UNIVERSITY OF ZULULAND

Dissertation submitted for the Degree of Masters in the field of Microbiology

The e**valuation of existing household water treatment system using World Health Organisation recommendations.**

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Abstract

Household water treatment technology coupled with nanotechnology research have been developed and managed to treat water at household level. This technology will help address United Nation Millennium Goals (UNMG) 2015 of access to safe drinking water within South African Development Countries (SADC). University of Zululand (UNIZULU) Hydrology Department has developed a low cost household treatment system (HWTS) using a traditional slip-cast process of inorganic and organic compounds mixed together and fired at high temperature to produce pores.

The filter has shown high strength, high chemical resistance, and thermal stability and proved very durable when subjected to harsh operational conditions. When the filter properties were compared to other low cost HWTS, it shows increased porosity of 69%, pore sizes of 0.3- 4µm and flow rate of 1-2L/h. The HWTS was initially impregnated with metal oxides such as silver oxide (AgO), copper oxide (CuO), zinc oxide (ZnO) and iron oxide (FeO) using capillary suction dried in kiln to improve viral removal. The impacts of these metal oxides were tested on selected bacteria, protozoa and bacteriophages. The selected bacteria show log reduction value (LRV) of 5, protozoa LRV of >6 and bacteriophages LRV 2.8 for AgO and CuO showing LRV of 4. Field testing was repeatedly performed over a period of one month. Cleaning of HWTS was only done at the end of testing. The results were as follows: somatic phages were reduced by 99.999% while F^- specific phages showed a reduction of 99.999%. This concludes that UNIZULU locally developed HWTS has met WHO performance standards and can be utilised to uplift the impact of safe drinking facing the SADC.

DECLARATION

I, Tlou Nelson Selepe, declare that:

- The research reported in this dissertation, except where otherwise indicated, is my original research.
- This dissertation has not been submitted for any degree or examination at any other university.
- This dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from those persons.
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Signed: …… Date ……

I am satisfied that I have given the candidate necessary supervision in respect of this dissertation and it meets the University's requirements in respect of postgraduate research dissertations.

I have read and approved the final version of this dissertation and it is submitted with my consent.

Signed: …… Date ……
JJ Simonis

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LIST OF ABBREVIATIONS

ATCC	American Type Culture Collection
Ag	Silver
AgO	Silver oxide
APHA	American Public Association
BF	Biosand Filter
CCP	Ceramic Candle Filter
CDC	Centres for Disease Control
CFU	Colony Forming Unit
CLED	Cysteine Lactose Electrolyte Deficient
Cu	Copper
CuO	Copper oxide
CWF	Ceramic Water Filter
DC	Direct current
DNA	Deoxynucleotide Acid
DWA	Department of Water Affairs
EHEC	Enterohaemorrhagic *Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
ETEC	Enterotoxigenic *Escherichia coli*
Fe	Iron
FeO	Iron oxide
FO	Forward Osmosis
GAC	Granular Activated Carbon
GLP	Good Laboratory Practice
HWTS	Household water treatment system
ICP	Inductively Argon Coupled Plama
LRV	Log Reduction Value
MF	Microscope Factor
MF	Microfiltraion
NaDCl	Sodium dichloroisocynorate

NaOCl	Sodium hypochloride
NF	Nanofiltration
Nm	Nonometer
NSF	National Sanitation Foundation
NTU	Nephelometric Turbidity Units
QMRA	Qualitative Microbial Risk Assessment
RO	Reverse Osmosis
RSA	Republic of South Africa
R-S-R	Disulphide linkage
SADC	South African Development Communities
SIPP	Silver Impregnated Porous Pot
SODIS	Solar Disinfection
SPEC-20	Spectrophotometer
UF	Ultrafiltration
UNICEF	United Nation International Children Fund
UNIZULU	University of Zululand
USEPA	United States Environmental Agency
UV	Ultraviolet
WHO	World Health Organisation
Zn	Zinc
ZnO	Zinc oxide

CHAPTER 1: BACKGROUND OF THE STUDY

1.1 INTRODUCTION

Water as a natural resource is essential for drinking, sanitation and economic development. Water is vital for the survival of almost every living organism on earth. It is therefore important to ensure that water sources are protected for generations to come. Unsafe drinking water, inadequate sanitation and insufficient hygiene accounts for about of 9.1% of the global burden of waterborne diseases and malnutrition (Prüss-Ustun *et al.*, 2008). The persistent poor quality of drinking water and the negative impact on public health hinder growth and development especially in developing countries (CDC, 2013). According to WHO and UNICEF (2012) statistics, 780 million people live without access to safe drinking water and approximately 2.5 billion people lack basic sanitation. The lack of water supply infrastructure worldwide contributes to one billion people living without potable water of which 84% are from rural areas (WHO, 2007). This problem according to Lantagne *et al*., (2006) contributes to a projected 1.87 million children dying worldwide per year as a result of diarrhoeal disease associated with contaminated water.

Safe drinking water in developing countries requires a variety of household water treatment systems (HWTS) to provide potable drinking water at a household level. The types of HWTS effectively used include boiling of water, use of chemical disinfectants, solar and filtration. Filtration is the most cost effective HWTS method. It includes a number of methods such as the biosand filter (BF); ceramic candle filter (CCF); bucket filter; and silver impregnated porous pot (SIPP) filter. These filters have been developed and implemented in many countries.

The ceramic water filter is one of the best methods as it reduces the turbidity and bacterial loads by more than 99%, however it is unsatisfactory for viral removal (Van Halem et al., 2007; Sobsey, 2002). These water filters have been used by developing countries to treat household water which is insufficiently disinfected or not purified (Clasen and Boisson, 2006). The locally developed ceramic filter from the University of Zululand (UNIZULU) Department of Hydrology was impregnated with silver oxide and has showed improvement in reducing waterborne protozoa and bacteria by more than 99.999% (Simonis and Basson, 2011).

1.2 PROBLEM STATEMENT

Unsafe drinking water contributes to waterborne diseases and high mortality rate in developing countries (Prüss-Ustun et al, 2008; Lantagne et al, 2006). Current methods used to purify bulk water, only reduces the turbidity and bacteria but do not significantly reduce viral load (Van Halem et al., 2007; Sobsey, 2002). Therefore this study was aimed at developing and improving an existing HWTS to meet the WHO performance standards and protocols for safe drinking water. Also to in future strive to meet the United States Environmental Protection Agency (USEPA) and National Sanitation Foundation (NSF) standards.

1.3 HYPOTHESIS

H_0: An improved existing HWTS will meet WHO performance standards, testing protocols versus.

H_1: An improved existing HWTS which will not meet WHO performance standard, testing protocols

1.4 AIM OF THE STUDY

The aim of this study was to improve an existing ceramic water filter (CWF) system for compliance with WHO recommendations regarding removal of microbial pathogens (bacteria, protozoa and viruses).

1.5 OBJECTIVES OF THE STUDY

The study objectives were as follows:

1.2.1 To identify the best techniques for improving CWF systems for microbial removal.

1.2.2 To identify and apply suitable metals and metallic oxides for coating an existing CWF.

1.5.2 To use selected microorganisms (bacteria, protozoa and viruses) for testing the improved CWF system for compliance with WHO recommendations.

1.5.3 To analyse the results and make recommendation for the best practice in improving the existing CWF system.

1.6 METHODOLOGY

Different types of methodologies that were employed are discussed below:

1.6.1 To identify the best techniques for improving CWF systems for microbial removal

The capillary suction technique (Simonis and Basson 2013) was used in improving the CWF and testing the efficiency to reduce waterborne pathogens (bacteria, protozoa and viruses) from filtered water. The selection was based on the cost analysis benefits and accessibility.

1.6.2 To identify and apply suitable metals and metallic oxides for coating an existing CWF

The following group of metals in their reducing and oxidizing form, silver ion (Ag^{2+}), zinc ion (Zn^{2+}), copper ion (Cu^{2+}), iron ion (Fe^{3+}), silver oxide (AgO), zinc oxide (ZnO), copper oxide (CuO) and iron oxide (FeO) were selected for coating an existing CWF and their impact was tested on selected microorganisms

i. Stock solution of suitable soluble metallic salts was prepared.
ii. Selected microorganisms (bacteria) were inoculated in nutrient broth containing stock solution.
iii. Inhibition by metallic salts on microorganisms was determined using a spectrophotometer (Spec20) at 620nm (Simonis and Basson, 2013).

A concentration of stock solutions was used to impregnate CWF using capillary suction technique developed and modified by Simonis and Basson (2014). The metals and metallic oxides were bonded to the CWF, dried and fired at 600°C in kiln. Leachate concentration test (metals leached from the CWF) was conducted using Inductively Coupled Argon Plasma (ICP).

1.6.3 To use selected microorganisms (bacteria, protozoa and viruses) for testing the improved CWF system for compliance with WHO recommendations.

The selected microorganisms (bacteria, protozoa and viruses) were utilised in testing the improved CWF for compliance with WHO recommendations. WHO (2011) recommended guideline lists of waterborne microorganisms which includes *Campylobacter jejuni* (bacteria), *Cryptosporidium* and *Giardia* (protozoa) and

Rotavirus (viruses). The unavailability of these pathogen microorganisms and safety standard of laboratory condition makes it difficult to utilise the above microorganisms as recommended, therefore the following groups of microorganisms are recommended to be used in this current research:

i. A group of bacteria was collected from Mhlathuze Water Board. This included *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Staphylococcus aureus Pseudomonas aeruginosa* and *Salmonella typhimurium*.
ii. Water samples containing protozoa were collected from the stream at Addison Park Empangeni, Mhlathuze River, and chicken manure from the chicken farm at the University of Zululand, KwaDlangezwa.
iii. Viruses – bacteriophages of somatic phages and F- specific phages.

The methods listed below were used in cultivating these selected waterborne microorganisms.

1.6.3.1 Estimation of bacterial counts using the Pour Plate method

The aim of this method was to cultivate selected bacteria for testing the existing HWT system.

a. Nutrient broth was prepared and selected bacteria were inoculated.
b. Inoculum were spiked in distilled water and filtered through existing coated and uncoated HTW system.
c. The colony forming units (CFU) were counted for both the pre- and post filtrate and the average CFU/ml was calculated (Frankhauser, 2005).

1.6.3.2 Estimation of protozoal using the Direct Microscopic Technique

The aim of this method was to concentrate protozoa from water polluted samples using centrifugation and then filter the water samples through existing coated HWT system.

a. The water samples were collected from still standing water containing protozoa.
b. The sample was separately filtrated through coated HTW system.
c. The pre-filtrate water sample (control) and post- filtrate sample were centrifuged.

d. Both the supernatant and pellet was microscopically observed for the presence of protozoa.
e. The protozoa were counted in 10 microscopic fields and the average quantity of protozoa per ml was determined. (Tortora *et al.*, 2007).

1.6.3.3 Estimation of phage density using the Double-Agar-Layer-plaque assay method

The aim of this method was to cultivate the phages using selected bacterial hosts such as *E. coli* (ATCC70078) and *S. typhimurium* (ATCC700030) while the phages are somatic and F^- specific phages.

a. Sets of coated HWT were prepared in duplicates and sterilised at 150^0C for three hours.
b. Both bacterial host cultures and phages were inoculated into nutrient broth and incubated.
c. The inoculum was centrifuged in order to retain the phages in the supernatant.
d. Tenfold serial dilutions of each phage and molten top phage agar were prepared, with the molten phage top agar placed in the water bath at 45-50^0C.
e. Each host was pipetted into the molten phage top agar together with phage dilutions, and allowed to absorb onto the host.
f. The inoculums were transferred on the phage agar plates and incubated.
g. Plaques were counted and expressed as phages/ml.
h. The phages/ml were diluted with sterile distilled water and filtered through the existing coated HWT system.
i. The cultivating procedures were repeated for the post filtrate samples to determine any viruses present (Grabow *et al.*, 1995).

The results were analysed and recommendation for the best practice in improving existing HWT system was made based on obtained results.

1.7 SIGNIFICANCE OF THE STUDY

There is a need to improve the existing cost effective HWTS, making it efficient against viruses. The provision of safe drinking water using HWTS will also help in reducing the occurrence of infectious diseases like hepatitis A, diarrhoea, cholera and gastroenteritis. The design of improved low cost efficient HWTS will provide poor families with effective means of removing viruses from contaminated water. The HWTS will reduce suspended solids and microorganisms.

1.8 EXPECTED OUTCOMES

a. Published papers in accredited journals
b. Oral and poster presentations at microbiological and hydrological national conferences.

1.9 INTELLECTUAL PROPERTY

Patent registration will be considered in line with correct procedures.

1.10 ETHICAL, HEALTH AND SAFETY

The study was approved by Faculty Board of Science and Agriculture and Higher Degrees Committee. The proposal and methodology were submitted to Ethical Committee to seek ethical clearance whereby it was agreed that selected microorganisms were mainly waterborne pathogens which do not pose a risk when general laboratory practices (GLP) were followed.

CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

The purpose of this study was to evaluate the existing locally developed household water treatment (HWTS) using World Health Organisation (WHO) recommendation. This chapter presents a literature review covering different technologies of HWTS, performance standards and testing protocols of HWTS, ceramic filters as the best HWTS, the use of metals and metallic oxides in coating the ceramic filter and acting as antimicrobial agents, different techniques for coating ceramic filters and selecting of microbial strains for testing HWTS.

2.2 HOUSEHOLD WATER TREATMENT SYSTEMS (HWTS)

Countries in The South African Development Community (SADC) still utilise unimproved water sources for drinking, with 72% reported in Democratic Republic of Congo, 71% for Madagascar and Mozambique, 55% reported for Tanzania, 62% Angola, 54% for Zambia, 23% reported in Malawi, Zimbabwe has 25%, 19% in Lesotho, 14% in Swaziland while Namibia and Botswana are on 12% and 10% respectively (WHO and UNICEF, 2010). According to the Department of Water Affairs (DWA), (2010) South Africa is still in search of the best reliable technology to serve 49% of its population living without safe drinking water.

This means that, South Africa still requires maintenance on existing infrastructure, building modest and suitable water systems to supply the population in need. This technology depends on:

1. Quality of raw water.
2. Availability of required materials and equipment.
3. Time frame in which it is used.
4. Customs, preferences, education levels of the local population.
5. Availability of personnel to provide the training and monitoring for its successful implementation.

Household water treatment systems such as bio-sand, ceramic filtration, appropriate chemical disinfection, solar disinfection and natural purifiers can be utilised to improve microbial quality of drinking water and decrease incidence of endemic diarrhoea caused by

waterborne pathogens (Murcott, 2006). According to United Children's Fund (UNICEF and WHO, 2009) HWT systems are used in under developed countries to ensure provision of clean and safe water. Sobsey (2002) identified thirty seven (37) different products, technologies and approaches used for microbiological treatment of drinking water in households. The technologies are seen as a superior, cost-effective purification method of treating raw water and poorly disinfected drinking water when compared with conventional centralised treatment and water reticulation systems (Lantagne, 2001; Clasen and Boisson, 2006). Figure 2.1 shows the effectiveness of different technologies in removing microbial pathogens from polluted drinking including viral removal which is technologically challenging (Bielefeldt *et al.,* 2010). These minor particle size of viruses (25–200 nm) makes it possible to remove them using expensive nano-filtration and reverse osmosis, which are not suitable or affordable on a domestic scale

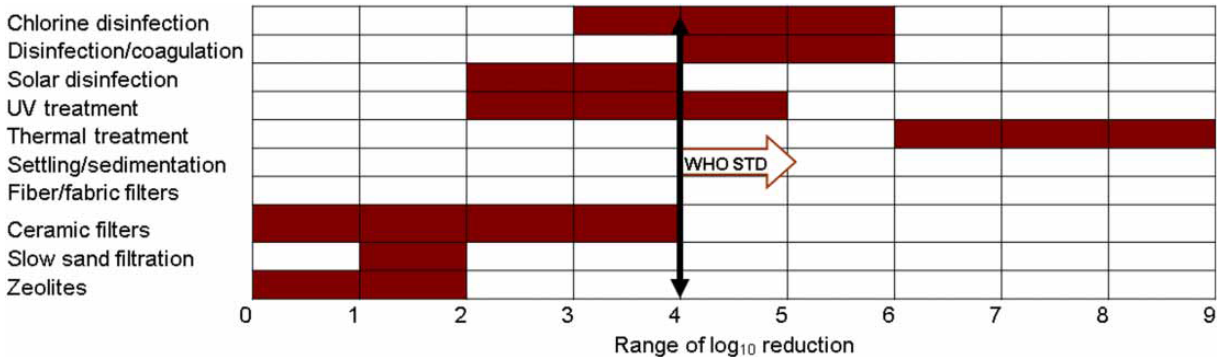

Figure 2.1: The effectiveness of different technologies to reduce polluted drinking water contaminated with viruses.

Where effectiveness is defined in terms of $\log_{10}$ reduction (LRV), as follows: LRV1 (reduction by 90%); LRV 2 (reduction by 99%); LRV3 (reduction by 99.9%); etc. The WHO now recommends a disinfection standard of LRV 4 (reduction equal to or greater than 99.99% (Simonis and Basson, 2011).

Mwabi *et al*. (2011) reiterated when testing these different technologies (HWTS), the obtained results had to be compatible with World Health Organisation (WHO) bacteriological standards, thus improving the quality of potable water at household level. However the shortfalls on the 37 discovered technologies was their performance on viral removal, which still needs further research (Bielefeldt *et al.,* 2010*).* WHO acknowledged

several point-of-use technologies that bridges these shortages of infrastructures. These technologies were thoroughly tested for microbiological performance and health impact. They include: boiling, solar, disinfection, chlorination and filtration.

2.2.1 Boiling

According to Sobsey (2002), boiling of water deactivates waterborne pathogens such as viruses and protozoan cysts that are chlorine resistant and is an effective water treatment method at the household level. Boiling managed to deactivate thermo tolerant coliforms rendering microbiologically safe drinking water (Rosa *et al*., 2010). The only disadvantage of boiling technique is lack of residual protection as the quality worsened during unsafe handling (Psutka *et al*., 2011; Oswald *et al*., 2007). According to Tagami and Uchida (2011) boiling does not remove chemical and radionuclides in drinking water but increases non-volatile concentration and evaporates water. Other limitation of this method is boiled water is not palatable, availability and affordability of energy is costly in some instances and also time consuming for mothers to collect fire woods which in turn leads to depletion of natural resources (Gupta *et al*., 2007). Care should be taken to prevent children from getting burnt as results of the fire. Furthermore boiling should be for specific time period in order to kill pathogens.

2.2.2 Solar disinfection

According to Reed (2004) solar disinfection (SODIS) is an effective method of disinfecting water under severely limited conditions. The method involves combination of thermal and ultraviolet (UV) radiation. It is effective in eliminating microbial pathogens and reducing diarrhoeal morbidity (Hobbins, 2004) as well as reduction of cholera epidemic (Conroy *et al.*, 2001). According to Hindiyeh and Ali (2010) application of SODIS is very simple with zero cost to individuals from various households. Small communities can use this technology during emergencies. Water was placed in a clear glass or plastic bottle with low turbidity (30<NTU) and exposed to the sun for a number of hours. Water was then used, resulting in substantial reduction of dysentery and diarrheal diseases. The process is accomplished under high level of motivation and compliance among the users (Du Preez *et al*., 2010). The motivation and compliance level is promoted through educational campaigns, training and marketing (Meierhofer and Landolt, 2009).

2.2.3 Chlorination process

Backer (2008) stated that chlorination is inexpensive and easily available in several forms. This process was developed by the community at household level after the Indonesian Tsunami (Gupta *et al*., 2007) and used sodium hypochlorite (NaOCl) to improve quality of stored water. There are numerous chlorine tablets such as sodium dichloroisocyanurate (NaDCC) being widely used in emergencies. The tablets offer pronounced advantages such as stability, safety, low capital investment, single package can be used and is lighter compared to NaOCl (Clasen and Edmondson, 2006; Lantagne *et al*., 2010). The shelf life of tablets is longer and cost of transportation is lower than liquid chlorine (Berg, 2010). Disadvantages of chlorination is that the water has a poor taste and health education is required to ensure correct dosage is applied to prevent over- or under-chlorination.

Mazumdar *et al*. (2010) have researched an innovative insoluble polymeric technology that kills microbes by releasing halogen. The mechanism has biocidal beads and provides enough residuals even during storage of drinking water. During disinfection biocidal beads are integrated into filtration trains for controlled flow rates and predictable performance. NaDCC tablets are favored in chlorination technology because they are easier to handle and are deployed rapidly in acute situations.

Dosage depends on factors such as temperature, turbidity, presence of natural organic matters and type of microorganisms (Abbaszadegan *et al*., 1997). The efficiency of NaDCC tablets in reducing diarrheal risks as shown in Zambia (48%), depends on contamination level of water (Jain *et al*., 2010). The only disadvantage of chlorination process is formation of dangerous disinfection byproduct (DBP). Lantagne *et al.* (2010) detected high levels of chloroform (84mg/L and 90mg/L) in a river subjected to NaOCL and NaDCC tablets.

According to Arnold and Colford (2007) a dose of 1.875mg/l with exposure time of 30 minutes allow free chlorine to inactivate more than 99,99% of enteric pathogens with the exception of *Cryptosporidium* and *Mycobacterium* species which require 2–3 mg/l to achieve 99.9%. Chlorination process affects the taste of the water.

2.2.4 Filtration

The filtration processes is constructed on the separation driving force such as pressure, temperature and osmotic differences across the membrane filter (Fane *et a*l., 2011). The membrane filter is pressure-driven and sub classified created on the pore size mainly as

microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) reverse osmosis (RO) and forward osmosis (FO) (Mulder, 2000; Fane *et al*., 2011). This process has a great advantage over other technologies as it operates under a variety of conditions such as temperature, pH and turbidity and is a non-chemical process. The process is easy to use and improves taste and odours if used in conjunction with activated carbon. Types of filtration technology are as follows:

i. Nano filtration

The membrane traps multivalent ions and dissolved organics. This requires energy to desalinate water (Fane *et al*., 2011). The Nano filter is coupled with a module that will generate energy required to pressurize the feeding system (Oh *et al*. 2000).

j. Reverse osmosis filtration

A reverse osmosis membrane filters a wide range of contaminants, including radionuclides. It has better flexibility in cases where the water quality is unclear. The membrane is effective in trapping radionuclides such as 238U, 234U, 137Cs and Ra (Tagami and Uchida, 2011). The disadvantage of this system is high energy consumption and it is only used when water source is absolutely contaminated.

ii. Ultrafiltration

The system reduces bacteria, viruses and turbidity (Laıˆne´ *et al*., 2000). An example of UF technology is Life Straw filter (Vestergaard), whereby raw water is sucked through an embedded straw to produce microbiologically safe drinking water (Clasen *et al*., 2009). The study conducted by Boisson *et al*. (2010) in Congo has shown reduction of diarrhea by 15% after using the Life Straw ultracentrifuge filter.

iii. Microfiltration

Microfilters are produced in small-scale systems globally, by firing a mixture of clay and flammable filler such as rice husk or sawdust which creates pores when fired (Archer and Elmore, 2010). Micro filters are available in different shapes such as a candle, pot and disc filter. Clasen and Boisson (2006) have produced commercial micro filters with a hollow core filled with Granular Activated Carbon (GAC) which was deployed in various countries.

The filters have shown a reduction in *E. coli* while the viral removal is less (Bielefeldt *et al*., 2010; Brown and Sobsey, 2010). The studies conducted by Brown et al. (2008) and Clasen *et al*. (2004) have shown 49% reduction of diarrheal cases in Cambodia and 70% in Bolivia where micro filters were used. In some cases, the filters were impregnated with silver in order to improve their bactericidal effects.

Van Halem *et al*. (2007) had proven that micro filters impregnated with silver particles can significantly increase the biocidal effect, however, some other studies have also argued that there is no significant difference in microbial reduction (Bielefeldt *et al.,* 2010; Brown and Sobsey, 2010). Simonis and Basson (2011) from the University of Zululand have produced a high quality ceramic filter impregnated with silver and showed improvement in decreasing waterborne protozoa by more than 99.9% and bacteria by more than 99.999%. The filter has met the WHO performance standard and testing protocol for HWTS while a further improvement for viral removal was required.

2.3 PERFORMANCE STANDARDS AND TESTING PROTOCOLS OF HWTS

According to Sobsey (2002), unsafe water supply to poor communities challenges the risk based performance guidelines and protocols for microbiological testing of the HWT systems. The efficiency of HWTS against waterborne microorganisms demands performance standards guidelines and microbiological evaluation protocols (WHO, 2011).

These microbiological performance standards and testing protocols are planned to inform, assess, protect and encourage technological improvement of the HWTS nationally and internationally. Although other international guideline standards and protocols such as United States Environmental Protection Agency (USEPA) and National Sanitation Foundation (NSF) provide for evaluation performance of the HWT devices, such testing requires sophisticated facilities and expertise. The USEPA and NSF specification for water treatment technologies must consistently satisfy the following criteria:

a. 3 log reduction value (LRV) for protozoa (USEPA, 1987; NFS, 2003).
b. 6 LRV for bacteria
c. 4 LRV for viruses

Such extreme standard guidelines and protocols are possible in developed countries, but cannot be applied to countries with poor and limited resources.

