ENUMERATION OF MICROORGANISMS IN DOMESTIC LOCAL WATER TANKER IN UESC-IGBO

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ENUMERATION OF MICROORGANISMS IN DOMESTIC LOCAL WATER TANKER IN IJEBU – IGBO

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CERTIFICATION

This is to certify that this research work was carried out by **AYOOLA PAMILERIN OPEYEMI (14/06/3100) and DUROJAYE MOJISOLA ELIZABETH (14/06/3164)** of Science Laboratory Technology, Abraham Adesanya Polytechnic, Ijebu – Igbo, Ogun State under my supervision.

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DEDICATION

This piece of research work is dedicated to the Almighty God, the giver of all understanding. The Alpha and Omega of all things, we ascribe all the glory to him, the King of Kings, Ancient of days, The I am that I am, the all sufficient God. Also to our parent **Mr. and Mrs. Durojaye, Mr. and Mrs. Ayoola** for their support and also to our siblings.

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We give all glory, honour and adoration to our father in heaven, the Lilly of the valley. Who has made this research work to be a successful one. The work was made possible through the will of God who has given us a great opportunity to pass through this stage of our carrier despite all sorts of odds and at the end He has made us who we are today.

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ABSTRACT

Many water utilities are required to monitor source water for the presence of total coliforms, fecal coliforms, or both. The Colilert system, an application of the defined substrate technology, simultaneously detects the presence of both total coliforms and Escherichia coli directly from a water sample. After incubation, the formula becomes yellow if total coliforms are present and fluorescent at 366 nm if E. coli is in the same sample. No confirmatory tests are required. The Colilert system was previously assessed with distribution water in a national evaluation in both most-probable-number and presence-absence formats and found to produce data equivalent to those obtained by using Standard Methods for the Examination of Water and Wastewater (APHA, 1985). The Colilert system was now compared with Standard Methods Multiple-Tube Fermentation (MTF) for the enumeration of total coliforms and E. coli from surface water. All MTF tubes were confirmed according to Standard Methods, and subcultures were made to identify isolates to the species level. Colilert tubes were sub-cultured to determine if color changes were specific to the target microbes. The Colilert system was found equally sensitive to MTF testing by regression, t-test, chi-square, and likelihood fraction analyses. Specificity of the Colilert system was shown by the isolation of a species of total coliform or E. coli after the appropriate colour change. The Colilert test can be used for source water samples when enumeration is required, and the benefits previously described for distribution water testing-sensitivity, specificity, less labor, lower cost, faster results, and no non-coliform heterotroph interference-are applicable to this type of water analysis. This study demonstrates that there is prevalence of microorganism can be found in water tanker and should be treated more regularly or often to prevent consumers or user from contacting these coliform disease which is harmful to human lung.

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CHAPTER ONE

1.0 INTRODUCTION

Water is one of the most important elements for all forms of life and is indispensable in the maintenance of life on Earth and essential for the composition and renewal of cells. Water represents 70% of our body, participates in the composition of our tissues, and transports the most diverse substances throughout our organism. Notwithstanding, human beings increasingly continue to pollute the reserves which still remain, provoking illnesses that can jeopardize the population (WHO, 2008).

Water is considered a vehicle for the propagation and dissemination of human associated bacteria. Safe tanker water is a fundamental human right and if contaminated with opportunistic pathogenic environmental bacteria, it may have health implications for consumers. Human health should therefore be protected by preventing microbial contamination of water that is intended for consumption. In rural communities, untreated surface water from rivers, dams, and streams is directly used for drinking and other domestic purposes. These unprotected water sources can be contaminated with microbes through rainfall runoff and agricultural inputs, mixing with sewage effluents and faeces from wild life, which render them unacceptable for human consumption (WHO, 2006). Faecal coliforms, Aeromonas and Pseudomonas, are used as indicators of faecal contamination in water and the presence of these pathogens may have Africa and the world at large, waste water reuse can form an important component of water demand management, but this waste water reuse may affect the quality of drinking water if proper treatment procedures are not implemented. Whereas waste water reuse has been extensively implemented in some European and African countries, yet in Nigeria, only a few

waste water reuse schemes have been documented and there is limited implementation of this alternative in communities. Severe health implications on consumers especially those that are immune compromised (WHO, 1976).

Water is unsafe for human consumption when it contains pathogenic or disease-causing microorganisms. Pathogenic microorganisms (and their associated disease(s)) may include bacteria, such as Salmonella typhi (typhoid fever), Vibrio cholerae (cholera), Shigella (dysentery, shigellosis), and viruses, such as poliovirus or Hepatitis A virus and protozoa such as Giardia lamblia (giardiasis) or Cryptosporidium parvum (cryptosporidiosis).A major challenge for water suppliers is how to control and limit the risks from pathogens and disinfection by-products. It is important to provide protection from pathogens while simultaneously minimizing health risks to the population from disinfection by-products (EPA, 2011).

In addition to bacterial-related health risks, faecal contamination carries the increased risk of viral contamination of the water source. Although viruses cannot multiply in water, some may remain static. This health risk is elevated in treated water (i.e. chlorinated tanks) where the faecal indicators may be absent. Many viruses have been identified as key etiological agents in outbreaks of drinking water derived gastrointestinal illness in the United States and Netherlands (Leclerc et al., 2002).

Alarming increases in the consumption of antibiotics through human therapy and agricultural processes have been reported and this extensive usage in both human and animal medicine has resulted in the development of antibiotic-resistant bacteria which affect the treatment of infections. Antibiotic resistance has therefore become a major public health issue and its presence in waste water, surface water, and drinking water is well documented. The hazard associated with the

pathogenicity of microbes is aggravated by its ability to resist destruction by antibiotics. Biological treatment processes in the waste water treatment plants may result in a selective increase of antibiotic-resistant bacteria and therefore increase the occurrence of multidrug-resistant organisms. Although microorganisms in drinking water are reduced by chlorination, they may survive the treatment process and enter the distribution system. Moreover, the presence of antibiotic resistance in microorganisms has been previously reported (WHO, 2008).

Considering the fact that the public health of a community may be related to the quality of treated waste water supplied and that public health can be protected by reducing the pathogenic microorganisms in drinking water, the present study was designed to isolate environmental bacteria from surface and drinking water in Mafikeng and identify the Pseudomonas and Aeromonas species using polymerase chain reaction (PCR). A further objective was to characterize the isolates using their antibiotic resistance profiles (WHO, 2006).

In developing countries including Nigeria, where the majority of people live in rural areas, rivers, streams, and more recently boreholes', serve as the main sources of water for drinking and domestic use. The underground water supplies are usually considered the safe provided they are properly located, constructed and operated according to the World Health Organization Guidelines for Drinking Water (WHO, 1971).

