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**CHAPTER TWO**

**INVESTIGATING THE BACTERIAL LOAD IN WATER SUPPLY IN THE POLY TANKS IN SOME SELECTED KNUST HALLS OF RESIDENCE**

**BY**

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**2.0 LITERATURE REVIEW**

**2.1 Background**

Access to clean and safe drinking water is essential for sustaining life and promoting public health. Water is one of the most important natural resources and necessary for any form of life to survive (Zhu & Shin, 2021).However, ensuring water quality remains a significant challenge, particularly in regions with limited access to adequate sanitation and water treatment infrastructure. Bacterial contamination poses a significant risk to water safety, leading to waterborne diseases and adverse health effects.

Water quality encompasses various chemical, physical, and biological parameters that determine the suitability of water for human consumption and other purposes (EPA., 2020). These properties are either dissolved or suspended in water and these water qualities can be influenced by both natural processes and human activities. The capacity of a population to safeguard sustainable access to adequate quantities and acceptable quality of water for sustaining livelihoods of human wellbeing as well as ensuring protection against pollution is regarded as water security (Luvhimbi et al., 2022). Bacterial contaminants, such as *Escherichia coli (E. coli), Salmonella* spp., and *Legionella* spp., can proliferate in water sources and pose serious health threats to individuals exposed to contaminated water (WHO., 2017).

Polyethylene (poly) tanks have emerged as popular choices for storing and distributing water due to their affordability, durability, and ease of installation (Hollis., 2018). The characteristics of poly tanks also guarantees its protection against sun rays and external agents such as dirt. However, poly tanks present unique challenges in maintaining water quality, as they are susceptible to microbial growth and contamination under certain conditions. Poly tanks present unique challenges in maintaining water quality, as they are susceptible to microbial growth and contamination under certain conditions (Jones & Brown., 2019). Factors such as temperature fluctuations, UV exposure, and the accumulation of organic matter can contribute to bacterial proliferation in poly tanks, jeopardizing the safety of stored water(Manga et al., 2021; Zlatanović et al., 2017).

Water quality is a crucial aspect of public health and sustainability. It refers to the chemical, physical and biological characteristics of water that determine its suitability for various uses, including drinking, recreation and agriculture (EPA., 2020). One of the primary concerns regarding water quality is the presence of bacterial contaminants which pose significant health risks if not properly controlled and managed (Boyd, 2015).

Water quality encompasses several parameters, including but not limited to pH, turbidity, dissolved oxygen, and the presence of pathogens such as bacteria, viruses, and protozoa (EPA., 2020). These parameters are essential indicators of water safety and suitability for human consumption.

**2.2 Water storage**

It is the collection and storing of water in containers or reservoirs. There are various reasons why water storage is important. It can provide a backup supply of water in case of emergencies or disruptions in the water supply such as natural disasters, power outages or equipment failures. Additionally, it can be used to collect and store rainwater for non-potable purposes like flushing toilets, washing cars and clothes and watering plants (Chalchisa et al., 2018). It is essential to take precautions when storing water to guarantee it stays pure and safe. This could involve cleaning and maintaining the storage container, treating the water with chemicals or filtration systems and guarding the container against contamination like insects or animal waste(Slavik et al., 2020)*.* In areas where irregular water supplies or water scarcity is an issue, water storage is a common practice.

**2.2.1 Methods of water storage**

Water conservation is a key element of any strategy that aims to mitigate water scarcity. By 2025, one-third of the population of the developing world will face severe water shortages (Keller et al., 2000). On an annual scale, water resources such as rainfall or rivers that vary seasonally may not supply enough water to meet demand as people need water always. Because of the sporadic spatial and temporal distribution of precipitation, the only way water supply can be controlled to match demand is through storage (Rosegrant et al., 2000). Various methods have been developed to store water, ranging from traditional practices such as rainwater harvesting and well drilling to modern technologies such as aquifer recharge. There are various methods of water storage. Water can be stored in storage tanks like polyethylene (poly) tanks, barrels mainly used to collect rain water in various homes and reservoirs (Wang et al., 2023). Each method has its own advantages and disadvantages, and the choice of storage method depends on factors such as cost, scale of storage required and feasibility of implementation.