WHO (2011) recommended guidelines for HWTS, which evaluates the microbiological performance target at the risk level of 1 million for reduction of bacteria, viruses and protozoa in HWT systems. Table 1 shows the WHO recommended LRV target performance for HWT systems.

Table 2.1: Health based HWT performance targets (WHO, 2011)

Microbes	USEPA & NRF (2003)	LRV WHO Requirement for HWST (2011)			Local ceramic filter	
		Highly protective	Protective	Interim	Uncoated	Coated
Protozoa	3	4	2	≥2	>5	>5
Bacteria	6	4	2	≥2	>4	>4
Viruses	5	5	3	Not required	1.5	>4

WHO (2011), suggested that evaluation of HWT technologies be done at appropriately certified laboratories. The standards and protocols should encourage improvement of the HWTS technologies and consider the local use and storage conditions of the treated water (Brown *et al*., 2007). The guidelines for testing the effectiveness of HWT devices in removing the microorganisms should meet the following requirements:

a. testing methods of both influent and effluents samples
b. standard analytical methods
c. procedures for sampling and treating the sample volume
d. replicates preparation of dilution factors
e. Specification of incubation times and detection limits (Brown *et al*., 2007 and WHO, 2011)

A summary of worldwide testing protocols on low HWT systems in developing countries from the period 2000-2010 is presented in Table 2.2.

Table 2.2: Bench mark standards for low cost ceramic filters (Simonis and Basson 2011)

REFERENCE	BACTERIAL TYPE	REDUCTION %	REDUCTION LRV (log_{10})	REMARKS
Benchmark standards for low cost CWF				
Dies (2000)	*Escherichia coli*	> 98		Katadyn filter
Sagara (2000)	*Escherichia coli*			Nepalese CWF
Lantagne (2001)	Giardia lamblia		4.6	
	Cryptosporidium parvum		4.3	
(Lantagne 2001; Roberts 2004; Duke et al. 2006).	*Escherichia coli*	GM 98		Brown (2007): 90- 99%
	diarrhoeal disease	mean 46		
Hwang (2002)	Total Coliform	94 - 99.9		Terafil filter (Low 2002)
Dies (2003)	*Escherichia coli*	88		White Clay CWF
	Escherichia coli	>98		White Clay CWF with CS
	Escherichia coli	>98		Hong Phuc CWF
Coulbert (2005)	*Escherichia coli*	99.8		Pozzani CWF
Franz (2005)	*Escherichia coli*	92 - 100		tested 5 types of commercial filters
McAllister (2005)	Bacteria	99		Potters for Peace with CS
	Virusses	20		
Clasen et al (2006)				mean reduction of 39 - 44% in diarrheal disease
Van Halem (2006)	*Escherichia coli*		3 - 6.8	
	Clostridium spores		3.3 -4.9	
Baumgartner (2007)	*Escherichia coli*	99.8		Filtron with CS
	Total Coliform	99.4		
WSP (2007)	*Escherichia coli*	UP TO 98	1.7	50% reduction in darrheal disease
Oyanedel-Craver & Smith (2008)	*Escherichia coli*	boiling: 99		
	Escherichia coli	≥ 97.8		
	Virusses	< 90		
Brown (2010)	*Escherichia coli*	mean 99	2.1 - 2.9	
	Bacteriophages MS2	90 -99	1.2 - 4.1	
USA and other standards for producing drinking water				
(USEPA 1987; NSF 2003),	Bacteria		6	Specification for filters
	Viruses		4	
	Protozoa		3	
Brazil ABNT NBR 14908	*Escherichia coli*		2	
	Virusses			No reduction required
WQA ORD0901	*Escherichia coli*		3	
	Bacteriophages MS3		3	

The test results from filters in Table 2.2 have shown that the use of low cost HWT systems where standard water treatment is not affordable, will significantly improve the quality of household water and protect the end-users (UNICEF and WHO, 2009).

2.4 CERAMIC FILTERS AS HWTS

The porous ceramic filters are HWT filtration technology with effective pore size diameters ranging from 33-54 µm. This reduces microbes by mechanical screening which includes physical straining, sedimentation, diffusion and absorption (Van Halem, 2006). The

selectivity of the filters is based on size exclusion, differences in diffusion coefficient, electric charge, solubility and adsorption (Lantagne, 2001). The pore sizes differ in dimension which allows water flow through the ceramic. The pore sizes and wall thickness of ceramic filters use surface filtration or sieving and depth filtration mechanisms in removing the microorganisms and other pollutants.

a. Surface filtration screens larger particles to pass through the pores and the mechanism involves:
    i. Direct interception or sieving mechanism: whereby larger particles cannot pass through small pore sizes, the retention of these large particles are complete.
    ii. Bridging mechanism: smaller particles are very minute to be captured; this results in simultaneous collusion of two or more particles against the walls of the filter forming a bridge across the pores. The bridged particles slowly adhere to each other forming filter cake that results in decreasing the flow rate of the filter.
    iii. Inertial impaction mechanism: the particle resist to flow through water due to their mass of inertia. They are captured within the surface barrier while water is flowing through the ceramic filter.
b. Depth filtration mechanism: Particles penetrate the ceramic filter and is captured throughout the walls of the candle filter. The mechanism is subdivided into the following categories in which the microorganisms and other contaminants can be trapped:
    i. Labyrinth mechanism: the filter walls depth intercepts particles with sizes smaller than the pores. Water traverse through a complex maze of labyrinths following a twisted path of turns through sharp angles.
    ii. Cluster formation mechanism: particles coagulate together to form a large cluster, that the dead end of filter cavities can trap.
    iii. Adsorption mechanism: smaller particles are adsorbed onto the wall of the ceramic filter by attraction of Van der Waal forces.

Table 2.3: The sizes of common pathogens in water on ceramic candle filter (water4life, 2014)

Microorganisms	Mode of action	Pore sizes
Viruses	Smallest and most complex to remove	0.02-0.10 (µm)
Bacteria	Most dominant	0.5-5 (µm)
Protozoa	May be able to form cysts	4-20 (µm)

Table 2.3 shows the mode action of ceramic filter on several pathogens found in water. The mode of action depends on both the size of filter pores and pathogens present. The application of ceramic filtration technology has increased in developing countries due to cost effectiveness. Chemical disinfection and boiling of water are not always practical and cost effective (Colwell *et al*., 2003). The filters manufactured in different countries show disparities in porosity and pore sizes. Mercury intrusion porosimetry measurement of a Cambodian filter has shown 43% porosity while Ghanaian filter has shown 39% porosity and the Nicaraguan filter showing porosity of 37%. Van Halem (2006) indicated that the pore sizes were measured as follows: Cambodia 25µm, Ghana 22µm and Nicaragua 17µm.

Comparing these filters with the newly developed ceramic filter from University of Zululand filter, the porosity and pore size is significantly different as shown on Table 2.4 (Simonis and Basson, 2011; 2012). This locally produced ceramic candle filter has physical size of 8cm length width of 5cm and flow rate of 1-2L/h which is acceptable to provide one day drinking water in a household (Simonis and Basson, 2011; 2013).

Table 2.4: Properties of the Potters for Peace filters of different countries as compared to locally developed ceramic filter at University of Zululand

Production	Porosity	Pore size
Cambodia	43	35
Ghana	39	22
Nicaragua	37	17
Unizulu filter	68	0.3-4

Ceramic technology exists in many forms, as seen in figure 2.2 where the ceramic pot filters is the most prevalent in developing countries and are promoted by the non-governmental organisation (NGO) named Potters for Peace (PFP) (Clasen and Mintz, 2004; Clasen *et al.*, 2006). The filter is gravity driven and often uses a nested bucket system to safely store treated water at a flow rate between 1-3 litres of water per hour (Brown *et al.,* 2007). According to Van Halem *et al. (*2007), PFP ceramic filter significantly removed *E. coli* and protozoan cysts but was unsatisfactory for viral removal. Bielefeldt *et al.,* (2010), states that the filter performance and removal efficiency of viruses (0.02-0.1µm) varies significantly between 63-99.6%. Larger particles (0.5 µm) have a less variable removal range of 95.1-99.6% while 99.6% of particles larger than 1.0 µm were removed.

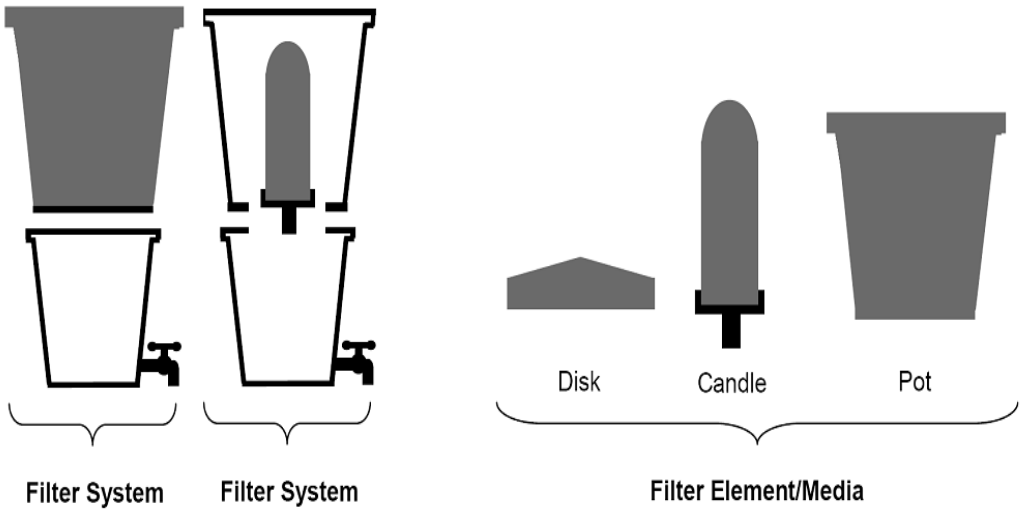

Figure 2.2: Different types of porous ceramic filter systems with their elements manufactured in different shapes such as disk, pot and candle (Simonis and Basson, 2011; 2013)

In order to increase the efficiency of the ceramic filters to high recommended LRV performance targets, colloidal silver is impregnated during the manufacturing (Lantagne, 2001) This involves tiny particles of silver mixed with water. Impregnation of colloidal silver, even at low concentration, enhances bactericidal and fungicidal properties on the ceramic filters.

The ceramic filter utilizes the following modes of action to deactivate microorganisms:

a. Catalytic oxidation: where the silver acts as a catalytic agent by absorbing oxygen and forms oxidation reaction. The absorbed oxygen reacts with sulfhydryl group on bacterial and viruses and removes the hydrogen atoms resulting in disulphide bond (R-S-R) bond which suffocate the microorganisms by blocking its respiration.
b. Catalytic reduction/oxidation reactions of colloidal silver react with the negative charge on the membrane proteins to deactivate the microorganisms.
c. Reaction of silver ions with bacterial cell membrane: attachment of silver ions on membrane radicals weakens cell respiration by blocking the energy transfer system. The Ag^{+} inhibits the specific enzyme required in certain biochemical activity. Silver binds with deoxynucleotide acid (DNA) the mechanism prevents the DNA of microorganisms from unwinding and halts cell replications.

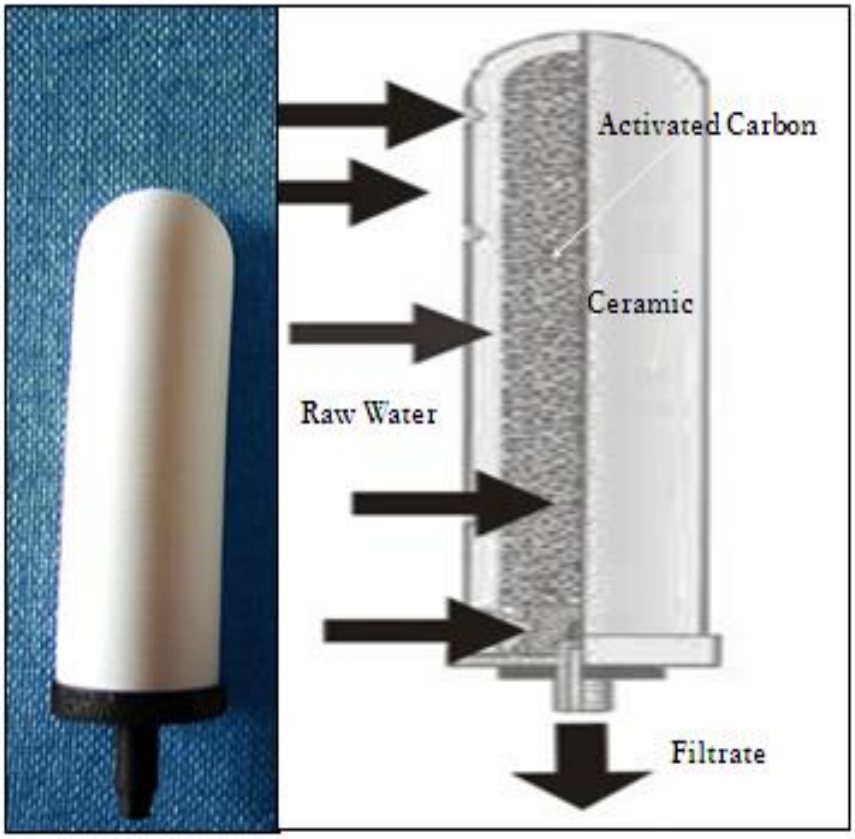

Figure 2.3: The mode of mechanism of activated carbon ceramic candle filter in filtering the water (Simonis and Basson, 2011)

Activated carbon ceramic filters as shown in Figure 2.3 only remove contaminants such as organic and residual of disinfectants such as chlorine and chloramines. The filter does not cope with high levels of bacteria in the water source, and uses two principal mechanisms of adsorption and catalytic reduction to remove contaminants from water.