Microorganisms of concern in contaminated water include the following bacterial agents of diarrhea and gastroenteritis namely Salmonnella sp., Shigella sp., Escherichia coli and vibrio cholera (Birmingham at el., 1997). Protozozooal agents of diarrhea include Entamoeba histolytica, Giardia lamblia, Balantidium coli and Cryptococcus pervum. Enteroviruses causing various clinical ailments,

not necessarily diarrhea, but are transmitted by water include Poliovirus, Rotavirus, Hapatitis A virus (Benjelloun et al. 1997).

The need to assess the microbiological quality of water has become imperative because it has a direct effect on the health of individuals. The recognition of the connection between pollution and the need to protect human health, recreation and fisheries production led to the early development of water quality regulations and monitoring methods (Anyanwu and Okoli, 2012). Pollution of groundwater stems from different sources that include insanitary condition during borehole construction, splashing of runoff into wells left uncovered, flooding at borehole site, leachate from old buried waste pit or latrine into the hole through cracks in aquifer and annular of the hole, closeness of boreholes to septic tanks especially where space is a constraint and as such boreholes are drilled at times at old garbage landfill site formations through which the wastewater is retrieved from the holes (Onwughara et al.,2013).

Worldwide, over one billion people lack access to an adequate water supply; more than twice as many lack basic sanitation (WHO/UNICEF, 2006). Unsafe water, inadequate sanitation, and insufficient hygiene account for an estimated 9.1 percent of the global burden of disease and 6.3 percent of all deaths, according to the World Health Organization (Prüss – Üstün et al.,., 2008). This burden is disproportionately borne by children in developing countries, with water-related factors causing more than 20 percent of deaths of people under age 14. Nearly half of all people in developing countries have infections or diseases associated with inadequate water supply and sanitation (Bartram et al., 2005).

1.1 The Effect of Water in the Body

A healthy sedentary adult living in a temperate climate should drink at least 1.5 liters of water per day1. This level of water intake balances water loss and helps keeping the body properly hydrated. The water you consume through food and drinks follows a very precise route to arrive in your cells, of which it is a vital constituent (Bartram, 2008).

After passing through the stomach, water enters the small intestine, where it is largely absorbed in the first sections, the duodenum and jejunum. The rest passes into the colon. It crosses the intestinal mucous membrane into the bloodstream, then into the interstitial tissues that make up the framework of every organ, to arrive in the cells. Blood brings nutritional elements to cells (minerals, vitamins, protein components, lipids and carbohydrates). Waste products are then removes through urines (Bartram 2005). Water plays also an essential function in helping the regulation of temperature. The following are the functions of water in the body;

1. **Cell life:** Water is essential for cells to function properly: it enters into the composition of the cells.
2. **Chemical and metabolic reactions:** By enabling hydrolysis reactions, water participates in the biochemical breakdown of what we eat (proteins, lipids and carbohydrates). This is one of many reactions in which water is involved.
3. **Transport of nutrients and removal of waste:** Water as a main constituent of blood contributes to the transport of nutrients to the cells. In deed the nutrients are transported by the blood. Water, as a carrier, also helps removing waste products through urines.
4. **Body temperature regulation:** Water has a large heat capacity which helps limit changes in body temperature in a warm or a cold environment. Water

enables the body to release heat when ambient temperature is higher than body temperature (2): we begin to sweat, and the evaporation of water from the skin surface cools the body very efficiently.

Water is at the heart of life. This is why a human being can survive no longer than few days without water. Drinking water every day (approximately 1.5 liters*), and at regular intervals, 8 times a day (before, during and in-between meals), without waiting until you're thirsty, is important as part of a healthy lifestyle, at every stage of life (Bartram, 2012).

1.2 Aim and Objectives of the Study

The aim of these present study was to investigate water in tankers, enumerate any microorganism in this water. The following objectives are to be met

- To study the microorganism that can be found in tanker's water
- To enumerate the microorganism that can be found in tanker's water

CHAPTER TWO

2.0 LITERATURE REVIEW

Water is unsafe for human consumption when it contains pathogenic or disease-causing microorganisms. Pathogenic microorganisms and their associated disease(s) may include bacteria, such as *Salmonella typhi* (typhoid fever), *Vibrio cholerae* (cholera), *Shigella (dysentery, shigellosis),* and viruses, such as poliovirus or Hepatitis A virus and protozoa such as Giardia *lamblia (giardiasis)* or *Cryptosporidium parvum (cryptosporidiosis)*.A major challenge for water suppliers is how to control and limit the risks from pathogens and disinfection by-products. It is important to provide protection from pathogens while simultaneously minimizing health risks to the population from disinfection by-products (EPA, 2011).

In addition to bacterial-related health risks, faecal contamination carries the increased risk of viral contamination of the water source. Although viruses cannot multiply in water, some may remain static. This health risk is elevated in treated water (i.e. chlorinated tanks) where the faecal indicators may be absent. Many viruses have been identified as key etiological agents in outbreaks of drinking water derived gastrointestinal illness in the United States and Netherlands (*Leclerc et al., 2002*).

Some micro-fungi are known to be opportunistic human pathogens. Airborne spores are an important potential source of micro fungi found in water storage reservoirs. It has also demonstrated conclusively that filamentous micro fungi grow and sporulate on the inner surfaces of water pipe and in soft sediments within the water distribution system (*Sammon et al.,., 2011*).

Worldwide, over one billion people lack access to an adequate water supply; more than twice as many lack basic sanitation (WHO/UNICEF, 2006). Unsafe water, inadequate sanitation, and insufficient hygiene account for an estimated 9.1 percent of the global burden of disease and 6.3 percent of all deaths, according to the World Health Organization (Prüss-Üstün et al.,., 2008). This burden is disproportionately borne by children in developing countries, with water-related factors causing more than 20 percent of deaths of people under age 14. Nearly half of all people in developing countries have infections or diseases associated with inadequate water supply and sanitation (Bartram et al., 2005).

The presence of E.coli in water is a strong indication of recent sewage or faecal contamination. Sewage may contain many types of disease causing organisms. E. coli comes from human and animal waste. During rainfalls, snow melts, or other types of precipitation, E.coli may be washed into creeks, rivers, streams, lakes, or groundwater. When these waters are used as sources of drinking water and the water is not treated or inadequately treated, E.coli may end up in the drinking water (Health Canada, 2008).

