p 687 - 692

15. Lehman, AF, Young, JO, “Experimental investigations of incipient and desinent cavitation”, Journal of basic Engineering, June, 1964, p 275 - 281

16. Li J W, *et al*, “Effect of Ultraviolet Irradiation on the Characteristics and Trihalomethanes formation potential of Humic Acid” Wat. Res., Vol. 30, No. 2, 1996, p 347 – 350

17. Lorenzo-Lorenzo, M J, *et al*, “Effect of Ultraviolet Disinfection of Drinking water on the Viability of *Cryptosporidium parvum oocysts*”, J. Parasitol., Vol. 79, No. 1, 1993, p 67 – 70

18. Lykins, B W and Griese, M H, “Using Chlorine Dioxide for Trihalomethane Control”, J. AWWA Vol. 78, NO. 9, 1989, p 18 – 28

19. Mead, DJ, Sutherland, RG and Verrall RE, “The effect of ultrasound on water in the presence of dissolved gases”, CAN. J. CHEM., Vol. 54, 1976, p 1114 - 1119

20. Mofidi AA, Baribeau H, Rochelle P A, De Leon R, Coffey BM, and Green J F, “ Disinfection of Cryptosporidium parvum with polychromatic UV light”, Journal AWWA, June, 2001, p 95 - 109

21. Moreno, B, *et al*, " The Disinfection of Wastewater by Ultraviolet light", Wat. Sci. tech., Vol. 35, No. 11 – 12, 1997, p 233 – 235

22. Neis U, "Ultrasound in water, wastewater and sludge treatment", Water21, April, 2000, p 36 – 59

23. Neppiras EA and Hughes DE, "Some Experiments on the disintegration of yeast by high intensity ultrasound", Biotechnology and bioengineering, Vol. VI, 1964, p 247 - 270

24. Noesen, M, *et al*, "Lamp Output Testing for Low Pressure UV Disinfection Systems", Water Environmental Federation, 1999, p 1 – 7

25. Oppenheimer, J A, *et al*, "Testing the equivalency of ultraviolet light and chlorine for disinfection of wastewater to reclamation standards", Water Environmental Research, Vol. 69, No. 1, Jan/Feb, 1997, p 14 – 24

26. Parrotta M J and Bekdash F, " UV disinfection of small groundwater supplies", Journal AWWA, Vol. 90, Issue 2, February, 1998, p 71 - 81

27. Ransome, M E¸ *et al*, ' Effect of Disinfectants on the viability of *Cryptosporidium parvum Oocysts*", Water Supply, Vol. 11, 1993, p 131 – 142

28. Ransome, M E¸ *et al*, ' Practical Implications of Disinfection", Water Supply, Vol. 13, No. 2, 1995, p 131 – 142

29. Rice E W and Hoff J C, "Inactivation of *Giardia lamblia Cysts* by Ultraviolet Irradiation", Appl. Environ. Microbiol., Vol. 42, 1981, p 846 – 547

30. Warne S, 'Energetic growth in UV sterilisation", Ultra-Pure Water, World Water, May, 1986, p 38 - 40

31. Winship, S, " Evaluation of different methods to produce free radicals for the oxidation of organic molecules in industrial effluents and potable water with reference to Cav-Ox®", WRC report No 388/1/99, July, 1995

32. Winship, S, “ Evaluating hydrodynamic cavitation for the treatment of raw water and effluents”, SA Waterbullitin, Sept/Oct, 1999,p 11 – 14

33. Wolfe, R L, “Ultraviolet Disinfection of Potable Water”, Environ Sci. Tech., Vol. 24, No. 6, 1991, p 768 – 773

APPENDIX A

LIST OF TABLES

THE EFFECT OF HYDRODYNAMIC CAVITATION ON THE CHEMICAL QUALITY OF WATER (TABLE A1 –A7)

I pH

Table A1: The effect of treatment with hydrodynamic cavitation on the pH of water tested.