The absorption effects results from negatively charged contaminants attracted to the granular surfaces of the activated carbon when water flows through the filter creating a principle referred to as magnetism.

The catalytic reduction process creates an electron transfer from the activated carbon within the ceramic filter which traps the microorganisms and other contaminants. The chemical reaction destroys the microorganisms, making the activated carbon a reducing agent.

USEPA and WHO have established that activated carbon filters of any type, even those impregnated with silver, failed performance targets of reducing microorganisms in drinking water. It is specifically for this reason that the ceramic candle filter applications ensure physical removal of microorganisms (WHO, 2011). The viral removal from polluted drinking water is technologically challenging (Bielefeldt *et al.*, 2010).

This challenge can be overcome by the modification of coating ceramic filters with various groups of metals and metallic oxides such as silver oxide, copper sulphate, iron chloride, zinc oxide, titanium dioxide, calcium oxide and others metals. The coating of the ceramic filters will reduce the pore sizes and increase the antimicrobial effects on viruses, thus improving the efficiency of the ceramic filters. The coating of metals and metallic oxides on the ceramic filters will demonstrate antimicrobial effects by binding to reactive groups on microorganisms, denaturing proteins and resulting in precipitation and inactivation of the microbes.

A locally developed filter at the University of Zululand is efficient in eliminating bacteria from polluted water but requires enhancement for elimination of viruses (Simonis and Basson, 2013; 2014). The filter development was shown in Figure 2.3, using the traditional slip-cast process with inorganic (ceramic) and organic (carbon) compounds mixed together, and fired at sufficiently high temperature to produce micron-sized pores for enhanced flow of water through the filter.

The filter's physical properties and performance standards has shown improvement as the filter was previously assessed using water inoculated with high concentrations of different bacterial cultures as well as with locally polluted streams. The effectiveness of these filters showed LRV > 4 (99.99% reduction efficiency) of bacteria and suspended solids found in polluted water (Simonis and Basson, 2011; 2012).

Table 2.4 has already shown comparison of superiority in physical properties of this locally developed ceramic filter (Simonis and Basson, 2011; 2013). With the precise cleaning and basic maintenance of this filter, it can effectively provide 1-2L/hr drinking water to rural families affected by polluted water sources. This could provide the solution for more than 340 million people in Africa living without access to clean drinking water. It can also addresses the 2015 United Nations Millennium Development Goals of reducing by half the number of people, worldwide, who lack access to safe water ((WHO/UNICEF, 2006).

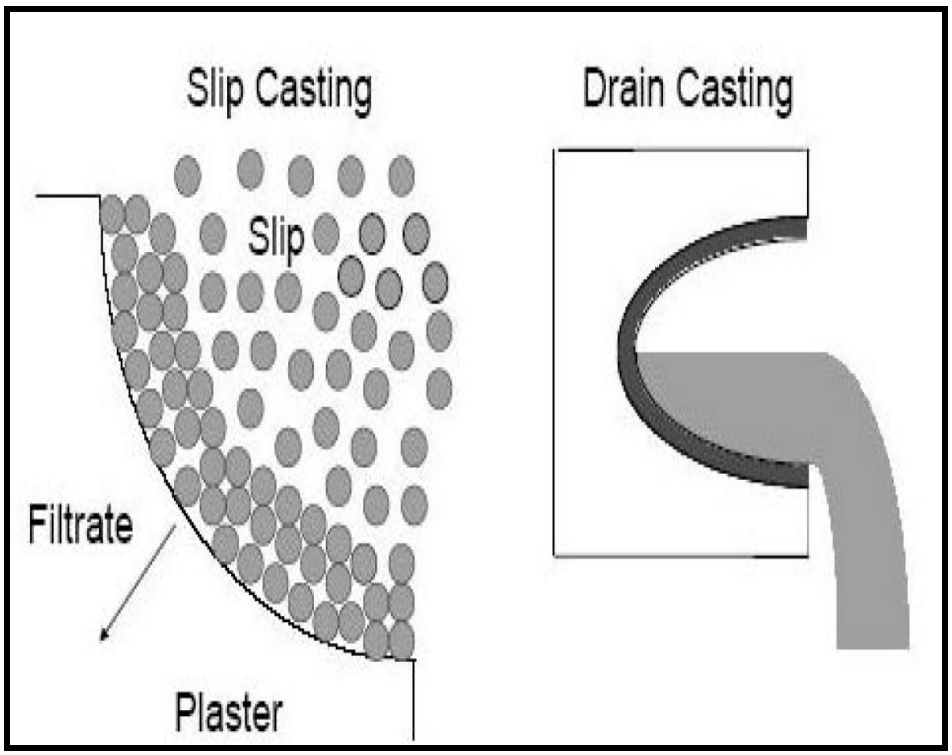

Figure 2.4: Locally developed ceramic candle filters using traditional slip-cast process Department of Hydrology Unizulu (Simonis and Basson, 2011)

2.5 USE OF METALS AND METALLIC OXIDES IN COATING CERAMIC WATER FILTERS

Metals and metallic oxides are commonly grouped into organic and inorganic. The inorganic metals and metallic oxides have the ability to endure adverse processing conditions (Whitesides, 2003). Currently the metallic metals and metallic oxides are extensively investigated and used as potential antimicrobials. The antimicrobial activity of these metals and metallic oxides is known to be a function of the surface area in contact with the microorganisms. The mechanisms involve small size and high surface to volume ratio whereby large surface area of the metals and metallic oxides boosts their interaction with the microbes aiding in the destruction of bacterial cell wall and specific targeted organelles (Whitesides, 2003).

Most metallic metals and metallic oxides have displayed antimicrobial activity when embedded and coated onto surfaces of water treatment devices, synthetic textiles, biomedical, surgical devices and food packaging (Gutierrez *et al*., 2010). The properties of different types of metallic metals and metallic oxides such as copper, silver and zinc, and

their mechanism of action as antimicrobial, antifungal and antiviral agents was emphasized in this study. The order of antimicrobial activity of metal oxides was demonstrated as follows AgO > ZnO > CuO > FeO (Azam *et al*., 2012).

2.5.1 Antimicrobial activity of silver metals and metallic oxides

Integration of silver metals and metallic oxides in health related products such as bandages and catheters prevent infection in hospitals (Ravishankar and Jamuna, 2011). According to Ip *et al.,* (2006) silver compounds treat burns, wounds and infections, with various salts of silver and their byproducts used as antimicrobial agents. Rai *et al.,* (2009) stated that nano-sized silver particles display antimicrobial properties with the use of several mechanisms; demonstrating their inhibitory effect on bacteria.

The high affinity of silver towards sulfur, abundant in the protein of bacterial cell membrane, and phosphorus containing amino acids inside and outside cell membrane are the key elements displayed in this antimicrobial effect. Silver metals and metallic oxides react with these compounds and affects cell viability (Kim *et al*., 2007).

According to Matsumura *et al.,* (2003) the silver ions (Ag+) released from silver metals and metallic oxides interact with phosphorus moieties in DNA, initiating inactivation of DNA replication, or react with sulfur-containing proteins, leading to the inhibition of enzyme functions. Ag^{+} ions separate the respiratory electron transport from oxidative phosphorylation, and hinder respiratory chain enzymes. The ions also interfere with the membrane permeability of both the protons and phosphate (Bard and Holt, 2005).

In broad-spectrum, less than 20nm diameter of Ag metals and metallic oxides is essential to create a greater porousness of bacterial cell membranes leading to death of the bacteria (Morones *et al.,* 2005). The dose dependent effect of silver metals and metallic oxides on the Gram-negative and Gram-positive microorganisms is within the size range of 10-15 nm (Shrivastava *et al.,* 2007).

2.5.2 Antimicrobial activity of Copper oxide metals and metallic oxides

The only disadvantage of using CuO as nano particle is that it requires large quantities to be used as bactericidal. The ionic nano-particulate properties provide CuO with the potential

application as an antimicrobial agent of high surface area and unusual morphology (Stoimenov, 2002).

Ren *et al.* (2009) stipulate that high concentrations of nano CuO express a bactericidal result in the killing range of bacterial pathogens especially in hospital acquired infections. CuO affects a negatively charged peptidoglycan of Gram negative bacteria such as *Pseudomonas aeruginosa* and *Proteus* spp. Rupareli *et al.* (2008) have shown high antimicrobial activity of copper metals and metallic oxides on the bacterial cells containing lot of amines and carboxyl groups. Copper ions released interact with DNA molecules and disrupt the biochemical process of the microorganisms.

2.5.3 Antimicrobial activity of zinc oxide as metals and metallic oxides

Stoimenov (2002) stated that the stability of zinc oxide under harsh processing conditions and its moderately low toxicity favors its application as antimicrobial agent. Further studies have shown ZnO selective toxicity to bacteria and minimal effect on human cells (Brayner *et al*., 2006). The recommended use of these metal oxides is found in agricultural and food industries (Zhang *et al*., 2007).

According to Jiang *et al*. (2009) the antimicrobial activities of zinc metals and zinc oxides have an influence on food related microorganisms such as *Bacillus subtilis, Escherichia coli* and *Pseudomonas fluorescens*. ZnO metals and metallic oxides (12nm) inhibit growth of *E. coli* by disintegrating the cell membrane and increasing the membrane permeability (Jin *et al*., 2009). The antimicrobial activity mechanism of ZnO generates hydrogen peroxide to inhibit bacterial growth and release Zn^{2+} ions which damages the cell membrane and interacts with intracellular contents (Brayner *et al.,* 2006).

2.5.4 Antimicrobial activity of iron oxide as metals and metallic oxides

Iron oxide has shown effectiveness against waterborne viruses, arsenic and lead (Kumar *et al*., 2009; Gutierrez *et al*., 2005). The charge surface of iron oxide in a solution detach a hydroxyl group that will bind and remove viruses from water using electrostatic interaction mechanisms resulting in formation of complexes (Ryan *et al*., 2002). According to Roden *et al*. (1996) when iron oxide metals and metallic oxides are coated on fiberglass, it demonstrates an improved antiviral potential while displaying a low antibacterial performance.

These metals and metallic oxides metal oxides coat ceramic water filters using the best techniques such as sputter deposition, brushing dipping, vacuum impregnation and capillary suction. Nano materials are at the leading edge of rapidly developing fields of nanotechnology with many of their applications.

2.6 TECHNIQUES FOR COATING MATERIALS WITH METALS AND METALLIC OXIDES

The following techniques were used to impregnate locally developed CWF with metals and metallic oxides to improve their efficiency in removal of microorganisms. Some of the techniques are costly. It is important to select the cheapest and easily assessable technique. They are classified as:

2.6.1 Sputter deposition technique

Sputter deposition is a physical vapour deposition method of depositing thin films by sputtering (ejecting) material from a source which then deposits onto a substrate. An inert gas is added to the vacuum where sputtered ions hover from the target in a straight line. The sputter ions impact energetically on the substrates (ceramic filter) or vacuum chamber having high gas pressure being created. The ions collide with the gas atoms that act as a moderator and they move diffusively, reaching the ceramic filter and then condensing after undergoing a random walk (Rossnagel, 2002).

Sputtering of metals and metallic oxides on the ceramic filter requires a sputtering system chamber consisting of platens, which both rotate and are water cooled, and magnetrons that are planar (three off-axis). The ceramic filter samples are coated at room temperature in a vacuum enclosure pumped to 1.3 × 10–4 Pa using metals and metallic oxides target.

The distance between the object and magnetron is adjustable for obtaining a homogeneous coating and rate of deposition. The only disadvantages of the technique is affordability as the machine is very expensive and coating only done on the outside of the filter.

2.6.2 Vacuum Pressure Impregnation technique

Vacuum impregnation technique seals the material without impacting on useful or dimensional characteristics of the material. The technique comprises use of pressure and vacuum (especially static or varied pressure at about 1 to 5 atm), to drive the coating solution deeply into pores with simultaneous discharging of trapped gas. This impregnation process forces a solution containing the metal coating through the pores of the ceramic filter under constant or varied positive (or negative) pressure using a piston or other type of pumps.