Faecal coliforms and E.coli are bacteria whose presence indicates that the water may be contaminated with human or animal wastes. Microbes in these waters can cause short-term effects, such as diarrhea, cramps, nausea, headaches, or other symptoms. They may pose a special health risk for infants, young children, some of the elderly, and people with severely Compromised Immune Systems (CDC, 2009).

Some bacteria are ubiquitous in soil, water and on surfaces in contact with soil or water such as Pseudomonas aeruginosa which is an opportunistic pathogen. P.aeruginosa is an opportunistic pathogen. It produces tissue damaging toxins and causes urinary tract infections, respiratory system infections, central nervous

system, endocarditis (P.aeruginosa infects heart valves establishes itself on the endocardium), dermatitis, soft tissue infections, bacteraemia, bone and joint infections, gastrointestinal infections and a variety of systemic infections, particularly in patients with severe burns and in cancer and AIDS patients who are immunosuppressed. Spread occurs from patient to patient on the hands of hospital personnel, by direct patient contact with contaminated reservoirs, and by the ingestion of contaminated foods and water (EHA, 2012).

The presence of faecal coliform in aquatic environments may indicate that the water has been contaminated with the faecal material of humans or animals. Faecal coliform bacteria can enter water bodies through direct discharge of waste from mammals and birds, from storm and agricultural runoff, and from human waste (Doyle and Erickson, 2006).

Pet wastes (cats, dogs) can contribute to faecal contamination of surface waters. Runoff from roads, parking lots, and yards can carry animal wastes to streams through storm sewers. Birds can be a significant source of faecal coliform bacteria. Birds (seagulls, geese, swans) can all elevate bacterial counts, especially in freshwater systems (wetland, rivers, lakes and ponds). Some waterborne pathogenic diseases that may coincide with faecal coliform contamination include ear infections, viral and bacterial gastroenteritis, dysentery, typhoid fever and hepatitis A. The presence of faecal coliform tends to affect humans more than it does aquatic creatures, though not exclusively (Walkerton, 2011).

Fungi are ubiquitous organisms that are widely distributed in nature. Several fungal genera have been shown to be allergenic, such as Aspergillus, Alternaria and Cladosporium (Black et al., 2000; Bowyer et al., 2006; Hedayati et al., 2007; Simon-

Nobbe et al., 2006). Several studies have suggested an important role for waterborne.

The United Nations (UN) set a goal in their Millennium Declaration to reduce the amount of people without safe drinking water by half in the year 2015 (UN, 2000). Safe drinking water for human consumption should be free from pathogens such as bacteria, viruses and protozoan parasites, meet the standard guidelines for taste, odour, appearance and chemical concentrations, and must be available in adequate quantities for domestic purposes (Kirkwood, 1998). However, inadequate sanitation and persistent faecal contamination of water sources is responsible for a large percentage of people in both developed and developing countries not having access to microbiologically safe drinking water and suffering from diarrhoeal diseases (WHO, 2002a; WHO, 2002b). Diarrhoeal diseases are responsible for approximately 2.5 million deaths annually in developing countries, affecting children younger than five years, especially those in areas devoid of access to potable water supply and sanitation.

Political upheaval, high numbers of refugees in some developing countries, and the global appearances of squatter camps and shanty rural towns, which lack proper sanitation and water connections, have contributed to conditions under which disease causing microorganisms can replicate and thrive (Leclerc et al., 2002). The people most susceptible to waterborne diseases include young children, the elderly, people suffering from malnutrition, pregnant woman, Immune compromised individuals, people suffering from chemical dependencies and persons predisposed to other illnesses like diabetes (Leclerc et al., 2002). Furthermore, an increasing number of people are becoming susceptible to infections with specific pathogens due to the indiscriminate use of antimicrobial drugs, which have led to the selection

of antibiotic resistant bacteria and drug resistant protozoa (WHO, 2002a; WHO, 2002b).

In developing countries, many people are living in rural communities and have to collect their drinking water some distances away from the household and transport it back in various types of containers (Sobsey, 2002). Microbiological contamination of the water may occur between the collection point and the point-of-use in the household due to unhygienic practices causing the water to become a health risk (Sobsey, 2002).

To improve and protect the microbiological quality and to reduce the potential health risk of water to these households, intervention strategies is needed that is easy to use, effective, affordable, functional and sustainable (Sobsey, 2002). Many different water collection and storage systems have been developed and evaluated in the laboratory and under field conditions (Sobsey, 2002). In addition, a variety of physical and chemical treatment methods to improve the microbiological quality of water are available (Sobsey, 2002). The aim of this study was to improve the microbiological quality of drinking water in rural households by the implementation of intervention strategies which include the use of traditional storage containers as well as the Center for disease control and prevention (CDC) safe storage container, with or without the addition of a sodium hypochlorite solution at the point-of-use.

2.1 Waterborne Diseases

Many infectious diseases are associated with faecally contaminated water and are a major cause of morbidity and mortality worldwide. Waterborne diseases are caused by enteric pathogens such as bacteria, viruses and parasites (Table 2.1) that are transmitted by the faecal oral route. Waterborne spread of infection by these

pathogenic microorganisms depends on several factors such as: the survival of these microorganisms in the water environment, the infectious dose of the microorganisms required to cause a disease in susceptible individuals, the microbiological and physico-chemical quality of the water, the presence or absence of water treatment and the season of the year (Leclerc et al., 2002).

Table 2.1 Waterborne pathogens and their associated diseases (Leclerc et al., 2002)

Pathogen		Diseases
Bacteria	*Campylobacter* spp.	Diarrhoea and acute gastroenteritis
	Enteropathogenic *Escherichia coli*	Diarrhoea
	Escherichia coli O157:H7	Bloody diarrhoea and haemolytic uremic syndrome
	Salmonella spp.	Typhoid fever, diarrhoea
	Shigella spp.	Dysentery, diarrhoea
	Vibrio cholera	Cholera, diarrhoea
	Yersinia spp.	Diarrhoea, gastrointestinal infections
Viruses	Adenoviruses	Diarrhoea, respiratory disease, conjunctivitis
	Astroviruses	Diarrhoea

	Coxsackie viruses (Enterovirus)	Respiratory, meningitis, diabetes, diarrhoea, vomiting, skin rashes
	Echoviruses (Enterovirus)	Meningitis, diarrhoea, myocarditis
	Enteroviruses 68-71	Meningitis, diarrhoea, respiratory diseases, rash, acute enteroviral haemorrhagic conjunctivitis
	Hepatitis viruses (A, E)	Hepatitis (jaundice), gastroenteritis
	Caliciviruses	Diarrhoea, vomiting
	Poliovirus (Enterovirus)	Poliomyelitis
	Rotaviruses	Diarrhoea, vomiting
	Small Round Structured viruses	Diarrhoea, vomiting
Protozoan parasites	*Cryptosporidium parvum*	Cryptosporidiosis, diarrhoea
	Entamoeba hystolytica	Amoebic dysentery
	Giardia	Giardiasis, diarrhoea
Helminths	*Dracunalis medinensis*	Guinea worm (Dracunculiasis)
Emerging opportunistic pathogens	*Actinobacter* spp.	Septicemia, meningitis, endocarditis
	Aeromonas spp.	Diarrhoea, gastroenteritis
	Cyclospora spp.	Diarrhoea, abdominal cramping, fever