**Tanks**

There are many different sizes and materials for water storage tanks including concrete, fiberglass and plastic. They are frequently used to store water for domestic, commercial and agricultural use and can be installed above or below the ground (Wang et al., 2023). The commonly used one is the polyethylene tanks popularly known as poly tanks. Poly ethylene tanks have some characteristics such as high resistance to pressure and chemical agents and exhibit great non-toxicity that make them particularly suitable for water storage. They guarantee a great ease of installation and can be placed in relatively confined spaces. However, Poly tanks can be susceptible to bacterial contamination if not properly maintained. Biofilm formation and temperature fluctuations are factors that can contribute to the proliferation of bacteria in poly tanks (Van Der Merwe et al., 2013).

**Reservoirs**.

Large bodies of water called reservoirs are made by damming rivers or streams. They are frequently utilized for recreational purposes, hydroelectric power production, and municipal water supply(Liming et al., 2020). Small reservoirs capture parts of surface runoff and make it available for later use.

**Barrels.**

Barrels are mostly used in collecting and storing rainwater for non-potable purposes like washing cars or watering plants. They can be fixed beneath a drainage system or other rainwater collection system and are typically made of plastic or metal(Chalker-Scott, 2023).

**2.2.2. Role of poly tanks in water distribution**

The sustainable Development Goals (SDG) 6 mandates all countries to achieve universal and equitable access to safe and affordable drinking water to all its citizens. As such, there is a need to bridge the gap of unequal water distribution in order to ensure easy access to sustainable water. Polyethylene (poly) tanks are commonly used for storing and distributing water in various settings due to their affordability, durability, and ease of installation. However, the use of poly tanks presents certain challenges in maintaining water quality, particularly concerning the proliferation of bacterial contaminants (Manga et al., 2021).

**2.2.3. Challenges associated with maintaining water quality in poly tanks**

Household water storage is fraught with many challenges which ultimately results in compromising the quality of water (Nnaji et al., 2020). Despite their advantages, poly tanks are susceptible to microbial growth and contamination, primarily due to factors such as temperature fluctuations, UV exposure, and the accumulation of organic matter(Manga et al., 2021).These challenges necessitate effective strategies for mitigating bacterial contamination and ensuring the delivery of safe drinking water to consumers.

**2.3 Potential bacteria that can be found in water**

Several types of bacteria can be found in water sources, with some species posing greater health risks than others. Common bacterial contaminants include *Escherichia coli (E. coli), Salmonella spp., and Legionella spp.,* (Cabral JP., 2010)total coliform and fecal coliform(Hassan Omer., 2020).

***2.3.1 Escherichia coli***

*Escherichia coli (E. coli)* is a well-known indicator of fecal contamination and is often used as a marker for water quality assessment (Water Science School, 2018). *E. coli* is a member of the Enterobacteriaceae family and has a rod-like structure. The majority of *E. coli* bacteria are found in both human and animal digestive tracts. *E. coli* have a fermentative and respiratory metabolism and are facultatively anaerobic. Some species have flagella for movement whereas others do not have it. Consumption of polluted water, raw vegetables, raw milk and raw or undercooked meals might spread the bacterium (WHO., 2018). *E. coli* is spread through the feces of humans and animals. *E. coli* enters water bodies through rain, snow and falls causing harm when used for domestic purposes.

***2.3.2 Salmonella* sp*.***

*Salmonella* spp. is a member of the *Enterobacteriaceae* family. They have a gram-negative stain, are rod-shaped, non-capsulated, non-sporing and have peritrichous flagella. They can develop with or without oxygen. The best selective media for it is Xylose Lysine Deoxycholate agar and the best enrichment media is Selenite F broth. *Salmonella* spp. has been identified as the most common cause of gastrointestinal illness in people who consume contaminated water and food. They are mostly found in the intestines of warm-blooded animals. They can tolerate stomach acid when consumed and can thus enter the epithelial cells of the host’s small and large intestines. *Salmonella* may endure in environments with pH values of 4.05 to 9.5. They can also endure and grow rapidly at temperatures ranging from 7°C to 48°C. *Salmonella* spp. are pathogenic bacteria associated with foodborne illnesses, but they can also be found in water sources contaminated with animal or human feces (Galán-Relaño et al., 2023).