	Klip River water		**Filtered water**	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	7.66	7.81	7.32	8.00
Maximum	8.08	8.09	8.27	8.33
Average	7.91	7.98	8.02	8.17
Mean	7.94	8.00	8.17	8.23

II Turbidity

Table A2: The effect of treatment with hydrodynamic cavitation on the turbidity (NTU) of water tested.

	Klip River water	**Filtered water**

	Control	After Treatment	Control	After Treatment
Minimum	1.29	1.60	0.66	0.63
Maximum	3.33	3.49	1.56	1.76
Average	2.22	2.64	1.05	1.12
Mean	2.03	2.86	0.99	1.05

III Conductivity

Table A3: The effect of treatment with hydrodynamic cavitation on the conductivity (mS/m) of water tested.

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	76.6	77.6	25.0	25.0
Maximum	83.1	82.2	35.0	34.5
Average	81.1	80.8	30.1	29.9
Mean	81.8	81.1	30.5	30.7

IV UV Absorbance

Table A4: The effect of treatment with hydrodynamic cavitation on the UV absorbance (%) of water tested.

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	70.16	70.16	73.25	73.12
Maximum	75.48	75.48	77.58	77.51
Average	73.25	73.43	75.28	75.27
Mean	73.47	73.58	75.14	75.13

V mo-Alkalinity

Table A5: The effect of treatment with hydrodynamic cavitation on the mo-alkalinity (mg/l $CaCO_3$) of water tested.

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	69.83	69.53	80.09	79.63
Maximum	75.08	73.89	106.29	104.53
Average	73.01	71.94	89.56	87.95
Mean	73.00	72.11	84.91	94.42

VI Hardness

Table A6: The effect of treatment with hydrodynamic cavitation on the hardness (mg/L $CaCO_3$) of water.

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	240.32	243.84	76.38	74.69
Maximum	260.09	260.67	111.38	100.71
Average	254.79	251.29	85.75	83.36
Mean	255.98	252.16	78.81	77.69

VII Chlorine demand

Table A7: The effect of treatment with hydrodynamic cavitation on the chlorine demand (mg/L Cl_2) of water.

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	-0.01	-0.02	0.01	0.00
Maximum	0.06	0.03	0.11	0.07
Average	0.02	0.01	0.04	0.03
Mean	0.01	0.01	0.03	0.03

THE EFFECT OF HYDRODYNAMIC CAVITATION ON THE BIOLOGICAL QUALITY OF WATER (TABLE A8)

Table A8: The effect of treatment with hydrodynamic cavitation on the microbiological quality of water tested.

	% Reduction				
	SPC 22°C	**SPC 37°C**	**TC**	**FC**	***Clostridium***
Klip River source water:					
Minimum	18.82	27.44	0.00	0.00	0.00
Maximum	66.67	90.42	44.12	31.43	29.63
Average	43.58	73.66	16.51	10.59	13.66
Median	49.60	83.91	17.24	6.61	12.50
Treated water:					
Minimum	10.68	10.79	15.52	49.33	17.14
Maximum	49.59	58.54	63.77	80.67	62.50
Average	30.32	35.24	44.10	63.83	42.74
Median	31.74	37.81	48.55	62.67	42.36

THE EFFECT OF HYDRODYNAMIC CAVITATION AND ULTRASOUND ON THE CHEMICAL QUALITY OF WATER (TABLE A9 –A15)

I pH

Table A9: The effect of treatment with hydrodynamic cavitation and ultrasound on the pH of water tested.

	Klip River water		**Filtered water**	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	7.21	8.11	8.03	8.03
Maximum	8.40	8.43	8.48	8.40
Average	8.07	8.33	8.16	8.16
Mean	8.27	8.38	8.14	8.13

II Turbidity

Table A10: The effect of treatment with hydrodynamic cavitation and ultrasound on the turbidity (NTU) of water tested.

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	3.47	4.04	0.66	0.61
Maximum	13.00	12.50	4.45	4.11
Average	6.53	7.24	1.80	1.86
Mean	3.93	5.56	0.88	1.22

III Conductivity

Table A11: The effect of treatment with hydrodynamic cavitation and ultrasound on the conductivity (mS/m) of water tested.