	Isospora spp.	Diarrhoea
	Legionella spp.	Legionnaires disease, Pontiac fever
	Microsporidia spp.	Gastrointestinal infections, diarrhoea
	Nontuberculosis *Mycobacteria*	Skin infections, cervical lymphadenitis, nontuberculosis mycobacterium disease
	Pseudomonas aeruginosa	Septicaemia, wound and eye infections

The survival of microorganisms such as bacteria in water environments depends on the presence of nutrients and the water temperature. The infectious dose of some bacteria ranges 107 to 108 cells, with some enteric bacteria able to cause infections at doses as low as 101 cells. Viruses cannot replicate outside living cells, but can survive for extended periods in the water. The infectious dose of viruses has been established to be as low as 1 to 10 infectious particles. Enteric protozoa such as Giardia and Cryptosporidium cannot replicate in water and are highly resistant to most disinfectants and antiseptics used for water treatment. The infectious dose for parasites depends on host susceptibility and strain virulence. The infectious dose for Giardia might be as low as 10 oocysts and for Cryptosporidium the presence of 30 oocysts might cause an infection (Leclerc et al., 2002).

2.2 The Microbiological Quality of Water

Water supplies in developing countries are devoid of treatment and the communities have to make use of the most convenient supply (Sobsey, 2002). Many

of these water supplies are unprotected and susceptible to external contamination from surface runoff, windblown debris, human and animal faecal pollution and unsanitary collection methods (WHO, 2000).

Detection of each pathogenic microorganism in water is technically difficult, time consuming and expensive and therefore not used for routine water testing procedures (Grabow, 1996). Instead, indicator organisms are routinely used to assess the microbiological quality of water and provide an easy, rapid and reliable indication of the microbiological quality of water supplies (Grabow, 1996).

In order for a microorganism to be used as an indicator organism of pollution, the following requirements should be fulfilled (WHO, 1993):

- The concentration of the indicator microorganism should have a quantitative relationship to risk of disease associated with exposure (ingestion/recreational contact) to the water;
- The indicator organism should be present when pathogens are present;
- The persistence and growth characteristics of the indicator organism should be similar to that of pathogens;
- Indicator organisms should not reproduce in the environment;
- The indicator organism should be present in higher numbers than pathogens in contaminated water;
- The indicator organism should be at least as resistant to adverse environmental conditions, disinfection and other water treatment processes as pathogens;
- The indicator organism should be non-pathogenic and easy to quantify;
- The tests for the indicator organism should be easy, rapid, inexpensive, precise, have adequate sensitivity, quantifiable and applicable to all types of water;

- The indicator organism should be specific to a faecal source or identifiable as to the source of origin of faecal pollution.

Although many microorganisms have desirable features to be considered as possible indicators of faecal pollution, there is no single microorganism that meets all of these requirements (Sobsey, 2002). Several studies have showed the limitations of some of the current indicator organisms, which include the following:

- Indicator organisms may be detected in water samples in the absence of pathogens.
- Some pathogens may be detected in the absence of indicator organisms. Vibrio cholera (V. cholera) persists in water exposed to solar disinfection well after E. coli was inactivated. Potable water supplies in Egypt contained bacteriophages, with zero total and faecal coliform counts, which indicated the possible risk of the presence of human enteric viruses.
- E. coli bacteria have a short die-off curve with temperature playing an important role.
- Injured coliform bacteria can be undetected due to several chemical and physical factors and were unable to grow on commonly used media.
- Improper filtration, temperature, inadequate disinfection and treatment procedures, biofilms and high Assimilable Organic Carbon (AOC) levels, could all be responsible for the regrowth of coliform bacteria in water samples.
- The prevalence of viruses in water may differ from that of indicator organisms.
- Low numbers of viruses are present in water samples compared to indicator organisms, viruses are only excreted for short periods of time while coliform bacteria is excreted continuously, and the structure, size, composition and

morphological differences between viruses and bacteria also had an influence on behavioural and survival patterns of these microorganisms.

In spite of the shortcomings of indicator microorganisms, it is better to use a combination of indicator microorganisms to give a more accurate picture of the microbiological quality of water Natural Resources Conservation Services (NRC, 2004). In general, every country has its own set of guidelines for drinking water. However, most of these guidelines are similar for different countries and the same indicator microorganisms to indicate the presence of pathogenic microorganisms are used. The water quality guidelines for South Africa are shown in Table 2.2.

Table 2.2 Microbiological Requirements for Domestic water in South Africa (Simon-Nobbe, et al., 2006)

Indicator organism	Units	Allowable compliance
Heterotrophic plate count	Colony forming units.1 ml-1	100
Total coliform bacteria	Colony forming units.100 ml-1	10
Faecal coliform bacteria	Colony forming units.100 ml-1	1
Escherichia coli	Colony forming units.100 ml-1	0
Somatic bacteriophages	Colony forming units.10 ml-1	1

Enteric viruses	Plaque forming units.100 l-1	1
Protozoan parasites (*Giardia/Cryptosporidium)*	Count.100 l-1	0

The most commonly used indicator microorganisms include heterotrophic plate counts, total coliform bacteria, faecal coliform bacteria, E coli, faecal enterococci, C. perfringens as well as somatic and male specific F-RNA bacteriophages (WHO, 2000). Each of these indicator microorganisms has advantages and disadvantages which will be discussed in more detail in the following sections.

2.2.1 Heterotrophic plate counts

Heterotrophic microorganisms or heterotrophs are naturally present in the environment and can be found in soil, sediment, food, water and in human and animal faeces (Collin et al., 1984). Broadly defined, heterotrophs include bacteria, yeasts and molds that require organic carbon for growth (WHO, 2002a). Although generally considered harmless, some heterotrophic microorganisms are opportunistic pathogens, which have virulence factors that could affect the health of consumers with suppressed immune systems (Bartram et al., 2005). Heterotrophic microorganisms can also survive in biofilms inside water distribution systems, water reservoirs and inside household storage containers. Therefore, heterotrophic plate counts can also be used to measure the re-growth of organisms that may or may not be a health risk (WHO, 2002b).