***2.3.3 Legionella* sp.**

*Legionella* spp. are pathogenic gram-negative bacteria that causes legionellosis. They are also responsible for causing Legionnaires' disease, a severe form of pneumonia typically contracted through inhalation of contaminated water droplets and also causes Pontiac fever; a mild flu-like disease (Maria woodie., 2018).

### ***2.3.4* Total coliform**

Total coliforms were identified after cholera was contracted due to drinking contaminated water from a well. Coliforms are bacteria that can be found in the intestines of both people and animals as well as in their feces. They are a group of rod-shaped, gram-negative, non-sporing bacteria that grow aerobically on bile salt-containing agar and ferment, creating gas and acid. Most of them spend 24 hours incubating at 35° to 37° (Cheesebrough, 1984; Anderson & Davidson., 2002). The presence of coliforms indicates unclean water but the absence of bacteria suggests microbial fecal pollution. Water can become contaminated by human waste, animal waste and sewage leading to the spread of total coliform. Typically, total coliform includes organisms that can survive and flourish in water (WHO., 2008). Water that contains coliform is contaminated and therefore potentially hazardous or polluted (Maier *et al.,* 1996). When treated with disinfectant, the presence of coliform in water storage and distribution systems indicates the potential for biofilm formation from the growth of elements such as plants (WHO., 2008).

***2.3.5* Fecal Coliform**

Fecal coliforms are type of bacteria that are commonly found in the intestines of warm-blooded animals including humans. They belong to the subclass of coliform bacteria which also includes environmental coliforms that are not fecal. Indicators of fecal contamination in water, soil and food include fecal coliforms(Kashem et al., 2022). This is because they are typically found in large numbers in the feces of warm-blooded animals and are easily detected using standard laboratory techniques. For instance, high levels of fecal coliforms in water may be a sign of contamination from human or animal waste which could be harmful to public health if consumed. The safety of water and food supplies must therefore be ensured by monitoring fecal coliform levels (Bhardwaj et al., 2021).

**2.4 Comparison of bacterial load in different water storage systems**

It is thought that piped water has a high quality, though it can be potentially deteriorated (biological and chemical) in distribution systems (Routt et al., 2008). Water storage tanks and reservoirs are critical component of distribution systems, yet can pose significant challenge for water utilities as they often have a negative impact on water quality. Total coliforms and fecal coliforms are considered as they are indicators of sanitary quality of water and pathogenic bacteria. The presence of colonies in drinking water before and after storage may show that the water either contaminated during distribution or the treatment process was not sufficient to eliminate microorganisms. Comparative studies have been conducted to evaluate the bacterial load in various types of water storage systems, including concrete tanks, metal tanks, and poly tanks, to identify the most effective option for ensuring water quality (Akinseye Janet et al., 2021) When comparing bacterial loads in different water storage systems, factors such as tank material, maintenance practices, environmental conditions and water sources play significant roles. Different water source determines bacterial loads, as water from untreated sources may contain higher levels of contaminants compared to treated supplies (Akinseye Janet et al., 2021) For instance, studies have shown that polyethylene tanks tend to support bacterial growth more than other materials like concrete or stainless steel due to their surface characteristics and potential for biofilm formation. Additionally, stagnant water, inadequate cleaning, and warmer temperatures can further increase bacterial proliferation in storage systems. Contrarily, well-maintained systems with regular cleaning and disinfection tend to have lower bacterial loads regardless of the material used in making the tank. (Akinseye Janet et al., 2021; Routt et al., 2008; Van Der Merwe et al., 2013)

Numerous studies have investigated bacterial contamination in different water storage systems using various methodologies such as;

• **Water Sampling and Analysis**: Water samples are collected from storage systems and analyzed in the laboratory for the presence and load of bacteria using either culture-based or molecular techniques. (Routt et al., 2008)

**• Biofilm Assessment:** A biofilm is an assemblage of surface-associated microbial cells that is enclosed in an extracellular polymeric substance matrix. Studies assess biofilm formation and characterize the bacterial species present. (Van Der Merwe et al., 2013)