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	78.40	79.60	25.80	25.30
Maximum	82.40	80.60	34.80	31.60
Average	81.17	80.33	29.49	28.45
Mean	81.30	80.35	29.61	29.28

IV UV Absorbance

Table A12: The effect of treatment with hydrodynamic cavitation and ultrasound on the UV Absorbance (%) of water tested

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	DNA[2]	DNA	51.21	50.22
Maximum	DNA	DNA	69.18	69.18
Average	DNA	DNA	62.20	61.78
Mean	DNA	DNA	64.08	63.28

V mo-Alkalinity

Table A13: The effect of treatment with hydrodynamic cavitation and ultrasound on the mo-alkalinity (mg/L $CaCO_3$) of water tested.

	Klip RIVER water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	73.15	67.87	77.68	77.78
Maximum	78.24	75.74	93.15	92.22
Average	75.48	72.68	84.91	81.88
Mean	75.14	72.64	82.68	77.72

VI Hardness

Table A14: The effect of treatment with hydrodynamic cavitation and ultrasound on the hardness (mg/L $CaCO_3$) of water tested.

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	233.44	228.20	74.13	70.95
Maximum	243.17	232.69	75.82	75.44
Average	236.95	230.17	74.93	73.68
Mean	236.34	229.88	74.79	73.88

VII Chlorine demand

Table A15: The effect of treatment with hydrodynamic cavitation and ultrasound on the chlorine demand (mg/L Cl_2) of water tested.

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	0.00	-0.05	-0.03	-0.01
Maximum	0.07	0.15	0.02	0.02
Average	0.03	0.03	0.00	0.00
Mean	0.02	0.03	0.01	0.00

[2] DNA – Data not available due to equipment failure

THE EFFECT OF HYDRODYNAMIC CAVITATION AND ULTRASOUND ON THE BIOLOGICAL QUALITY OF WATER (TABLE A16)

Table A16: The effect of treatment with hydrodynamic cavitation and ultrasound on the microbiological quality of the water tested.

% Reduction					
	SPC 22°C	**SPC 37°C**	**TC**	**FC**	***Clostridium***
Klip River source water:					
Minimum	3.26	0.68	0.00	0.00	5.08
Maximum	62.35	22.02	39.02	66.67	32.20
Average	23.93	8.36	19.25	28.92	17.50
Median	21.57	5.58	18.93	22.50	17.95
Treated water:					
	SPC 22°C	**SPC 37°C**	**FC**	**Bacterio-phages**	***Clostridium***
Minimum	0	0.68	60.61	18.03	13.01
Maximum	17.95	84.80	84.38	44.27	37.08
Average	5.96	29.80	72.21	44.27	37.08
Median	4.00	24.72	70.79	41.37	33.11

THE EFFECT OF HYDRODYNAMIC CAVITATION, UV AND ULTRASOUND ON THE CHEMICAL QUALITY OF WATER (TABLE A17 –A23)

I pH

Table A17: The effect of treatment with hydrodynamic cavitation, UV and ultrasound on the pH of water tested.

	Klip River water		**Filtered water**	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	7.79	7.58	7.34	7.33
Maximum	7.93	8.00	8.16	8.23
Average	7.87	7.91	7.82	7.87
Mean	7.86	7.94	7.98	8.08

II Turbidity

Table A18: The effect of treatment with hydrodynamic cavitation, UV and ultrasound on the turbidity (NTU) of water tested.

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	68.70	79.30	0.43	0.39
Maximum	86.90	98.00	3.34	3.54
Average	77.73	87.76	1.91	1.91
Mean	77.95	87.65	1.79	1.82

III Conductivity

Table A19: The effect of treatment with hydrodynamic cavitation, UV and ultrasound on the conductivity (mS/m) of water tested.