The forced solution rapidly and deeply dislodges entrapped air into the filter thereby completely and uniformly coating the surfaces of the pores. The vacuum pressure operates at about 0.2 to 1 atm gage. The pressure frequency variation of this coating technique may be on the order of less than one cycle per hour from ultrasonic frequencies. The technique is used commercially whereby the law of fluid mechanics govern the flow and remove the air from the pores using air compressor pump. The deposition process involves replacement metal coating solution and ceramic filter in chamber and evacuated until the pressure reaches 5-10 bar.

The generated vapour from coated solution in chamber forms a coating on the ceramic filter surfaces, whereby it diffuses and condenses on the filter surfaces. Although vacuum pressure deposition technique is clean, easily reproducible and ensuring high quality of coating, the costs become a problem in utilizing this technique in the research as existing developed filter target poor communities with less resources. (Li *et al*., 2010; Yanez, 2009,)

2.6.3 Capillary suction technique

The capillarity suction was identified as the suitable technique for improving HWT system for viral removal (Simonis and Basson, 2014).The capillarity suction technique results from two forces; the mutual attraction (cohesion) and the molecular attraction (adhesion) between water and ceramic material.

2.6.4 Brushing and dipping

Brushing is one of the methods of coating the ceramic filters with metals or metal oxides. The only limitation with brushing is uniformity in the distribution of ions on the filter surface and apply more labour intensive. It requires continuous brushing to cover the wide area of filter and concentrate the pores with metals whereby some of potential metal segregate to the filter surface during drying creating a concerns (Brown *et al*., 2007).

Dipping is one of the method of presenting metals and metallic oxides into the porous filter material. The method involves wet chemistry where untreated ceramic filter units were immersed in a 1 mg l^{-1} metals solution for 30 min (Van Halen *et al*., 2009). The metals were then removed from the solution, air dried and heated to 600ºC in an oxidising or a reducing environment, to produce an artificial deposit coating on the ceramic material, respectively (Wegmann *et al*., 2008).

The only disadvantage of this technique is that it requires constant stirring of the solution as to maintain equal distribution of ions within the solution, generates potential large volume of waste and is difficult to determine the amount of metal absorbed by each filter. Dipping method has concern as potential metals segregate to the filter surface of the ceramic filter during drying (Brown *et al*., 2007).

2.7 SELECTED MICROBIAL STRAINS

The selected microbial strains were based on quantitative microbial risk assessment (QMRA) which represents a coherent relationship between the pathogen levels in drinking water and waterborne diseases. The QMRA approximate the health impact of drinking water and its quality control measures (WHO, 2011). These selected strains are different in their physicochemical and biological properties in terms of their resistance to various treatment technologies. They are present in drinking water sources of both developed and developing countries mostly found in children and elderly people where the immune system is compromised (Lavin, 2009).

WHO selected strains such as *Campylobacter jejuni,* viruses (*rotavirus)* and the protozoa *Cryptosporidium* are used as QMRA with respect to their occurrences, concentration and health impact (WHO, 2011). The microorganisms are present in humans and faecal contaminated water that can be utilized by QMRA models to estimate the potential health effects after their ingestion. The locally produced HWT system will be used for testing these surrogate microbes to the required WHO recommended performance standard. The surrogate microbes involve the following selected microbial strains: *Escherichia coli* ATCC (25922), *Enterococcus faecalis* ATCC (29212*), Klebsiella pneumoniae* ATCC (31488*), Salmonella typhimurium,* ATCC (700030*) Staphylococcus aureus* ATCC (25925), coccoides and somatic and F^{-} specific phages.

2.7.1 Escherichia coli

The bacterium *E. coli* is a Gram negative, facultative anaerobe, non-spore forming bacillus with the width of 0.5µm and 2µm length. It is a member of the total coliform group of bacteria. This bacterium is found in the faeces of humans and other animals. Its presence in the water indicates faecal contamination, with gastrointestinal symptoms such as diarrhoea and nausea. Most of these bacteria are not pathogenic, only six groups of the *E. coli* are pathogenic and their pathogenicity is based on epidemiological evidence, phenotypic traits, clinical features of the disease and specific virulence factors. According to APHA (1978) the following groups of *E. coli* can be characterised as enterohaemorrhagic (EHEC), enterotoxigenic (ETEC) and enteroinvasive (EIEC). The enterohaemorrhagic strain causes bloody diarrhoea and abdominal cramps when contaminated water is consumed (Bruce-Grey-Owen Sound Health Unit, 2000).

Enterotoxigenic E. coli (ETEC) Strains result in infantile gastroenteritis in developing countries due to lack adequate clean water and poor sanitation. Strains isolation is performed in children below 5 years of age, and account for several hundred million cases of diarrhea and l tens of thousands deaths each year (Scheutz *et al*., 2005).

Enterohemorrhagic E. coli (EHEC) Strains infection is due to the consumption of contaminated foods, such as raw or undercooked ground meat products and raw milk, as well as water contaminated by human sewage or cattle faeces. The symptoms are abdominal pain, bloody diarrhea, and hemolytic uremic syndrome which last for 7–10 days. Shiga-like toxins with incubation period is 3–4 days, can result in acute renal failure (WHO, 2010).

Enteroinvasive E. coli (EIEC) Strains invade and multiply in the intestinal epithelial cells of the distal large bowel in humans. The strain is like shigellae with symptoms of abdominal cramps, diarrhea, vomiting, fever, chills and generalized malaise. Blood and mucus is found in the stools of infected individuals.

2.7.2 Enterococcus faecalis

E. faecalis is a streptococcus and its presence in potable water is used as a traditional indicator of faecal pollution. The microorganism has a teichoic acid group antigen of Lancefield group D and their normal habitat is the intestines of humans or animals (Sleigh and Timbury, 1981). It is a Gram positive spherical and oval coccus. It occurs in pairs or in chains with a diameter of 0.7-0.9 µm. The culture grows well in the blood agar enriched with blood serum or glucose and ferment lactose.

2.7.3 Klebsiella pneumoniae

K. pneumoniae is a significant member of genus *Enterobactericea*e is a Gram negative, non-motile encapsulated lactose fermenting, facultative anaerobe, and rod shaped bacterium. The bacteria inhabit animal and human intestines. Some of the strains are saprophytes and found in the soil, water and vegetation and survive well in moist environments (Ryan and Ray, 2004). When the strains of *Klebsiella pneumoniae* are isolated they produce mucoid colonies on several media such as blood agar, Cysteine Lactose Electolyte Deficient (CLED) agar and MacConkey agar.

2.7.4 Salmonella typhimurium

S. typhimurium is an animal pathogen bacterium which can also cause disease in humans. Meat products from animal sources are an important vehicle in the transmission of the salmonellosis. The isolation of *S. typhimurium* gives pale non – lactose fermenting colonies. The bacterium is a facultative anaerobe, Gram negative, flagellated, cylindrical rod shaped bacterium with the size of 2-3 x 0.4 - 0.6µm (Montville and Matthews, 2008). The identification is based on motility, biochemical tests and also serological identification which are antigenic characteristics where the O-antigen is surface capsular polysaccharides, H-antigen is flagellar and VI is for virulence.

2.7.5 Staphylococcus aureus

S. aureus is a facultative anaerobe, non-spore forming, catalase positive, coagulase positive and Gram positive spherical shaped coccus with the size of about 1µm. They are usually arranged in grapelike irregular clusters. The genus of Staphylococcus contains 15 different species associated with human diseases. The culture grows at an optimal temperature of 37^0C with pigment varying from orange to white colonies. The culture is also salt tolerant (Sleigh and Timbury, 1981).

The heat stable staphylococcal enterotoxin causes gastrointestinal diseases characterised by vomiting, diarrhoea, fever, abdominal cramps electrolyte imbalance and loss of body fluids. The microorganism is mainly found on the skin mucous membrane. The organism can be detected in the gastrointestinal tract and raw sewage. The mode of transmission into the water environment is through swimming from ponds and leakage of sewage into water supplies (LeChavallier and Seidler, 1980).

2.7.6 Pseudomonas aeruginosa

The microorganism is one of the *Pseudomonadaceae* family members. It is Gram negative, rod shaped and found in water, soil and faeces of animals. The microorganism utilises a low nutrient environment for growth and can gain access to treated water. It is proliferated by using nutrients in the water or construction material of the distribution system such as pipes, bottled water and water vending machines. *Pseudomonas aeruginosa* causes nosocomial infection because of its resistance to several antibiotics and disinfection. It has the ability to grow in distilled water and dilute disinfectant. Their capability to colonise aquatic low nutrient environments leads to taste and odour problems in water (Pelczar *et al*., 1988).

2.7.7 Protozoa

Protozoa are parasitic species with varying sizes greater than 4µm, transmitted through water environment causing gastroenteritis in humans (Sunderland *et al*., 2007). Protozoa infects young children (Yoder and Beach, 2010) and immune compromised adults resulting in high mortality rates (Rose, 1997).

According to Tien and Earn (2010), the mode of transmission for cysts is through contamination of food or water. The genus *Giardia* has flagella and inhabits the small intestine of humans and animals. They are pear shaped with two nuclei and one pair of flagella. The organisms can attach to mucosa of the small intestine where they absorb their nourishment. Cysts can survive more than three months in water. Disinfectants such as chlorine cannot kill cysts and they can only be inactivated by boiling.

Baldursson and Karanis (2011) reported that *Cryptosporidium* is responsible for half of disease outbreaks resulting from drinking water. It is a major concern in the national public health and environmental agencies as it causes diarrhoeal diseases that can last for several days and even a few weeks. *Cryptosporidium* is classified into several types:

a. *Cryptosporidium parvum* usually classified as Type 1. It infects human only but not agricultural and laboratory animals while *Cryptosporidium parvum* Type 2 has a broader host range such as cattle, sheep and laboratory animals.

b. *Cryptosporidium meleagridis* infects animals and birds. *Cryptosporidium felis* infect cats, cattle and humans.

c. *Microsporidia*: have distinctive coiled polar tube spores. The species causes chronic diarrhoea especially in immune-compromised individuals as a result it infects a range of agricultural animals. The route of transmission is through water contaminated with human waste.
d. *Toxoplasma*: parasitic oocysts found in cats, it forms cysts by infecting the secondary host. The life cycle is completed when the primary hosts consumes the secondary hosts. Improperly uncooked meat infects human. The sporulated oocytes are very resistant to both environmental conditions and purifiers.
e. *Entamoeba hystolytica*: causes amoeba dysentery resulting in abscesses formation in the liver and other tissue organs with the morphology of these species being identical to non-pathogenic *Entamoeba dispar*. Waterborne infection arises when contaminated food and water is consumed.

2.7.8 Bacteriophages

Bacteriophages are viruses which infects bacteria. They are easily and rapidly cultured in laboratories with unsophisticated facilities, and equipment (Pelczar *et al*., 1988). The genetic property of phages marks a new development of science such as molecular biology and medicine. Phages have a genome surrounded by a protein coat (capsid). Morphologically the capsid is made of subunits called capsomeres which consist of a number of protein subunits or molecules called protomers. Some phages have lipid, and other structures such as tails and spikes.

Bacteriophages resemble human viral pathogens in shape, size, composition and their response to water treatment process. The phages are characterised into various families namely; F-RNA coliphages (Family *Leviviridae*) resembles enteroviruses such as polio viruses (Family *Picornaviridae*) which both have an icosahedral capsid with a diameter of about 25 nm and a single strand (ss)-RNA genome, thus both F-RNA coliphages and enteroviruses excreted by humans, uses the F pili to attack the host.

a. *Microviridae*: (φX 174 and S13) is a single stranded DNA. The size is 30nm with large tail capsomeres and uses the cell wall to attack the host.

b. *Myoviridae:* ($T_{2,}$ T_4 and T_6) are double stranded DNA with the sizes of 94 X 65nm and contractible tails. The phages use the cell wall to attack the hosts.

c. *Siphoviridae:* (T_5 and ʎ) is a double stranded DNA with the long contractile tail and the 34nm size. The phage attacks the hosts by the F^+ pilli (Leclerc et al., 2000).

The phages withstand environmental stresses like disinfection, heat, ultra-violet light and sunlight (Grabow, 2001).

CHAPTER 3: METHODOLOGY

3.1 INTRODUCTION

This chapter outlines the different methods used in the project. The methods involve literature review on techniques for improving HWTS, coating methods, Leachate tests and effect of coated filters on various microbial strains.

