GROUNDWATER QUALITY ASSESSMENT AND THE IMPACT OF SILVER BIONANOPARTICLES ON FAECAL INDICATOR BACTERIA IN IFAKARA TOWN, MOROGORO, TANZANIA

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CERTIFICATION

The undersigned certify that he has read and does hereby recommend for examination by the Open University of Tanzania a thesis titled **"Groundwater quality assessment and the impact of silver bio nanoparticles on faecal indicator bacteria in Ifakara town, Morogoro, Tanzania"** in fulfilment of the requirements for the degree of Master of Science in Environmental Sciences (Health) of the Open University of Tanzania.

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DECLARATION

I **Alex Absalom Mlwisa,** do hereby declare that except for reference to other people's work which have been dully cited, this thesis submitted to the Faculty of Science, Technology and Environmental Studies is the result of my own investigation, and has not been presented for any other degree elsewhere.

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Signature

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Date

DEDICATION

This study is dedicated to my late Mother, Rose Mkupala; Father, Absalom Mlwisa; my wife, Elinjiwanza Mshiu; and my children, Ariel and Aric.

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ABSTRACT

This study investigated the groundwater quality in Ifakara town, South-eastern Tanzania and developed a nano-disinfectant to deactivate faecal indicator bacteria. Physico-chemical parameters and faecal indicator bacteria were assessed in groundwater samples from 54 hand-pump borewells using standardized methods. The sanitation conditions around the borewells were evaluated using a WHO-approved questionnaire, and a nano-disinfectant was generated through a straightforward and environmentally friendly procedure. While all borewells (100%) met Tanzania Bureau of Standards (TBS) and World Health Organization (WHO) guidelines for physico-chemical parameters, 46% of the borewells exceeded the permissible limits for faecal indicator bacteria. The presence of faecal indicator bacteria in the groundwater was significantly correlated ($r=0.5$-0.6, $P<0.05$) with poor sanitation conditions around the borewells. The study also observed distinct relationships between faecal indicator bacteria and faecal markers of human, pig, and ruminant, indicating that these animals were not the sources of faecal contamination in groundwater. Silver nanoparticles generated in this study effectively eliminated high levels of fecal indicator bacteria ($>10^5$ cfu/100 mL) in surface water to undetectable levels within 4 hours at a concentration of >9.37 mg/mL, underscoring their potential for water purification. The study recommends improving sanitation practices around borewells and utilizing effective disinfectants to enhance the microbiological quality of groundwater.

Keywords: *Physico-chemical Parameters*; *Microbial Parameters*; *Microbial Source Tracking*; *Sanitation Practices, Silver Bionanoparticles, Water Treatment*.

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LIST OF ABREVIATIONS AND ACRONYMS

AFM	Atomic Force Microscopy
APHA	American Public Health Association
CA-FL	*Cassia abbreviata* Fresh Leaf
CA-RB	*Cassia abbreviata* Root Bark
CA-SB	*Cassia abbreviata* Stem Bark
CCA	Chromocult Coliform Agar
CFU	Colony Forming Unit
Ct	Cycle Threshold
DNA	Deoxyribonucleic Acid
dsDNA	Double Stranded Deoxy Ribonucleic Acid
EC	Electrical Conductivity
EPA	Environmental Protection Agency
FIB	Faecal Indicator Bacteria
KN	Feldspathic micaceous sandstones and conglomerates with grey and maroon shales and siltstones.
M	Feldspathic micaceous sandstones.
MIC	Minimum Inhibitory Concentration
MST	Microbial Source Tracking
n	Number of borewells
NBS	National Bureau of Statistics
ND^{+}	Not Detected
ND^{++}	No hand pump wells falls in this category

Ng	Coarse gravel with rounded pebbles
NRZ	Pale non-alluvial sands.
Nz	Red –brown and light sandy earths
Nzf	Ferruginized cemented sands and gravels
Nzs	Grey cemented sands
Nzsp	Grey cemented sands and gravels with rounded pebbles
PCR	Polymerase Chain Reaction
PFU	Plaque Forming Unit
qPCR	Quantitative Polymerase Chain Reaction
SBNs	Silver Bionanoparticles
SPSS	Statistical Package for Social Sciences
SRP	Surface Plasma Resonance
TBS	Tanzania Bureau of Standards
USA	United State of America
WHO	World Health Organisation

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Groundwater constitutes 97% of global freshwater (Kretschmer *et al*., 2023). This vital resource has been utilized by mankind for many generations. It is reported that out of the total global water supply, only 2.4% is distributed on the mainland (Varotsos *et al*., 2023), and within this distribution, only a mere 0.3 to 0.5% is available as freshwater while 11% of the global population has no access to clean drinking water, particularly in regions like sub-Saharan Africa and Oceania (De Luka *et al*., 2024).