2.2.2 Total coliform bacteria

Total coliform bacteria are defined as aerobic or facultative anaerobic, Gram negative, non-spore forming, rod shaped bacteria, which ferments lactose and produce gas at 35°C (APHA, 1998). Total coliforms include bacteria of known faecal origin such as E. coli as well as bacteria that may not be of faecal origin such as *Klebsiella spp, Citrobacter spp, Serratia spp and Enterobacter spp* which are found in nutrient rich water, soil decaying vegetation and drinking water with relatively high levels of nutrients (WHO, 1971). The recommended test for the enumeration of total coliforms is membrane filtration using mEndo agar and incubation at 35°C to 37°C for 24 h to produce colonies with golden green metallic shine (APHA, 1995).

2.2.3 Faecal coliform bacteria

Faecal coliform bacteria are Gram negative bacteria, also known as thermo tolerant coliforms or presumptive E. coli (APHA, 1995). The faecal coliform group includes other organisms, such as Klebsiella spp, Enterobacter spp and Citrobacter spp, which are not exclusively of faecal origin (APHA, 1995). Escherichia coli are specifically of faecal origin from birds, humans and other warm blooded animals (WHO, 1971). Faecal coliform bacteria are therefore considered to be a more specific indicator of the presence of faeces.

2.3 Human and Animal Faecal Pollution in Water

Water polluted with human and animal faeces may contain potentially pathogenic microorganisms that can cause diseases in consumers (Sobsey et al., 2002). The most commonly used faecal indicator microorganisms which include the total coliform bacteria, thermo tolerant coliform bacteria, E. coli and faecal

enterococci bacteria, are found in both human and animal faeces, but do not differentiate between the origins of faecal pollution. Human viral pathogens such as Calici virus, Hepatitis E virus, Reo viruses, Rotaviruses, somatic bacteriophages and male specific FRNA bacteriophages also infect other animals which can serve as reservoirs (NRC, 2004).

Consequently, these animals can be important potential sources of contamination of water sources because the release of microorganisms into aquatic environments by animal hosts could lead to human exposure (NRC, 2004). Poor communities in developing countries share their water sources with cattle and other domestic animals, therefore, the risk of waterborne transmission of zoonotic pathogens to humans, increases (Leclerc et al., 2002).

However, water contaminated with human faeces is regarded as a greater risk to human health since it is more likely that it would contain human specific enteric pathogens (Sinton et al., 1999). Although various microbial and chemical indicators have been described to identify the origin of faecal pollution in water supplies, different levels of success have been.

2.3.1 The use of microorganisms to determine the origin of faecal pollution

Several microorganisms have been suggested and tested to distinguish between human and animal faecal pollution in domestic drinking water supplies. Various factors can have an effect on the specificity of microorganisms that can be used as indicators to determine the origin of faecal pollution, such as:

(1) Specific bacteria, viruses and protozoan parasites can have multiple hosts (not species specific) (Sinton et al., 1999);

(2) different microorganisms can have similar biochemical reactions in the environment, especially within the same species or genus (Sinton et al., 1999) and (3) interspecies gene transfer may occur which include small pieces of DNA (e.g. plasmids and integrons) and transposons that are carried from one bacteria to another

2.3.2 The use of chemicals to determine the origin of faecal pollution

Several chemical indicators have been used to identify the source of faecal pollution in various water supplies (Sinton et al., 1999). However, expensive equipment and high concentrations of the chemical in the water sample is needed for accurate identification of the origin of faecal pollution (Sinton et al., 1999).

2.4 Source Water Supplies

The World Health Organization (WHO) classifies source water supplies as either improved or unimproved (WHO, 2000). Improved water sources include public standpipes, household connections, boreholes, protected dug wells, protected springs, bore holes and springs connected via a pipe system to a tap, as well as rainwater collection (WHO, 2000). Unimproved water sources include unprotected wells, unprotected springs, vendor-provided water, rivers as well as tanker truck provision of water (WHO, 2000).

Several studies carried out in developing countries have determined the microbiological quality of these improved and unimproved water sources and depending on the water source, different results were obtained (Sobsey et al., 2002). Studies conducted in Iran and in northern Sudan have both showed that water at communal taps were microbiologically of a better quality than untreated irrigation canal water. Contrary to these findings, a study in Burma has showed that tube well

and shallow well water supplies were microbiologically of a better quality than municipal tap water and pond water source supplies.

In addition three recent studies conducted in the Vhembe region of the Limpopo Province in South Africa indicated that rivers and fountains used by rural communities for domestic water were all contaminated by enteric pathogens including E. coli, Plesiomonas shigelloides, V. cholera, Enterobacter cloacae, Shigella spp, Salmonella spp, Aeromonas hydrophila, Aeromonas caviae and Campylobacter spp (Obi et al., 2004). Escherichia coli isolates obtained from the different rivers during this study were typed using molecular techniques to determine the presence of virulent genes (Obi et al., 2004). Enterotoxigenic E. coli isolates (11.8%) contained heat stable and heat labile genes; Shigatoxin producing E. coli (4.4%) isolates contained stx1 and stx2 genes; Necrotoxigenic E. coli (35.6%) contained cnf1 and cnf2 genes and Enteropathogenic E. coli (34.1%) isolates contained BfpA and EaeA genes (Obi et al., 2004). Necrotoxigenic E. coli may play a role in possible zoonotic transmission since it has been shown that human and animal strains share similar serogroups and carry the same genes coding for fimbrial and afimbrial adhesion. All of these studies indicated that the water sources used by communities in developing countries are microbiologically contaminated and pose a health risk to the consumers (Obi et al., 2004).

Very few studies have investigated the microbiological quality of water during collection and transportation. In a study in Rangoon, Burma the water at the source and during collection were analyzed and indicated that the faecal coliform counts in the collection samples were higher than the counts in the source water samples (Hänninen et al., 1998). The increase in faecal contamination of the water in the collection containers after collection from the source could have been due to

unhygienic handling of the water and posed a potential health risk of diseases to the consumers (Sobsey, 2002). In a study in Sri Lanka, it was found that only 5% of tube well water samples were contaminated if the pump was sterilized prior to collection of the sample compared to 50% if the pump was not sterilized. This implied that the taps were contaminated by hands or animals during collection (Sobsey, 2002).

2.5 Points-of-Use Water Supplies in the Household

Source water contamination is likely to have a wide effect on the community because it can introduce new pathogens in the home environment (Sobsey, 2002). However, several studies have reported that the microbiological quality of the water deteriorate after collection, during transport and during storage at the point-of-use due to secondary contamination factors. Due to the distances and unavailability of piped water supplies on the dwelling or inside the households in many developing regions of the world, people are forced to store their drinking water (Sobsey, 2002).