3.2 LITERATURE REVIEW TO IDENTIFY THE MOST SUITABLE TECHNIQUES FOR IMPROVING HWT SYSTEM FOR VIRAL REMOVAL.

The techniques are discussed as follows:

3.2.1 Sputter technique

The coating procedure was as follows: Cleaning of the chamber with acetone to reduce contamination. A metals and metallic oxides target were placed into the DC magnetron and ceramic samples were loaded together with one metal and metallic oxide as substrate choice. Vacuum was applied for a period in excess of 2 hours (pump down). Argon gas was applied to the chamber. The magnetron was powered at 80W for 60 s at a deposition pressure of 3 × 103 Pa. The chamber was then de-pressurised with air and the magnetron powered off (Rossnagel, 2002).

3.2.2 Dipping technique

Dipping as another technique of impregnation was to introduce metals or metallic oxides onto the porous filter material. The untreated ceramic filter units were immersed in a 1 mg l^{-1} of metal solutions for 30 min (Van Halen *et al*., 2009). They were then removed from the solution, air dried and heated to 600 ºC in an oxidising or a reducing environment, to produce a superficial deposit of either metals or metallic oxides on the ceramic material, respectively (Wegmann *et al*., 2008).

3.2.3 Capillary suction impregnation technique

The capillarity suction was identified as the suitable technique for improving HWT system for viral removal (Simonis and Basson, 2013). The technique results from two forces: the mutual attraction (cohesion) between water molecules and the molecular attraction (adhesion) between water and ceramic material.

Impregnation of the ceramic filter involved dissolving soluble copper and silver salts as stock solutions. The ceramic was impregnated with the stock solutions (0.1, 0.5 and 1mg l^{-1}

concentrations) using a capillary suction technique. The filters were dried and fired (kiln) in an oxidizing (metallic oxide) environment.

The flow of water and metal ions was renewed by a simple cleaning process of brushing the outer surface the top layer of ceramic and contaminants allowing a new layer to be made available. This process can be repeated many times before the ceramic material is exhausted (Simonis and Basson, 2011).

Section 1.01 TO IDENTIFY AND APPLY THE BEST METALS AND METALLIC OXIDES FOR COATING AN EXISTING CWF

3.2.1 Testing the impact of selected metals on microorganisms.

In selecting the most effective metals to coat locally developed CWF the following compounds were tested: copper sulphate, copper chloride, iron nitrate, iron chloride silver nitrate, zinc sulphate, zinc carbonate, titanium oxide, titanium chloride, zirconium oxide and zirconium chloride.

Hundred mg l^{-1} of stock solutions of each metal were prepared (where necessary, heat was applied as to completely dissolve the metallic compounds). Dilutions of 10mg l^{-1} and 1mg l^{-1} prepared from the stock solution. One millilitre of selected microorganism cultured cell density was inoculated in each test tube of the nutrient broth containing 1ml of 1mg l^{-1} and 10mg l^{-1} stock solutions respectively.

The inoculum of selected microorganisms and dilution of metal solutions were thoroughly mixed and incubated overnight at 37ºC. Nutrient broth together with the different metal solutions were used to adjust the spectrophotometer (λ= 620nm) to zero absorbance. Nutrient broth and culture was used as control. Absorbance from the inoculums with metals was read and the results were tabulated (Reynolds and Farinha, 2005).

3.2.2 Coating process of an existing HWT using capillary suction technique

The following metals (Ag, Cu, Fe and Zn) were selected for impregnation of locally developed CWF. Two grams of the selected metals were weighed and dissolved in 100ml sterile distilled water.

Capillary suction technique was carefully chosen in impregnating the selected metals. CWF was immersed in the prepared metal solutions. The impregnated CWF were dried and fired at 600ºC either in reducing or oxidizing state to develop either metallic or metal coatings.

Leachate tests were performed by putting a fixed volume of purified water through the ceramic and testing the filtrate using Inductively Coupled Argon Plasma (ICP). The results were tabulated and compared with WHO requirements for HWTS (Simonis and Basson, 2014).

3.3 SELECTION OF MICROORGANISMS (BACTERIA, PROTOZOA AND VIRUSES) ACCORDING TO WHO RECOMMENDATION FOR TESTING THE LOCALLY DEVELOPED CERAMIC FILTERS.

WHO recommended the following lists of waterborne microorganisms for evaluation of CWF.

i. Protozoa – *Cryptosporidium*
ii. Bacteria – *Campylobacter jejuni*
iii. Viruses – Rotavirus

The microbial prevalence in South Africa and the laboratory safety made it difficult to utilise the microorganisms as recommended by WHO for HWTS. The following waterborne microorganisms were selected for use in the research:

List of selected local waterborne microbes:

i. Protozoa - water samples from standing water and chicken manure.
ii. Bacteria – *E coli*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Staphylococcus aereus*, *Salmonella typhimurium* and *Pseudomonas aeruginosa*
iii. Viruses – somatic phages and F^- specific phages.

3.3.1 Pre- and post-filtration of bacteria using the Pour Plate method (Laboratory testing)

The microorganisms were collected from the Mhlathuze Water Board. Selected bacteria were inoculated into 100ml nutrient broth and incubated overnight at 35ºC. The 100ml incubated inoculums were thoroughly mixed in 500ml sterile distilled water and filtered through pairs of coated filters, Ag^{+} AgO, Zn^{+} and ZnO, Cu^{2+} and CuO and Fe^{2+} and FeO. In each case the metal concentration was set at 1mg l^{-1}.

Tenfold serial dilutions of pre filtrate were prepared from 10^{-1} to $10^{-7}$and pour plates prepared. The 10 fold serial dilution of post filtrate was prepared from 10^{-1} to 10^{-3} and pour plates were prepared. The plates were incubated at 37ºC for 24 hours. The colonies were counted for both pre and post filtrate. The average colony forming units (CFU) was calculated per ml (Frankhauser, 2005). Results were tabulated.

3.3.2 Filtration of protozoa using the Direct Microscopic Technique.

Water samples containing protozoa were collected from the following three sources:

a. Naturally occuring ponds on the floodplain of the Mhlathuze River.
b. A stream in the Addison sports ground in Empangeni, RSA.
c. Chicken manure from University of Zululand chicken farm at KwaDlangezwa, RSA.

The samples were filtered through the coated pairs of filters. Hundred ml of the raw water samples from the ponds and stream were used as control (pre- filtrate). These polluted water samples were concentrated for protozoa using centrifuge (3500rpm for 5min). Twenty µl of both the sediments and supernatant of pre and post filtrate were observed microscopically for the presence of protozoa through counting 10 microscopic fields (Tortora *et al*., 2007).

The average count per microscope field was multiplied with MF (microscope factor) and the results were expressed as protozoa ml^{-1}. The microscope factor was calculated as follows:

MF= ab $(\pi r^2)^{-1}$ where MF is microscopic factor a is the surface area of the slide, b is the reciprocal of the volume used, πr^2 is the surface area of one microscope fields, r is the radius of one microscope field.

3.3.3 Pre- and post-filtration of phages using the Double-Agar-Layer-Plaque Assay Method.

Phages were cultivated using *Escherichia coli* (ATCC 77004) as selected bacterial hosts. Both hosts and phages were inoculated into nutrient broth and incubated at 35ºC overnight. Fifty millilitres inoculum was centrifuged at 10000 rpm for 5 m to retain the phages in the supernatant and then transferred into sterile McCartney bottles and stored at 5ºC.

Tenfold serial dilution of the phage suspension and the molten top phage agar were prepared in the McCartney bottles with the molten top phage agar placed in the water bath at 45ºC -50ºC. One ml host and 0.1ml of each tenfold dilution of phage were pipetted into each McCartney bottles containing molten phage agar and the phages were allowed to absorb onto the host for at least 10 min.

The inoculums were transferred on phage agar plates and allowed to solidify and were incubated at 35ºC overnight. Plaques were counted and expressed as phage ml^{-1}. The phage ml^{-1} was diluted into 1000ml sterile distilled water and 100ml of pre filtrates were filtered into the existing uncoated and coated ceramic filters. The cultivation procedure was repeated for post filtrate samples to determine the presence of phages in the post filtrate. The plaques present were counted and average phage ml^{-1} were calculated for pre- and post filtrates (Grabow *et al.*, 1995).

3.3.4 Field testing of the CWF

The coated and uncoated filters were daily syphoned through each of the coated CWF for a period of 1 month using 10l of water per day. The gravity pull from the tube suspended below the ceramic filter causes water to initially rise into the small pore diameters of the ceramic to a height above the water level in the container. All the pores in the ceramic filters are of capillary size, and as a result water is pulled upward into the ceramic filter until all the pores are completely saturated.

On saturation every pore becomes simultaneously involved in the filtration process causing the water to flow and therefore increasing both the effectiveness of the surface and depth

filtration of the ceramic. The process that will continue until the last drop of water has been removed from the container.

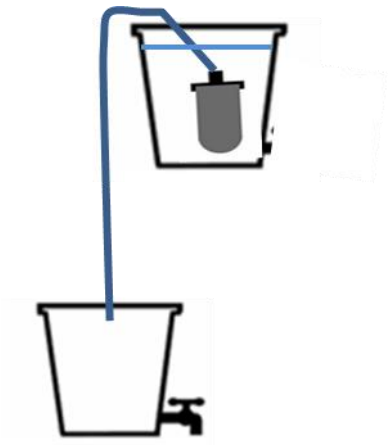

Figure 3.1: Syphoning process

Testing of selected coated ceramic filters against bacteriophages for pre and post-filtrate was done on day 1, 7, 14, 21 and 30 as well as a cleaning step (brushing the outside surface). The 10 l of water was inoculated daily with 10ml of phage suspension. Post filtrate was cultured using the Double Agar-Layer-Plaque Assay Method using both somatic and F – specific phages from different hosts (*Escherichia coli* ATCC (13706) Umgeni Water Board, *Escherichia coli* ATCC (700730) and *Salmonella typhimurium* ATTCC (14028) from University of North West for comparing the pre- and post-filtrate phage concentration and for calculating the LRV (Simonis and Basson, 2011). Results were tabulated.

CHAPTER 4: RESULTS

4.1 INTRODUCTION

This chapter outlines results on inhibition effect of different metals on selected bacteria, oligodynamic action of silver-coated ceramic using sputter technique in comparison with wet chemical impregnation, impact of metals and metallic oxides impregnated ceramic filters on water infected with selected bacteria, protozoa and impact of metal impregnated ceramic on bacteriophages. Leachate results from three different concentrations of Ag_2O and CuO, field testing techniques using three different concentrations of Ag_2O and CuO on coated ceramic filters for viral testing are also presented in this chapter. The results of sputter coating image of ceramic filters when coated with silver and the structure of micron sized pores of locally developed ceramic filter captured using electron microscope also form part of this section.

4.2 PRESENTATION OF RESULTS

A group of selected metallic compounds were tested on a group of Gram negative and Gram positive bacteria (Table 4.1). Findings show that two Gram positive bacteria *E. faecalis* and *S. aureus* were effectively inhibited by $CuSO_4$ while all Gram negative bacteria *Escherichia coli, K. pneumoniae* and *S. typhimurium* were effectively inhibited by $FeNO_3$.

Table 4.1: Inhibition effect of metal on selected bacteria

Metal	*E. coli* Indicator presence of coliforms in polluted water. Gram negative, spherical shaped	*K.pneumoniae* Gram negative rod shaped bacteria	*S.typhimurium* Gram negative, flagellated, rod shaped bacteria	*E. feacalis* Gram positive, spherical coccus	*S. aureus* Gram positive, grape like, irregular clusters
$AgNO_3$	X			X	
$CuCl_2$	X				
$CuSO_4$				X	X
$FeCl_2$		X	X	X	
$FeNO_3$	X	X	X		
$ZnCO_3$		X			
$ZnSO_4$		X	X		X

key: x- represent inhibition of bacteria by selected metals

$FeCl_2$ and $ZnSO_4$ only managed to inhibit two Gram negative bacteria namely *K. pneumoniae* and *S. typhimurium* and also showed some inhibitory effects on Gram positive bacteria *E. faecalis* and *S. aureus*. With the exception of *Escherichia coli* which was only inhibited by $AgNO_3$ and $CuCl_2$ while $ZnSO_4$ showed an inhibitory effect on *Klebsiella pneumoniae*.

The results of oligodynamic action of silver-coated ceramic filters using sputter technique in comparison with wet chemical impregnation (dipping technique) are shown in Table 4.2.

Table 4.2: Oligodynamic effect of silver-coated ceramic filters using sputter technique

Microorganism	Sputter coated Ag^+	Wet chemical impregnation Ag^+	Wet chemical impregnation Ag_2O
E.coli	11	11	21
P.mirabilis	5	14	15
P.aureginosa	10	11	18

Both the $Ag^+$and Ag_2O are shown to be effective in relation to the magnitude of the zones of inhibition for the microbes tested of sputter coating and wet chemicals results (Table 4.2). The oligodynamic effects of silver-coated ceramic filters have shown significant zones of inhibition for *Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa.* The wet impregnated ceramic filter have shown increased in zone of inhibition on both Ag_2O and Ag^+as compared to sputtered coated ceramic filter. This impregnation process is a much easier and cheapiest method of incorporating Ag_2O and Ag^+on active surfaces in the porous ceramic while the sputter technique coat the outer surfaces only and involves expensive and sophisticated equipment.