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	28.20	27.80	28.20	27.80
Maximum	32.50	31.40	32.50	31.40
Average	30.60	29.06	30.60	29.06
Mean	30.70	28.85	30.70	28.85

IV UV Absorbance

Table A20: The effect of treatment with hydrodynamic cavitation, UV and ultrasound on the UV absorbance (%) of water tested.

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	74.98	72.94	74.98	72.94
Maximum	82.35	79.92	82.35	79.92
Average	77.54	76.51	77.54	76.51
Mean	76.33	76.51	76.33	76.51

V mo-Alkalinity

Table A21: The effect of treatment with hydrodynamic cavitation, UV and ultrasound on the mo-alkalinity (mg/L $CaCO_3$) of water tested.

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	189.63	172.79	80.28	76.76
Maximum	198.06	196.75	92.59	88.24
Average	194.10	187.61	84.34	81.16
Mean	185.60	183.61	82.36	79.40

VI Hardness

Table A22: The effect of treatment with hydrodynamic cavitation, UV and ultrasound on the hardness (mg/L $CaCO_3$) of water tested.

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	189.63	172.79	75.51	72.07
Maximum	198.06	196.75	76.38	74.32
Average	194.10	187.61	75.04	73.15
Mean	194.50	188.42	74.97	73.20

VII Chlorine demand

Table A23: The effect of treatment with hydrodynamic cavitation, UV and ultrasound on the chlorine demand (mg/L Cl_2) of water tested.

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	-0.14	-0.16	-0.01	-0.02
Maximum	0.21	0.17	0.02	0.01
Average	0.06	0.06	0.00	0.00
Mean	0.06	0.09	0.00	0.00

THE EFFECT OF HYDRODYNAMIC CAVITATION, UV AND ULTRASOUND ON THE BIOLOGICAL QUALITY OF WATER (TABLE A24)

Table A24: The effect of treatment with hydrodynamic cavitation, UV and ultrasound on the microbiological quality of the water tested.

	% Reduction					
	SPC 22°C	**SPC 37°C**	**TC**	**FC**	**Bacterio-phages**	***Clostri-dium***
Klip River source water:						
Minimum	1.43	17.69	58.33	32.73	90.57	21.59
Maximum	69.00	98.63	95.12	94.44	100.00	48.84
Average	27.48	57.41	77.02	72.95	95.76	30.40
Median	24.98	57.27	71.01	76.67	96.44	29.21
Treated water:						
	SPC 22°C	**SPC 37°C**	**TC**	**FC**	**Bacterio-phages**	***Clostri-dium***
Minimum	85.22	95.30	92.87	52.00	98.33	62.14
Maximum	99.82	99.52	96.87	97.86	100.00	87.33
Average	96.23	97.73	94.64	90.92	99.71	78.73
Median	97.95	97.97	94.67	95.82	99.97	81.12

THE EFFECT OF HYDRODYNAMIC CAVITATION AND UV ON THE CHEMICAL QUALITY OF WATER (TABLE A25 –A31)

I pH

Table A25: The effect of treatment with hydrodynamic cavitation and UV on the pH of the water tested.

	Klip River water		**Filtered water**	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	8.02	8.09	7.83	7.87
Maximum	8.34	8.22	8.09	8.15
Average	8.20	8.16	7.99	8.04
Mean	8.18	8.17	8.03	8.08

II Turbidity

Table A26: The effect of treatment with hydrodynamic cavitation and UV on the turbidity (NTU) of the water tested

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	13.10	14.00	0.62	0.62
Maximum	19.77	24.40	1.46	1.58
Average	16.96	18.57	0.92	1.00
Mean	17.59	18.72	0.80	0.94

III Conductivity

Table A27: The effect of treatment with hydrodynamic cavitation and UV on the conductivity (mS/m) of the water tested.

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	58.70	56.20	28.30	27.20
Maximum	61.20	61.20	31.60	30.90
Average	60.09	59.35	29.45	28.78
Mean	60.40	59.50	29.25	28.65

IV UV Absorbance

Table A28: The effect of treatment with hydrodynamic cavitation and UV on the UV absorbance (%) of the water tested.