**2.5. Factors Influencing Bacterial Growth in the water found in the poly tanks**

Poly tanks are widely used for water storage due to their durability, affordability, and ease to use. However, like any water storage system, polyethylene tanks can be susceptible to bacterial contamination if not properly maintained. Various intrinsic and extrinsic factors can influence bacterial proliferation in water stored in poly tanks, including environmental conditions, nutrient availability, and the presence of biofilms (Hull et al., 2001). The physical, chemical, and biological composition of water is affected key determinants which include the physicochemical properties of water such as temperature, pH and nutrient availability (Hassan Omer, 2020). The number of years the tanks have been used with frequency of water circulation in the water tanks is one of the main concerns because a longer the period of water tank use, the higher the chance of microbial growth in the water tanks especially when the environmental conditions (especially high ambient air temperature above 35◦C) promotes microbial growth. Material composition and potential to release organic compounds are some intrinsic factors of polyethylene tanks that plays a vital role in the proliferation of bacteria. Also, external factors such as sunlight and temperature variations can impact bacterial dynamics. There are four main factors that affect bacterial growth are warmth, moisture, pH levels and oxygen levels (Khan & Almadani, 2017; Kumar et al., 2021; Saalidong et al., 2022; USGS, 2019; Van Der Merwe et al., 2013).

**2.5.1 Temperature.**

Temperature has a variety of effects on water quality. How quickly gases like oxygen and carbon dioxide dissolve in water is influenced by temperature. At lower temperatures, water may hold more dissolved gas and at higher temperatures, it can hold less. The temperature affects how bacteria grow and survive in water. In general, warmer water is better for bacterial growth than colder water and vice versa. Additionally, some compounds and contaminants may or may not dissolve in water depending on the temperature. This could affect the distribution of these substances and their environmental impact (Lai & Dzombak, 2021; Zlatanović et al., 2017)

**2.5.2 *pH***

The pH of the water is one of its most important characteristics. It is referred to as the negative logarithm of the hydrogen ion concentration (Spellman, 2017; Edzwald, 2010). The pH of water functions as a barometer for how acidic or basic the water is. Pure water is neutral with a pH of about 7.0 at 25 °C (Tchobanoglous *et al*., 1985; Tomar, 1999). The pH of typical precipitation is 5.6 which is mildly acidic (*APHA, 2005*). The pH range for drinking water that is safe for domestic use and the needs of living things is 6.5 to 8.5 (EPA, 2016). Water having a pH of 5 is 100 times more acidic than water that has a pH of 7 and water that has a pH of 7 is 10 times more acidic than water that has a pH of 8. There are two methods for calculating pH: electrometric and colorimetric(USGS, 2019) Extremely high or low pH levels can harm how well water is used. Food with high pH levels tastes sour and chlorine disinfection is less effective requiring the use of more chlorine (DeZuane, 1997), (Saalidong et al., 2022).

**2.5.3 *Turbidity***

Turbidity is the term used to describe cloudy water (APHA & APHA-AWWA, 2005; Tomperi et al., 2022). It is caused by particles suspended in water such as clay, silt, organic debris, plankton and other particles (Alley ER, 2007*).* Turbidity affects the composition of water clarity. High turbidity water can be yellow, brown, or greenish and has a hazy or muddy appearance. High turbidity levels can negatively impact water quality, disinfectant efficiency and equipment making it difficult to remove harmful bacteria and pathogens. Additionally, they may clog filters and pumps in water treatment facilities increasing the risk of waterborne diseases. As a result, water filtration might be less efficient and cost more money. Maintaining the levels of turbidity low to preserve the safety and quality of drinking water(Tomperi et al., 2022).

**2.5.4 Nutrient availability**

Microorganisms in water distribution system can thrive if provided with organic and inorganic nutrients that promote growth (USEPA). Organic compounds, particularly carbon and nitrogen compounds, can stimulate bacterial growth and proliferation in drinking water causing both public health and aesthetic water quality problems (Epa.Gov File). Several studies have investigated the link between microbial growth and nutrient levels. The main factor governing bacterial growth was the presence of bacterial nutrients (Van der Kooij and Hijnen (1982) and Servais *et al*. (1995)). The USEPA is concerned with relatively high nutrient levels in water because nutrients may lead to drinking water problems such as increased levels of microbes, in the bulk water, as well as in the pipe biofilm and sediments, unreliability of total coliform sampling due to increased growth of heterotrophic bacteria, resulting in false-positives or false-negative coliform tests. Coliform sampling may also become unreliable due to stimulated growth on pipe biofilms and sediments.