Figure 4.1: Oligodynamic action of coated silver ceramic filter

The impact of the coated ceramic filter on selected bacteria is presented in Table 4.3. These ceramic filters were coated with metals in both reducing and oxidising state (Ag^{+}, Cu^{2+}, Zn^{2+}, Fe^{2+}, AgO, CuO, FeO and ZnO) at concentrations of 1mg l^{-1} using capillary suction technique. Post filtration the coated filters have shown huge antimicrobial activity on both Gram negative and Gram positive bacteria. Both metals and metal oxides have high antibacterial efficacy above WHO recommendations for HWTS. The inhibitory pattern on metals are indicated as follows $Ag^{+} > Fe^{2+} > Cu^{2+} > Zn^{2+}$ and the metal oxide inhibitory pattern as AgO > CuO >ZnO > FeO with average LRV of 5.1. Both the metals and metal oxides LRV were above highly protective LRV 4 of WHO requirement for HWTS (refer to Table 2.1). Gram positive bacteria *S. aureus* and *E. faecalis* have shown greatest LRV of 7 and 6. Gram negative bacteria *E coli* and *K. pneumoniae* showed LRV of 5 while *S typhimurium* and *Pseudomonas aeruginosa* showed LRV of 4 which was within the highly protective limit value of WHO requirement for HWTS. The obtained LRV results where compared with WHO standards target see Table 2.1

Table 4.3: Impact of metal impregnated ceramic on water infected with selected bacteria

Metals	*E.coli (CFU/ml) ATCC770034*	*S.aureus (CFU/ml) ATCC25925*	*K.pneumoniae (CFU/ml) ATCC 31488*	*E.faecalis (CFU/ml) ATCC29212*	*S.typhimurium (CFU/ml) ATCC700030*	*P. aeruginosa (CFU/ml) NCTC10662*	LRV AVE
Control	74 000	9 000 000	110 000	2 290 000	8 900	5 300	
Non-coated (LRV)	5	5	4	6	4	4	4.6
Ag+	5	7	5	6	4	4	5.1
AgO	5	7	5	6	4	4	5.1
Cu++	5	7	5	6	2	4	4.8
CuO	5	7	5	6	4	4	5.1
Fe++	5	7	5	6	4	4	5.1
FeO	5	7	5	6	4	4	5.1
Zn++	5	6	2	6	4	4	4.5
ZnO	5	7	5	6	4	4	5.1
						AVE	5.0

The performance of locally developed coated ceramic filter was above 99.999%, above USEPA and NSF standards and satisfy WHO performance standard for HWTS. Impregnation had improved the performance of a locally developed filter on bacterial removal by 99.999% as compared 99.99% reduction results on uncoated filter previously tested by Simonis and Basson (2012). The uncoated filter showed average LRV of 4.6 slightly lesser than the average LRV of 5.1 of coated filters

Table 4.4 depicts the filtrate value (protozoa. ml^{-1}) obtained through filtration of water samples collected from various sample points. Using both metal and metallic oxide impregnated ceramic filters the results shows how the filters impact water infected with protozoa. Both coated and uncoated ceramic filters trapped protozoa from water samples collected from various sample points. Post filtrate from both impregnated and uncoated ceramic filters have no results, although control samples have shown a high amount of protozoa present. It means both coated and uncoated ceramic filters managed to completely remove protozoa. The LRV was therefore greater than 6 which is above the WHO recommendations for HWTS. The results prove that both coated and uncoated ceramic water filter can be utilised to trap Cryptosporidium oocytes and Giardia species to above

WHO requirements for HWTS which are problematic to other treatment technologies such chlorination and boiling (WHO, 2011).

Table 4.4: The impact of metal impregnated ceramic filters on water samples infected with protozoa

Samples	Mlathuze Pond A (protozoa ml^{-1})	Mhlathuze Pond B (protozoa $ml^{-1)}$	Addison Park (protozoa $ml^{-1)}$	Chicken manure (protozoa $ml^{-1)}$
Control	54 542	65 236	45 986	50 264
Non-coated	0	0	0	0
Ag^{+}	0	0	0	0
AgO	0	0	0	0
Cu^{++}	0	0	0	0
CuO	0	0	0	0
Fe^{++}	0	0	0	0
FeO	0	0	0	0
Zn^{++}	0	0	0	0
ZnO	0	0	0	0

The effects of the filtrate LRV obtained through filtration of bacteriophages using ceramic filters impregnated with both metal and metallic oxides with the results showing how impregnated filters impact water infected with bacteriophages are defined in Table 4.5. The obtained LRV results were compared with WHO standards.

Table 4.5: Impact of metal impregnated ceramic on bacteriophages

Sample	Bacteriophages Per ml	LRV	Virus reduction (%)
Control	620 000		
Non-coated	20 500	1.5	98
Ag^{+}	7 500	1.9	99
AgO	1 000	2.8	99.8
Cu^{++}	130 000	0.7	79
CuO	40	4.2	99.99
Fe^{++}	19 000	1.5	96.9
FeO	12 500	1.7	97.9
Zn^{++}	17 500	1.5	98
ZnO	160 000	0.6	74

Table 4.5 indicates how 0.1mgl^{-1}concentration of both metals and metal oxides impregnated ceramic filter impacted the bacteriophages with minimal trapping of bacteriophages while the control samples have shown high concentration 620000 bacteriophages present. Comparing the log reduction values ZnO and Cu^{++} have the least LRV of 0.6 and 0.7 even less than uncoated ceramic filters with LRV of 1.5. It means the ceramic filter coated with these two metals ZnO and Cu^{++} shows little effect on trapping the phages, therefore we may further increase the concentration of coating as to boost the efficiency of these metals and metallic oxides.

The LRV's on metals and metallic oxides came close to reaching the WHO recommended performance target for HWTS. The Ag_2O has improved the trapping with LRV of 2.8 (± 99.9%), which is just less than the WHO recommended performance target. CuO shows the highest performance with LRV 4.2 which displays a reduction efficiency of 99.99% on removal of phages. The improved antiviral effect on the phages can be due to the fact that copper as metals and metallic oxides, has exhibited a range of potentially useful physical and chemical properties, is cheaper and easily mixed in stable form with polymers thus rendering a wide application.

The Zn^{++} and Fe^{++} LRV is the same as the uncoated ceramic filter having LRV of 1.5. Most of the metals and metallic oxides (Ag^{+}, $Cu,^{2+}$ $Zn,^{2+}$ $Fe,^{2+}$, FeO and ZnO) have failed to meet the performance target as stated by WHO requirements for HWTS, with exception of CuO and Ag_2O (LRV 4.2 and 2.8) were therefore selected for further testing (higher concentration and field testing) for bacteriophages removal. Leachate was detected on these coated ceramic filters.

The leachate results obtained from ICP instrument after filling the coated ceramic filters with distilled water. Leachate results from different concentrations were tabled and compared with WHO detection limits for drinking water as shown in Table 4.6.

Table 4.6: Leachate results from different concentrations of Ag_2O and CuO using the ICP

Coating Concentration [mg l^{-1}]	Filtrate Leachate Concentration [µg l^{-1}]						WHO Drinking Water Limits [µg l^{-1}]
	Day 1		Week 1		Week 4		
	CuO µg l^{-1}	Ag_2O µg l^{-1}	CuO µg l^{-1}	Ag_2O µg l^{-1}	CuO µg l^{-1}	Ag_2O µg l^{-1}	
Ag^+							50 - 100
0.1		<17		<17		<17	
0.5		<17		<17		<17	
1.0		<17		<17		<17	
Cu^{2+}							2 000
0.1	<13		27		19		
0.5	25		27		19		
1.0	42		27		20		

Note: Ag^+ < 17 (concentration was below detection limits)

Both the CuO and Ag_2O have shown minimal leachate development after coated ceramic filters were subjected to field testing for a month period as specified by the WHO recommendations for testing HWTS. Ag_2O coated filter have concentration of less than 17µg l^{-1} Ag^+ (below detection limit) leached which is below the WHO drinking water limits of 50-100µg l^{-1}. The leachate result from three concentrations of Ag_2O shows uniformity throughout the test. The CuO coated filter have shown high concentration of leachate after the first day of filtration and as expected reduced with time for all the three concentrations. The metal leachate was within WHO standard (200µg l^{-1}).

Table 4.7 indicates the filtrate LRV obtained through filtration using different concentrations of metallic oxides ceramic filters. The results show how different concentrations of metallic oxides ceramic filters impact water infected with bacteriophages after day 1, 7, 14, 21 and 30 as well as a cleaning step filtration. The obtained LRV were compared to WHO performance standard of viruses.

Table 4.7: Field testing Techniques using both coated and uncoated ceramic filters for viral testing

Bacteriophages			*E coli*: NWU	*E coli*: NWU (LRV)	*E coli*: Umgeni	*E coli*: Umgeni (LRV)	*Salmonella typhimurium*	*Salmonella typhimurium* (LRV)
Pre- filtrate(phage 10000ml^{-1})			230		8200		12000	
Day 1	1	AgO(0.1mgl^{-1})	0	>6	0	>6	55	2
	2	AgO(0.5mgl^{-1})	0	>6	0	>6	0	>6
	3	AgO(1.0mgl^{-1})	0	>6	0	>6	0	>6
	4	CuO(0.1mgl^{-1})	0	>6	0	>6	3670	1
	5	CuO(0.5mgl^{-1})	0	>6	0	>6	1000	1
	6	CuO(1.0mgl^{-1})	0	>6	0	>6	0	>6
Day 2	1	AgO(0.1mgl^{-1})	0	>6	0	>6	0	>6
	2	AgO(0.5mgl^{-1})	0	>6	0	>6	0	>6
	3	AgO(1.0mgl^{-1})	0	>6	0	>6	0	>6
	4	CuO(0.1mgl^{-1})	0	>6	0	>6	0	>6
	5	CuO(0.5mgl^{-1})	0	>6	0	>6	0	>6
	6	CuO(1.0mgl^{-1})	0	>6	0	>6	0	>6
Week 2	1	AgO(0.1mgl^{-1})	0	>6	0	>6	0	>6
	2	AgO(0.5mgl^{-1})	0	>6	0	>6	0	>6
	3	AgO(1.0mgl^{-1})	0	>6	0	>6	0	>6
	4	CuO(0.1mgl^{-1})	0	>6	0	>6	0	>6
	5	CuO(0.5mgl^{-1})	0	>6	0	>6	0	>6
	6	CuO(1.0mgl^{-1})	0	>6	0	>6	0	>6
	Control	Uncoated	707	0	707	1	707	1
Week4	1	AgO(0.1mgl^{-1})	0	>6	0	>6	0	>6
	2	AgO(0.5mgl^{-1})	0	>6	0	>6	0	>6
	3	AgO(1.0mgl^{-1})	0	>6	0	>6	0	>6
	4	CuO(0.1mgl^{-1})	0	>6	0	>6	0	>6
	5	CuO(0.5mgl^{-1})	0	>6	0	>6	0	>6
	6	CuO(1.0mgl^{-1})	0	>6	0	>6	0	>6
	Control	Uncoated	20	1	20	3	20	3
Cleaning	1	AgO(0.1mgl^{-1})	0	>6	0	>6	0	>6
	2	AgO(0.5mgl^{-1})	100	0	20	3	0	>6
	3	AgO(1.0mgl^{-1})	0	>6	0	>6	0	>6
	4	CuO(0.1mgl^{-1})	0	>6	0	>6	0	>6
	5	CuO(0.5mgl^{-1})	0	>6	0	>6	0	>6
	6	CuO(1.0mgl^{-1})	0	>6	0	>6	0	>6
	Control	Uncoated	20	1	20	3	20	3

The result of field testing Techniques using both coated and uncoated locally developed ceramic filters for viral testing (Table 4.7) with somatic phages from both North West University and Umgeni Water hosts shown LRV > 6 throughout post filtrate field testing, except the LRV on 0.5mg concentration of Ag_2O after cleaning of filters which has shown decrease in phages on post filtrate. The F^- specific phages from *Salmonella typhimurium* host have shown a variation in LRV. This variation depends on the concentration of metal oxide impregnated on the filters. The 0.1mg concentration of CuO and Ag_2O has shown the LRV 1 and 2 while 0.5mg of CuO has shown LRV 1 on day 1 of post filtrate. The full

period of testing (1month) have shown a log reduction value in excess of 6. This means metal oxide impregnation can be used as a long term treatment method of viruses as phages represent most of the enteric viruses. After a month of usage of the filters leachate was minimal as the filter efficiency still shows higher LRV removal of the post filtrate. Even after cleaning of the coated filters still showed reduction values in excess of the WHO recommendations for HWTS.