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	79.38	79.63	77.96	77.96
Maximum	82.91	82.91	82.13	83.45
Average	81.39	81.58	80.34	80.22
Mean	81.68	81.99	80.81	79.98

V mo-Alkalinity

Table A29: The effect of treatment with hydrodynamic cavitation and UV on the mo-alkalinity (mg/L $CaCO_3$) of the water tested

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	53.69	52.79	97.37	94.89
Maximum	61.31	55.86	104.30	104.40
Average	55.78	54.22	101.32	99.68
Mean	54.92	54.22	101.87	101.18

VI Hardness

Table A30: The effect of treatment with hydrodynamic cavitation and UV on the hardness (mg/L $CaCO_3$) of the water tested

	KLIP RIVER water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	192.18	188.26	102.94	100.98
Maximum	2002.20	197.85	125.05	114.29
Average	196.27	192.99	109.31	106.77
Mean	196.09	191.69	109.30	108.61

VII Chlorine demand

Table A31: The effect of treatment with hydrodynamic cavitation and UV on the chlorine demand (mg/L Cl_2) of the water tested.

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	0.00	0.00	0.01	0.00
Maximum	0.11	0.09	0.11	0.07
Average	0.03	0.03	0.04	0.03
Mean	0.02	0.01	0.03	0.03

THE EFFECT OF HYDRODYNAMIC CAVITATION AND UV ON THE BIOLOGICAL QUALITY OF WATER (TABLE A32)

Table A32: The effect of treatment with hydrodynamic cavitation and UV on the microbiological quality of the water tested.

	% Reduction				
	SPC 22°C	**SPC 37°C**	**TC**	**FC**	***Clostridium***
Klip River source water:					
Minimum	25.31	25.66	30.78	50.00	23.68
Maximum	88.10	94.91	78.79	100.00	53.66
Average	64.02	69.15	56.03	81.20	41.94
Median	77.72	82.78	60.00	84.72	43.86
Treated water:					
	SPC 22°C	**SPC 37°C**	**TC**	**FC**	***Clostridium***
Minimum	62.50	91.75	34.67	40.00	20.00
Maximum	99.52	99.25	90.00	94.20	66.67
Average	91.89	97.30	69.14	67.02	48.69
Median	98.82	98.84	73.33	63.67	50.00

THE EFFECT OF ULTRASOUND ON THE CHEMICAL QUALITY OF WATER (TABLE A33 –A3)

I pH

Table A33: The effect of treatment with ultrasound on the pH of the water tested.

	Klip River water		**Filtered water**	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	7.79	7.86	8.17	8.19
Maximum	7.98	8.04	8.26	8.25
Average	7.90	7.94	8.22	8.21
Mean	7.91	7.95	8.24	8.21

II Turbidity

Table A34: The effect of treatment with ultrasound on the turbidity (NTU) of the water tested.

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	65.70	53.40	0.84	0.76
Maximum	78.20	94.60	2.35	2.80
Average	71.35	74.39	1.57	1.39
Mean	70.65	71.85	1.49	1.05

III Conductivity

Table A35: The effect of treatment with ultrasound on the conductivity (mS/m) of the water tested.

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	66.10	64.00	24.30	24.40
Maximum	68.60	66.60	30.00	28.90
Average	67.18	65.56	26.25	25.64
Mean	67.05	65.80	25.50	24.65

IV UV Absorbance

Table A36: The effect of treatment with ultrasound on the UV absorbance (%) of the water tested.

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	60.16	56.37	75.23	68.41
Maximum	64.81	63.16	81.37	79.96
Average	61.87	60.36	77.42	74.44
Mean	61.07	61.12	76.64	74.20

V mo-Alkalinity

Table A37: The effect of treatment with ultrasound on the mo-alkalinity (mg/L $CaCO_3$) of the water tested.