**2.5.2. Role of biofilms and microbial communities in poly tanks**

Biofilms, which are complex microbial communities encased in a matrix of extracellular polymeric substances (EPS), play a significant role in bacterial colonization and persistence in poly tanks. These biofilms can serve as reservoirs for pathogenic bacteria and contribute to the deterioration of water quality over time. Biofilm plays essential role in nutrient cycling, environmental processes and microbial interaction (Sekoai TP., 2020).

**2.5.2.1Biofilm in nutrient cycling**

Biofilm is a thin but robust layer of mucilage adhering to a solid surface and containing a community of bacteria and other microbes (Donlan RM., 2002). Biofilms form on a variety of surfaces, both biotic and abiotic, and stable and robust attachments, making removal difficult and hence enhancing survival. Biofilms, which are complex microbial communities encased in a matrix of extracellular polymeric substances (EPS), play a significant role in bacterial colonization and persistence in poly tanks. Development of biofilms occurring on the inner surface of storage tanks offers a suitable medium for the growth of microorganisms and consequently contributes to the deterioration of treated drinking water quality in homes (Donlan RM., 2002)*.*Biofilms can cycle and recycle nutrients, which can support the growth and survival of the micro-organisms in the community. This nutrient cycling is enhanced by enzymes that are produced by the microorganisms in the biofilm, as well as physical and chemical interactions between the cells and the matrix. The process of nutrient cycling can occur both within a single isogenic community or between species (Bamford et al., 2023; Seyram et al., 2016).

**2.6. Methods for Assessing Bacterial Load in Water Supply Systems**

Various methods and techniques are available for assessing bacterial load in water supply systems, including sampling techniques, laboratory-based analyses, and molecular biology approaches. A very important role in determining the quality of water is the assessment of its microbial quality. Methods for assessing bacterial load in water supply systems typically involve sampling. Some methods include Plate Count (HPC) where water samples are cultured on agar plates and incubated to allow bacterial colonies grow(APHA & APHA-AWWA, 2005). Presence or Absence (P/A) tests where selective media is used to detect the presence or absence of specific bacterial indicators, such as coliform bacteria or *Escherichia coli (E. coli).* Others include culture methods, flow cytometry, luminometry and membrane filtration in which water samples are passed through a membrane filter with a defined pore size to traps bacteria. The filter is then placed on a nutrient agar plate and incubated, allowing for the enumeration of bacteria colonies( Aziz et al., 2017; Kumar et al., 2021).

**2.6.1. Challenges and limitations of current methodologies in assessing microbial load**

One problem is that some bacteria, many viruses, and many protozoa show greater resistance to many conventional treatment methods than do fecal coliforms, so assessments of the safety of treated water are sometimes too optimistic. A limitation is that an increasing number of such pathogens as *Giardia* and *Legionella* are surfacing that can originate from sources other than human fecal material(Amalfitano et al., 2018; Harwood, 2014). Thus, the fecal indicator strategy is less relevant for these types of microorganisms. Despite their utility, current methodologies for assessing microbial load in water supply systems have certain limitations, including the time and cost associated with sample processing, the need for specialized equipment and expertise, and the potential for false-positive or false-negative result (Amalfitano et al., 2018; Harwood, 2014; Kumar et al., 2021). Traditional methods, such as culture-based techniques, require extensive incubation and manual work, making it time consuming and resource-intensive. Culture-based methods may not detect all viable microorganisms as they can only detect microorganisms grown in the laboratory, leading to an underestimation of microbial load. Also, variability between methods such as PCR-based methods, culture-based methods and traditional-based methods can yield different results, complicating comparison of data. Current methodologies primarily focus on quantifying microbial abundance which may not enough details about the metabolic activities or virulence of the bacteria present (Manga et al., 2021).

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