Figure 4.2 demonstrates the structure of micron sized pores of locally developed ceramic filter captured at the university of Zululand Physics Department using Carl Zeiss Σigma VP Electron microscope and the filter was fir**e**d at 1200ºC.

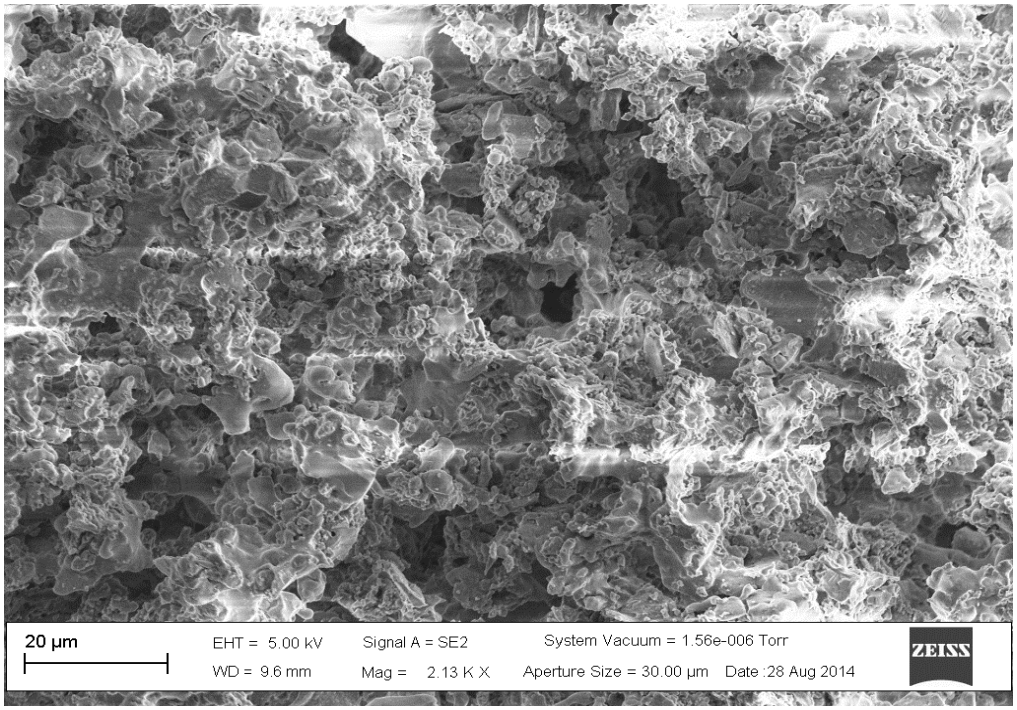

Figure 4.2: An electron microscope picture of micron sized pores of ceramic filter captured using Carl Zeiss Sigma VP system at University of Zululand

The pores of locally developed ceramic filters were structured after the traditional slip cast process (Figure 4.2). The filter showed a significant superiority in pore sizes when scanned by an electron microscope. The electron microscope demonstrated how the bi-modal interacts to form the pores, with larger pores resulted from this bimodal interaction

having the size of approximately 5 micron after firing and the smaller pores resulted from the necks (contact areas) between the larger pores with a pore size of 0.3 micron.

The ‘necking’ traps the bacteria and viruses and therefore becomes the determining factor for microbial protection. The distribution of these bi-modal pore sizes recommended why these filters acted as an effective micro-sieve in filtering out microbes and other suspended material from polluted drinking water. The clearly visible structured pore sizes on an electron microscope verified how the larger pores predominated with smaller pores still visible within the matrix after firing.

This locally developed ceramic filter still had a diameter ranged from 03- 4 µm, more superior as compared to other locally developed filters from other countries with pore sizes ranging from (17 µm - 35 µm) (Simonis and Basson, 2011; 2012) and (Van Halem, 2006). The filter was capable of providing the flow rate of 1-2L/h which was acceptable to provide one day drinking water in a household (Simonis and Basson, 2011; 2013).

Figure 4.3 displays the sputter coating image of ceramic filters when coated with silver where the diameter of coating varies between 40 and 50 nm. A homogeneous image of ceramic filter sputter coated with silver using the AFM, where the diameter of coating varies between 40 and 50 nm. The technique was a disadvantaged as sputter technique coats only the outer surface of the ceramic filter and the equipment is very sophisticated and expensive to maintain.

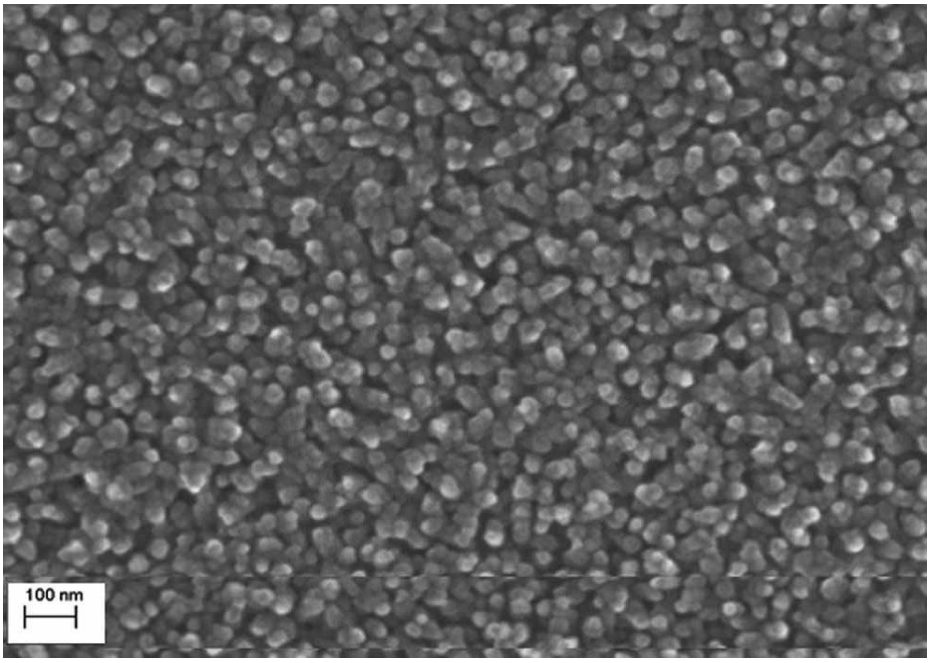

Figure 4.3: AFM image of the silver-coated ceramic surface where the diameters of the nano-particles vary between 40 and 50 nm

CHAPTER 5: DISCUSSION OF RESULTS

5.1 INTRODUCTION

This chapter is based on the discussion of results of the inhibition effect of various low concentrations (1 mg), metallic solutions on a number of bacterial morphologies and is compared to other studies. From the results obtained the most effective metallic solutions were selected for impregnation of the ceramic filters to further test against microbes. Oligodynamic testing of silver-coated ceramic using sputter technique in comparison with wet chemical impregnation. Further testing was done on selected metallic oxide (Ag_2O and CuO) of different concentrations using bacteriophages. Leachate testing was conducted after impregnated ceramic filters were subjected to a month period field testing.

5.2 DISCUSSIONS BASED ON RESULTS

Coating locally developed ceramic filters in this study with nanoparticles have improved removal of bacteria, protozoa and viruses by 99.999% as compared to the studies of (van Halem 2010; Bielefeldt *et al.*, 2010) were removal efficiency of bacteria and protozoa being 99.9% while the virsus removal was very unsatisfactory on uncoated PFP ceramic filter. This conclude the coating of locally used ceramic filter with nanoparticles will create some antimicrobial activity as alluded by Premanathan *et al.* (2011) and Xie *et al*. (2011) who reported that the small particle size properties of the nanoparticle gave it the bactericidal and bacteriostatic characteristics.

The locally coated developed ceramic filter in the study had shown LRV>6 target performance for bacteria, protozoa and viruses which above Health based HWT performance targets as confirmed by WHO in Table 2.1 (WHO, 2011). This locally coated ceramic filter has outperformed the other local produced filter as shown in (Table 2.2). Filters such as Katadyn filter, Nepalese filter and Potters of Peace (Dies 2000; Sagara 2000 and McAllister 2005) have shown less LRV or low removal efficiency on bacteria, protozoa and viruses as compared to 99.999% or LRV >6 from the results of this study. The results of the study filter is at par with the performance standard required by USEPA and NSF with the LRV of >6 (USEPA, 1987; NFS, 2003).

The local coated filter has shown superiority in their porosity and pore size as compared to other produced filters from other countries as confirmed by (van Halem 2006). Nanoparticles will render the filter negative charge on which will trap positive charge bacteriophages aiding in destruction of viruses. Bard and Holt (2005) and Azam et al. (2012) have confirmed antimicrobial and antiviral activity of the metal oxide as the results have shown their strength on inhibition and log reduction in the order of AgO > CuO > ZnO > FeO.

The Leachate testing of nanoparticle in the study was within the WHO recommended standard limits for drinking water that is 50-100µg l^{-1}as confirmed on Table 4.7. The field testing results on Table 4.7 has confirmed the strength of this locally developed filter which has a diameter range from 03- 4 µm, more superior as compared to other developed filters from other countries with pore sizes ranging from (17 µm - 35 µm) (Simonis and Basson, 2011; 2012) and (Van Halem, 2006). The filter was capable of providing the flow rate of 1-2L/h which was acceptable to provide one day drinking water in a household (Simonis and Basson, 2011; 2013) and (WHO 2011).

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1 INTRODUCTION

This chapter presents the conclusion and recommendations of the study. Different issues were raised in the study whether the hypothesis was supported or unsupported.

6.2 CONCLUSIONS

H_0: An improved existing HWTS will meet WHO performance standards and testing protocols. The testing hypothesis was supported by the outcome of the results. This is because the locally developed existing ceramic water filter met the WHO performance standards testing protocol for HWTS.

H_1: An improved existing HWTS which will not meet WHO performance standard and testing protocols. The null hypothesis was not supported because the locally developed existing ceramic water filter met the WHO performance standards and testing protocol for HWTS.

In this study the selection and application of metals and metallic oxides coating on the porous ceramic water filter has shown tremendous improvement in the effective filtration of microorganisms. The changing in diameter of the metals and metallic oxides and increased thickness of the nano-coating could further increase the effectiveness, bringing the locally developed HWTS in line with the WHO recommendations for viral protection (WHO, 2011)

The high surface area of the ceramic filter ($5m^2\ g^{-1}$) was believed to increase the metals and metallic oxides impregnation concentration from 1 to 2 mgl^{-1}. This could further improve the effectiveness, bringing this existing HWTS in line with the WHO (2011) recommendations for viral protection (Mpenyana-Monyats *et al*., 2013).

These coated ceramic filters offer a way out of adverse HWTS conditions as have shown reduction efficiency of 99.999% for most of the microorganisms, including the viruses which have been problematic in previous studies of filters such as PFP and many more (Bielefeldt *et al.,* 2010; Van Halem *et al.* 2007). They also have the advantage of avoiding harmful chemical by-products resulting from the use of chemicals to disinfect water (Lantagne et al. 2010).

The highest significance LRVs for bacteria, protozoa and viruses and it is in agreement with the WHO, USEPA and NSF performance standards (WHO, 2011; USEPA, 1987 and NSF, 2003) was observed. Therefore the locally developed ceramic filter meets and exceeds WHO highly protective performance targets for protozoa, bacteria and viruses (Table 2.1).

It was also proven that the antibacterial and antiviral strength of metals and metallic oxides coated on a ceramic filter can improve water treatment system and help in addressing the problem we were experiencing with disinfectant resistance by bacteria such as *Campylobacter jejuni*, viruses such as *Rotaviruses* and protozoa such as *Cryptosporidium* and *Giardia* (WHO, 2011).

The field testing condition on the filter has shown remarkable results and complimenting that the use of existing developed HWT systems where standard water treatment is not affordable, would significantly improve the quality of household water and protect the end-users (UNICEF and WHO, 2009). This locally developed ceramic filter will uplift the global burden of waterborne disease and malnutrition (Prüss-Ustun *et al.*, 2008) and provide better living conditions for many people including families living in remote rural areas and urban slums, where their lives are affected by poverty and diseases. The study concludes that the filter can be utilized by everybody even communities within urban areas to improve their access to clean, safe potable water.

6.3 RECOMMENDATIONS

The coated ceramic filters must be considered for local usage as it addresses the life of disadvantageous communities in the SADC region who lack safe drinking water. With good manufacturing practice (GMP) this impregnated ceramic filter can meet the challenging risk based on performance guidelines and protocols for microbiological evaluation.

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