Transmission of microorganisms inside the household can occur through several routes. The most important transmission routes include water, food, person-to-person contact, unhygienic behaviour (e.g. intra-household transmission of faeces), the storage conditions of the water storage containers at the point-of-use and the abstraction conditions of water from the storage container. In addition, a number of studies suggested that inadequate storage conditions increased the risk of contamination, which can lead to infectious diseases (Sobsey, 2002).

The literature study has showed that depending on water collection and storage practices, deterioration of the microbiological quality of the water may occur before the water is actually consumed, mostly due to secondary contamination at the point-of-use. Reviews by Sobsey (2002).

More point-of-use intervention field studies must be conducted. The bacteriological evidence in their studies showed that improved storage containers may be effective at reducing microorganisms in stored water if the sources were of good microbiological quality or uncontaminated. However, many of the point-of-use interventions mentioned in the literature review, especially the physical and chemical treatment interventions, are impractical because of costs and sustainability and therefore not suitable for impoverished rural households in developing countries such as South Africa (Sobsey, 2002).

In addition, the literature study has also showed that improving the microbiological quality of water before consumption would reduce diarrhoeal disease together with sanitation and hygiene education. However, many of the studies have used indicator microorganisms to assess the effectiveness of interventions. The literature review has indicated that most of the currently used indicator microorganisms used to evaluate the microbiological quality of water have shortcomings and will only give an indication of the potential risk associated with the transmission of waterborne diseases (Sobsey et al., 1995).

Several potentially pathogenic microorganisms in water polluted by human and animal faeces could cause diarrhoeal diseases in consumers (Leclerc et al., 2002). Little information on the origin of faecal contamination in the traditional and CDC safe storage containers are presently available. Literature has showed that microbiological and chemical indicators can be used to distinguish between human and animal faecal pollution in water (Sinton et al., 1999). However, no single microorganism or chemical determinant could reliably distinguish human from animal faecal contamination and therefore, the use of a combination of chemical

and microbial determinants together may provide the best solution for identifying the origin of faecal pollution at the point-of-use (Sinton et al., 1999).

Consequently, the literature study has indicated that the best interventions available that will be applicable to conditions in rural communities in South Africa included the use of the CDC safe storage container together with a chemical treatment such as sodium hypochlorite solution. The aim of this study was therefore to improve the microbiological quality of drinking water in rural households at the point-of-use by the implementation of intervention strategies which included the use of traditional storage containers as well as the CDC water storage container, with or without the addition of a sodium hypochlorite solution. The results obtained from this study would be used to provide information to the DOH and DWAF, which can be used in future water and health policy formulations to prevent waterborne outbreaks in these rural communities.

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Study Area

The study area was carried out in Ijebu-Igbo a relatively small town in Ijebu-North Local Government area of Ogun state, Nigeria. Ijebu-igbo is located on the tropical rain forest belt with hot and humid climate condition. Temperature increases to a high range during the dry season when compared with temperature in the rainy season. Dry season consist of a short period of harmattan between December and February.

3.2 Sample Collection

A total of 10 water samples were collected from10 water tankers in Ijebu-Igbo at different locations which are Oke-Agbo, Oke-sopin, Ojowo, Atikori, Japara, Oke-Alafia, Oke-Ife, Oke-Alafia (muulu), Abusi, Egbe. The water was collected in the morning and was taken to the Laboratory immediately for microbial analysis. Micro-organisms in the water were enumerated.

3.3 Collection Method

Water sample was carried out from each tank. Collection was done for about 45-60 min. different part of the tanks were visited, water collection was done using a white 1litter keg. The water were then transported to the Laboratory of Lagos State Environmental Protection Agency (LASEPA).

3.4 Bacteria Identification

The defined substrate technology, applied to water analysis as many water utilities are required to monitor source water for the presence of total coliforms, fecal coliforms, or both. The Colilert system, an application of the defined substrate

technology, simultaneously detects the presence of total coliforms directly from a water sample. After incubation, the formula becomes yellow if total coliforms are present and fluorescent at 366 nm. No confirmatory tests are required. The Colilert system was previously assessed with distribution water in a national evaluation in both most-probable-number and presence-absence formats and found to produce data equivalent to those obtained by using Standard Methods for the Examination of Water and Wastewater (Standard Methods). The Colilert system was now compared with Standard Methods multiple-tube fermentation (MTF) for the enumeration of total coliforms from surface water. All MTF tubes were confirmed according to Standard Methods, and subcultures were made to identify isolates to the species level (APHA, 1985).

Colilert tubes were subcultured to determine if color changes were specific to the target microbes. The Colilert system was found equally sensitive to MTF testing by regression, t test, chi-square, and likelihood fraction analyses. Specificity of the Colilert system was shown by the isolation of a species of total coliform after the appropriate color change. The Colilert test can be used for source water samples when enumeration is required, and the benefits previously described for distribution water testing-sensitivity, specificity, less labor, lower cost, faster results, and no non-coliform heterotroph interference-are applicable to this type of water analysis.

3.5 Procedure for Microbial Tests

Water samples were collected, transported, and stored in strict accordance with the guidelines described by Standard Methods (APHA, 1985).

3.5.1 Sterile Polymethylpentene

Glass flasks were used to collect the samples, Source water was diluted with sterile, dechlorinated tap water to result in a final total-coliform count in the range of 1 to 20 total coliforms per 100 ml so that meaningful statistical comparisons could be made. All comparative analyses were performed with split samples from the same water or origin Defined substrate technology. The Colilert system was used with 100-ml samples (Access Analytical Systems, Branford, Conn.). It was formatted in a 10-tube MPN arrangement (Caldwell, 1988).

The water samples were added to the Colilert tubes. Each tube contained enough Colilert powder to receive 10 ml of water. The contents of the tubes were shaken to dissolve the powdered formula. A colorless solution resulted. The vessels were then placed in a 35°C (+2°C) incubator for 24 hours (Caldwell, 1988).

A yellow color in the vessel after incubation denoted the presence of total coliforms. Any positive total-coliform test tube was exposed to a hand-held fluorescent (366 nm) light.

Fluorescence in the test tube indicated the presence of light. Therefore, a separate result was obtained for total coliforms. The number of coliforms per 100 ml was estimated from a 10-tube MPN table. No confirmatory or completed tests needed to be performed. At least one positive Colilert test tube from each positive water sample was sub-cultured, and the colonies were identified by species by the API 20E system (Analytab Products, Plainview, N.Y.), with supplementary tests as necessary.