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	84.16	81.11	70.18	67.78
Maximum	86.39	84.26	81.20	80.37
Average	85.41	83.46	75.08	71.83
Mean	85.60	83.79	74.40	69.77

VI Hardness

Table A38: The effect of treatment with ultrasound on the hardness (mg/L $CaCO_3$) of the water tested.

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	192.44	181.21	65.52	63.84
Maximum	200.49	195.44	76.94	73.01
Average	195.79	189.83	68.47	67.31
Mean	194.69	190.57	66.36	65.80

VII Chlorine demand

Table A39: The effect of treatment with ultrasound on the chlorine demand (mg/L Cl_2) of the water tested.

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	-0.34	-0.01	-0.02	-0.01
Maximum	0.37	0.43	0.02	0.02
Average	0.07	0.12	0.00	0.00
Mean	0.02	0.05	0.00	0.00

THE EFFECT OF ULTRASOUND ON THE BIOLOGICAL QUALITY OF WATER (TABLE A40)

Table A40: The effect of treatment with ultrasound on the microbiological of the water tested.

% Reduction						
	SPC 22°C	**SPC 37°C**	**TC**	**FC**	**Bacterio-phages**	***Clostri-dium***
Klip River source water:						
Minimum	43.98	48.39	20.93	45.76	42.86	10.53
Maximum	83.21	69.22	80.63	76.47	83.33	43.42
Average	62.96	57.55	57.81	60.40	66.07	24.23
Median	63.72	56.95	63.79	60.66	69.05	22.37
Treated water:						
	SPC 22°C	**SPC 37°C**	**TC**	**FC**	***Clostri-dium***	
Minimum	97.82	97.25	89.67	74.00	50.00	
Maximum	99.82	99.71	99.68	99.76	75.00	
Average	99.28	99.03	96.63	89.31	64.54	
Median	99.56	99.52	98.64	90.90	66.67	

THE EFFECT OF UV ON THE CHEMICAL QUALITY OF WATER (TABLE A41 –A47)

I pH

Table A41: The effect of treatment with UV on the pH of the water tested.

	Klip River water		**Filtered water**	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	7.89	7.85	8.15	8.15
Maximum	7.97	7.89	8.26	8.24
Average	7.92	7.91	8.17	8.19
Mean	7.93	7.91	8.18	8.19

II Turbidity

Table A42: The effect of treatment with UV on the turbidity results (NTU) of the water tested.

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	45.30	61.20	0.90	1.02
Maximum	81.5	80.80	3.93	3.81
Average	65.88	67.69	1.96	1.92
Mean	67.25	64.65	1.62	1.81

III Conductivity

Table A43: The effect of treatment with UV on the conductivity results (mS/m) of the water tested.

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	66.60	61.40	23.20	22.90
Maximum	67.30	66.30	28.20	24.80
Average	66.94	65.09	25.34	23.82
Mean	66.90	65.60	24.60	23.95

IV UV Absorbance

Table A44: The effect of treatment with UV on the UV absorbance (%)of the water tested.

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	54.78	55.15	69.99	98.17
Maximum	62.47	60.58	80.94	80.43
Average	59.44	57.84	76.83	79.25
Mean	59.57	57.97	78.19	79.33

V mo-Alkalinity

Table A45: The effect of treatment with UV on the mo-alkalinity (mg/L $CaCO_3$) of the water tested.

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	83.98	72.96	68.7	68.15
Maximum	86.02	85.00	81.02	77.96
Average	84.87	81.75	74.49	72.10
Mean	84.67	83.15	75.70	71.94

VI Hardness

Table A46: The effect of treatment with UV on the hardness (mg/L $CaCO_3$) of the water tested

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	192.25	177.28	62.90	63.09
Maximum	197.12	193.38	76.56	66.46
Average	194.92	188.83	66.50	64.69
Mean	194.97	190.10	65.33	64.77

VII Chlorine demand

Table A47: The effect of treatment with UV on the chlorine demand (mg/L Cl_2) of the water tested.