3.5.2 Multiple-Tube Fermentation Test

The multiple-tube fermentation test was performed as a 10-tube MPN test, with each tube containing 10 ml of double-strength lactose tryptose broth (Difco Laboratories, Detroit, Mich).

Positive tubes were confirmed in brilliant green bile lactose broth (Difco) the number of coliforms per 100 ml was estimated from a 10-tube MPN table. Only confirmed lactose tryptose broth tubes were included in the data base for comparison with the Colilert system. Heterotrophic-plate-count bacteria. Noncoliform heterotrophic- plate-count bacteria were determined for each water sample with R2A agar (Difco) incubated at 35°C for 48h (APHA, 1985).

CHAPTER FOUR

4.0 RESULT AND DISCUSSION

TABLE 4.1

S/N	PHYSICAL	RESULT SAMPLES					W.H.O STANDARD	REMARKS
A	MICROBIOLOGY	A	B	C	D	E		
	Total plate count	60	40	60	100	80	100cfu/ml	
	Total coliform count	19	>201	165	165	59	NIL	
	E.coli	0	0	0	0	0	NIL	

KEY: NA= NOT ANALYSED NS= NOT SPECIFIED ND= NOT DETECTED

COMMENSTS: Quality of water sample is unsatisfactory due to presence of coliform. Please improve on treatment process.

TABLE 4.2

S/N	PHYSICAL	RESULT SAMPLES					W.H.O STANDARD	REMARKS
A	MICROBIOLOGY	F	G	H	I	J		
	Total plate count	60	120	60	TNTC	TNTC	100cfu/ml	
	Total coliform count	22	201	>201	>201	109	NIL	
	E.coli	0	0	0	0	0	NIL	

KEY: NA= NOT ANALYSED NS= NOT SPECIFIED ND= NOT DETECTED

COMMENSTS: Quality of water sample is unsatisfactory due to presence of coliform and high bacteria load. Please improve on treatment process.

4.3 DISCUSSION

Water samples were collected from 10 regions and tested for microbial analysis. The important water quality parameter as isolation. The water is not standard for drinking purpose which is exceeded to a great extent are shown by the result above, which are not acceptable in term of the tanker water sample analyzed.

The result of this study showed the presence of coliform organism in water tanker. The prevalence of this organisms is observed to be outstanding.

From the result of this study, Coliform organism were found on inhabiting the water in the water tankers (carrier), this could be attributed abundance and fluffiness to human intestine. Coliform bacteria inhabit the intestine and colon of

mammals, which could as well make the region more conducive for the parasite to thrive.

Although the water used in this study were in storage for short period of time, the high prevalence of organism parasite on them is an indication that this type of organism are not only related to neatness or handling. The organism found in these water are of the bacteria type, this may account for mortality, reduced growth and efficiency of animals or mammals that feed on them.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

The research work focused on the water tankers in Ijebu-igbo, Oru, Ago in Ijebu-North Local government Area. This research work helps to ascertain "enumerate the microorganism present in domestic water tanker".

5.1 Conclusion

In conclusion this study demonstrates that there is prevalence of microorganism can be found in water tanker and should be treated more regularly or often to prevent consumers or user from contacting these coliform disease which is harmful to human lung. The organism population is likely to be influenced by the nutrient substrates available and by the ability of individual bacteria to survive in a dilute environment. The water used in health center areas and in pharmaceutical industries should be periodically analyzed as a preventive measure against the spreading of microorganisms, allowing measures of improvement to be taken rapidly, as required. The analysis of treated water for heterotrophic bacteria including Pseudomonas species is valuable in the prevention of the formation of biofilms and in the reduction of the amount of pyrogen.

5.2 Recommendation

From the above observed results of our study it is recommended that appropriate treatment control measures have to be practiced to mitigate the effect of Coli form in water transported or stored in the tanker. Therefore the washing of (storage tanks) reservoirs, deionization columns, reverse osmosis membranes, as well as the sanitation of distribution circuits should be carried out by the

determination of a schedule established for quality control (bacteriological and chemical) of water systems in the risky areas.

REFERENCE

American Public Health Association APHA (1985) Standard methods for the examination of water and wastewater, 16th ed. American Public Health Association and Washington D.C.

American Public Health Association APHA (1995) Standard methods for examination of water and waste water. 17th Edition, American Public Health Association and Washington D.C.

American Public Health Association APHA (1998) Standard methods for examination of water and waste water, 24th Edition. American Public Health Association and Washington D.C.

Anyanwu, C. U. and Okoli E. N. (2012). Evaluation of the bacteriological and physicochemical quality of water supplies in Nsukka, Southeast, Nigeria. Afr. J. Biotechnol., 11(48), 10868-10873.

Bartram J. (2008) Flowing away: water and health opportunities. Bull World Health Organ 86(1):2.

Bartram J and Cairncross S (2005) Hygiene, sanitation and water: forgotten foundations of Health. Plos Med 7(11): e1000367. doi:10.1371/journal.pmed.1000367.

Bartram J and Jones E.E. (2012) Getting wet, clean, and healthy: why households matter, The Lancet, Volume 380, Issue 9837: 85-86.

Benjelloun. S., B. Bahbouhi, N. Bouchart, L. Chericaoni, N. Had and J Mahjour. Valley Lake Region. Lancet 349: 981-988.

Bensumane (1997) Seroepidemiological study of an acute Hepatitis E outbreak in Morocco. Research Virology 148: 279-283.

Birmingham, M.E., L. A. Lea, N. Ndayiminje, S. Nkurikiye, B. S Hersh, J.G. Wells and M .S. Ijeming (1997) Epidemic cholera in Burundi, Patterns of transmission in the Gadat Rift Valley Lake Region. Lancet 349: 981-983.

Black P. N., Udy A. A. and Brodie S. M. (2000): Sensitivity to fungal allergens is a risk factor for life-threatening asthma. Allergy, 55:501-504.

Bowyer P, Fraczek M. and Denning D. W. (2006): Comparative genomics of fungal allergens and epitopes shows widespread distribution of closely related allergen and epitope orthologues. BMC Genomic, 7:251.

Caldwell, B. A., and R. Y. Morita (1988) Sampling regimes and bacteriological tests for coliform detection in groundwater. Project summary, EPA/600/S2-87/083. U.S. Environmental Protection Agency, Cincinnati.

Centers for Disease Control and Prevention, CDC (2009): E. coli 0157:H7 and Drinking Water from Private Wells. www.cdc.gov/healthywater/drinking/.../e_coli.html.

Collins, R.S. Mansell Bicki, T.J., R.B. Brown, M.E., and D.J. Rothwell. (1984) Impact of On-Site Sewage Disposal Systems on Surface and Groundwater Quality. Report to Florida Department of Health and Rehabilitative Services, Institute of Food and Agricultural Science, University of Florida, Gainesville, FL.

Doyle, M. P. and M. C. Erickson. 2006. "Closing the door on the faecal coliform

assay" Microbe 1:162-163. ISSN 1558-7460.

Environmental Protection Agency (EPA) "National Primary Drinking Water Regulations: Drinking Water Regulations for Aircraft Public Water Systems." Final rule. Federal Register, 74 FR 53590, 2010-10-19.

Environmental Protection Agency EPA (2011). "NPDES Permit Writers' Manual." Chapter 6. Document No. EPA-833-K-10-001.

Environmental Health Agency EHA (Environmental and Public Health Consulting Group) (2012): What is pseudomonas aeruginosa? www.ehagroup.com/.

Glenn T. Eskew, But for Birmingham: The Local and National Struggles in the Civil Rights Movement (University of North Carolina Press, 1997). p. 301.

Grabow, W.O. (1996) waterborne diseases: Update on water quality assessment and control. In: Water SA 22:193-202.

Grabow W. O., Clay C. G., Dhaliwal W., Vrey M. A. and Muller E.E. (1999) Elimination of viruses, phages, bacteria and Cryptosporidium by a new generation Aquaguard point-of-use water treatment unit. In: Zentralbl. Hyg. Umweltmed. 202:399-410.

Hänninen M. L., Niskanen M. and Korhonen L. (1998) Water as a reservoir for Campylobacter jejuni infection in cows studied by serotyping and pulsed-field gel electrophoresis (PFGE). Zentralbl. Veterinarmed. B 45:37-42.

Health Canada (2008): Drinking Water Contaminants - Escherichia coli, E. coli. www.freedrinkingwater.com/water.../ecolibacteria-r.

Hedayati M. T., Pasquallotto A. C., Warn P. A., Bowyer P. and Denning D. W.

(2007): Aspergillus flavus: human pathogen, allergen and mycotoxin producer. Microbiology, 153:1677—92, Appleton and Lange, Norwalk. Connecticut.

Kelly, P. B., K. S. Ndubani, P. Nchito, N. A. Luo, R. A. Feldman and M. J. Farthing (1997) Cryptosporidiosis in adults in Lusaka, Zambia and its relationship to oocyst contamination of drinking water and Journal of Infectious Diseases 176: 1120-1125.

Kirkwood, A. (1998) Safe water for Africa. DFID Conference features. Africa Health, 2, 9-11.

Leclerc H., L. Schwartzbrod and E. Del-Cas. (2002) Microbial agents associated with Waterborne diseases. Crit. Rev. Micro. 28(4), 371-409.

Leclerc H. and Moreau, A. (2002) Microbiological safety of natural mineral water. FEMS Microbiol. Rev. 26(2), 207–222.

Leclerc H., Mossel D. A., Edberg S. C. and Struijk C.B. 2001. Advances in the bacteriology of the coliform group: their suitability as markers of microbial water safety. In: Annu. Rev. Microbiol.55:201-234.

National Research Council (NRC National Research Council (NRC). 2004. Indicators for waterborne). 2004. Indicators for waterborne pathogens. National Academy Press, Washington, DC

Obi C., Green E., Bessong P., D. E. Villiers, B. Hoosen, A. Igumbor and E. Potgieter

(2004) Gene encoding virulence markers among Escherichia coli isolates from diarrheic stool samples and river sources in rural Venda communities of South Africa. Water South Africa, 30, 37-42.

Onwughara and Gundry (2013). Physicochemical Studies of Water from Selected Boreholes in Umuahia North Local Government Area, in Abia State, Nigeria. Int. J. Pure App. Biosci., 1(3), 34-44.

Prüss-Üstün A., Bos R., Gore F. and Bartram J (2008) safer water, better health: Costs, benefits and sustainability of interventions to protect and promote health. Geneva: World Health Organization.

Sammon N. B., Harrower K. M., Fabbro L. D. and Reed R. H. (2011): Three potential sources of microfungi in a treated municipal water supply system in sub-tropical Australia. Int JEnviron Res Public Health, 8(3):713-732.

Sobsey, M.D. and Shin, G.A. (2002) Reduction of Norwalk virus, poliovirus 1 and coliphage MS2 by monochloramine disinfection of water. Wat. Sci. Tech. 38(12), 151–154.

Simon-Nobbe B., U. Denk, V. Pöll, R. Rid and M. Breitenbach. (2006). The spectrum of fungal allergy. Int Arch Allergy Immunol. 145(1): 58-86.

Sinton L.W., Finlay, R. K. and Lynch P.A. (1999) Sunlight inactivation of fecal bacteriophages and bacteria in sewage-polluted seawater. Appl. Environ. Microbiol.65, 3605–3613.

United Nations Development Programme UNDP-World Bank. (2000) Reuse of

Human Wastes in Aquaculture: A Technical Review. Water and Sanitation report no. 2. UNDP-World Bank, Washington, DC.

United Nation Educational, Scientific Cultural Organization UNESCO, World Health Organization WHO, United Nations Environment Program UNEP (2006). Water quality assessment – A guide to use of biota, sediments and water in environmental monitoring.

Walkerton, T. (2011): Escherichia coli. From Wikipedia, the free encyclopedia, en.wikipedia.org/wiki/Escherichia_coli.

World Health Organization WHO (1971) Guideline for Drinking Water Quality World Health Organization Geneva.

World Health Organization WHO (1976) Surveillance of drinking-water quality. Geneva, World Health Organization. "Freedom-Now" Time, May 17, 1963;

World Health Organization WHO (1993) Guidelines for Drinking Water Quality. 2nd Edition, World Health Organization, Geneva.

World Health Organization WHO (2000). Guidelines for Drinking Water Quality: Incorporating First Addendum. 3rd ed. Vol. 1 WHO; Geneva, Switzerland: 2006.

World Health Organization WHO (2008). Guidelines for Drinking-water Quality, Third Edition, Volume 1, 2008, Geneva, pp. 2-7.

World Health Organization WHO, (2002a) The world health report: Reducing risks, promoting healthy life. World Health Organization, Geneva. Internet URL: http//www.who.int/whr/en (accessed on 27th October, 2016).

World Health Organization WHO, (2002b) Water for development: A practical advocacy guide for world water day 2002 3rd edition. Internet URL: http//www.worldwaterday,org/advocacy/adv.html (accessed on 27th October, 2016)

World Health Organization WHO, 2006. Guidelines for drinking water quality. 4th edition, Technical Report, World Health Organization, Geneva.