	Klip River water		Filtered water	
	Control	**After Treatment**	**Control**	**After Treatment**
Minimum	0.00	-0.11	-0.01	-0.06
Maximum	0.15	0.08	0.03	0.03
Average	0.05	0.02	0.00	0.00
Mean	0.06	0.01	0.00	0.00

THE EFFECT OF UV ON THE BIOLOGICAL QUALITY OF WATER (TABLE A48)

Table A48: The effect of treatment with UV on the microbiological quality of the water tested.

% Reduction			
	SPC 22°C	**SPC 37°C**	***Clostridium***
Klip River source water:			
Minimum	32.71	45.63	5.56
Maximum	77.68	88.33	23.08
Average	59.28	64.51	15.15
Median	61.98	56.88	16.67
Treated water:			
	SPC 22°C	**SPC 37°C**	***Clostridium***
Minimum	88.44	79.65	14.77
Maximum	96.84	9.95	25.00
Average	93.65	87.67	18.11
Median	95.11	88.44	17.37

THE EFFECT OF UV AND ULTRASOUND ON THE CHEMICAL QUALITY OF WATER (TABLE A49 –A55)[3]

[3] The experiments on the treatment with UV and ultrasound was only conducted on filtered water, due to problems experience with the transport of Klip River water and from the other experiments it was concluded that the treatment of Klip River water is less effective than the treatment of filtered water.

I pH

Table A49: The effect of treatment with UV and ultrasound on the pH of the water tested.

	Filtered water	
	Control	**After Treatment**
Minimum	8.00	8.03
Maximum	8.24	8.21
Average	8.13	8.15
Mean	8.13	8.16

II Turbidity

Table A50: The effect of treatment with UV and ultrasound on the turbidity (NTU) of the water tested.

	Filtered water	
	Control	**After Treatment**
Minimum	0.60	0.68
Maximum	1.17	1.56
Average	0.92	0.99
Mean	0.95	1.00

III Conductivity

Table A51: The effect of treatment with UV and ultrasound on the conductivity (mS/m) of the water tested.

	Filtered water	
	Control	**After Treatment**
Minimum	23.50	22.90
Maximum	25.30	25.30
Average	24.39	24.23
Mean	24.15	24.55

IV UV Absorbance

Table A52: The effect of treatment with UV and ultrasound on the UV absorbance (%) of the water tested.

	Filtered water	
	Control	**After Treatment**
Minimum	66.40	66.07
Maximum	78.74	79.33
Average	73.93	75.23
Mean	76.64	75.48

V mo-Alkalinity

Table A53: The effect of treatment with UV and ultrasound on the mo-alkalinity (mg/L $CaCO_3$) of the water tested

	Filtered water	
	Control	**After Treatment**
Minimum	72.87	70.92
Maximum	75.65	75.65
Average	74.44	73.18
Mean	74.72	73.38

VI Hardness

Table A54: The effect of treatment with UV and ultrasound on the hardness (mg/L $CaCO_3$) of the water tested.

	Filtered water	
	Control	**After Treatment**
Minimum	66.27	65.71
Maximum	73.20	68.52
Average	67.95	66.90
Mean	67.67	66.55

VII Chlorine demand

Table A55: The effect of treatment with UV and ultrasound on the chlorine demand (mg/L $CaCO_3$) of the water tested

	Filtered water	
	Control	**After Treatment**
Minimum	-0.10	-0.02
Maximum	0.04	0.04
Average	-0.01	0.01
Mean	0.00	0.01

THE EFFECT OF UV AND ULTRASOUND ON THE BIOLOGICAL QUALITY OF WATER (TABLE A56)

Table A56: The effect of treatment with UV and ultrasound on the microbiological quality of the water tested.

% Reduction						
	SPC 22°C	**SPC 37°C**	**TC**	**FC**	**Bacterio-phages**	***Clostri-dium***
Treated water:						
Minimum	88.27	92.44	95.80	96.27	90.00	42.86
Maximum	99.67	99.18	99.41	99.39	100.00	75.00
Average	95.49	95.60	97.46	97.34	97.75	56.45
Median	96.84	95.07	97.24	96.80	99.88	54.55