The groundwater in Africa is currently receiving significant attention (Gurmesa *et al*., 2022; MacDonald *et al*., 2021). In the past, this essential global resource was largely neglected, misunderstood, and misused. Cobbing (2020) noted that in Sub-Saharan Africa, there is a moderate yield of groundwater available at accessible depths, which is adequate for areas with a small population. In Tanzania, while most drinking water comes from surface sources, there is a growing reliance on groundwater in peri-urban and urban areas (Nyika and Dinka, 2023; Smiley, 2020). For example, in Dar es Salaam, Morogoro, and other urban and peri-urban regions of Tanzania, the use of groundwater is increasing due to rapid population growth and inadequate water distribution networks (Olarinoye *et al*., 2023). This has led many individuals to turn to deep and shallow wells, and in some cases, to sell water from their wells (Munro and Kweka, 2021). Groundwater is considered to be more stable and microbiologically superior to surface water (Ferreira *et al*., 2023), prompting

numerous communities in rural and urban areas to use it as an alternative drinking water source. However, the extraction of groundwater from shallow wells or unprotected boreholes increases the risk of groundwater contamination (Masindi and Foteinis, 2021).

Inadequate sanitation, along with improper sewage treatment, fertilizer use, and frequent flooding, has a significant impact on groundwater quality (Akhtar *et al*., 2021). Various studies in Tanzania have shown that the levels of chemical and microbial contaminants in groundwater can surpass the recommended standards set by the World Health Organization (WHO) and the Tanzania Bureau of Standards (TBS). For instance, elevated nitrate levels have been documented in Dar es Salaam (up to 477.6 mg/L), Dodoma (up to 441.1 mg/L), Tanga (exceeding 100 mg/L), and Manyara (180 mg/L) (Elisante and Muzuka, 2017). Makoba and Muzuka (2019) also found elevated fluoride levels above 1.5 mg/L in the volcanic region of Tanzania, surpassing WHO and TBS guidelines. Additionally, nitrate and fluoride have been identified as significant concerns in other parts of Tanzania (Alex *et al*., 2021; Malago *et al*., 2020).

Similarly, microbial contaminants have been found in groundwater in urban areas (De lambert *et al*., 2021; Ripanda *et al*., 2023) such that high counts of *Escherichia coli* (>10^3 cfu/100 mL), were detected in groundwater abstraction facilities located within 10 to 20 meters from toilets and septic tanks (Nayebare *et al*., 2020). In addition, Halla *et al*., (2022) reported high faecal pollution in the groundwater of peri-urban areas of Dar es Salaam that correlated with the sanitary condition of the

groundwater catchment, while Kwikima (2025) in a study conducted in Dodoma reported *E. coli* in shallow wells with a mean level of 40 cfu/100 mL. While these studies report high microbial contaminants in Tanzanian groundwater, they did not indicate whether these microbial contaminants come from human faeces or animal faeces, taking into consideration that human faeces harbor a significant number of pathogenic microorganisms causing diseases to humans compared to animal faeces. Therefore, there is a need to conduct an integrated study that will determine the levels and sources of faecal pollution in the groundwater so that a complete picture regarding the safety of groundwater can be obtained and relevant water quality policies, treatment procedures, and remediation plans can be proposed.

In a study on groundwater microbial quality, De Lambert *et al*., (2021) identified anthropogenic activities as the primary sources of contaminants leading to elevated levels of faecal indicator bacteria in groundwater. Various studies have highlighted the close connection between water quality and urbanization (Sarker *et al*., 2021; Ketadzo *et al*., 2021; Ayad *et al*., 2021). For instance, Zhang *et al*., 2020) demonstrated that both population size and growth have adverse effects on groundwater quality, while Fisher *et al*., (2016) found that household size influences groundwater quality. Poor-quality drinking water poses significant health risks and associated costs (Krishan *et al*., 2023), particularly in developing countries where waterborne diseases are prevalent.

Ifakara town, similar to many other towns in Tanzania, has fertile floodplains suitable for agriculture, rivers for fishing, and pasture land for livestock. These

features attract a large population from different parts of Tanzania, leading to environmental degradation and water contamination. Ifakara serves as a commercial center for districts such as Ulanga, Malinyi, and Mahenge, with ongoing development activities like trade and rice mills negatively impacting groundwater catchment. Previous research has indicated that groundwater catchments receive increased waste from households, industries, and agriculture (Sarker *et al*., 2021; Singh *et al*., 2025; Arif *et al*., 2021). Despite the known connection between catchment and groundwater quality (Gao *et al*., 2021), there is a lack of comprehensive data on pollution levels in the groundwater of Ifakara town. This lack of information hides the risks faced by residents who consume contaminated water and the potential for waterborne diseases like typhoid fever and cholera to spread. Groundwater is typically found in areas protected by igneous or sedimentary rocks, providing a natural barrier against pollution (Hunter *et al*., 2011). However, contamination can occur when pollutants from the catchment area seep through rock fractures during drilling processes or from abandoned wells and boreholes. Additionally, chemical substances from the geogenic environment can also contaminate groundwater (Li *et al*., 2023).

Detection of contamination is performed by measuring faecal indicator bacteria and physico-chemical parameters, but it is uncertain whether groundwater in Ifakara town is influenced by human activities or natural processes. This lack of clarity complicates groundwater quality management and hinders the ability to predict and model risks associated with drinking water from underground sources (Zhang *et al*., 2022).

On the other hand, numerous communities in Tanzania are compelled to consume untreated water due to their inability to afford costly water treatment technologies, their impoverished living conditions, and their restricted access to basic and affordable treatment methods. As a result, waterborne diseases are common in these communities, leading to the deaths of many individuals, especially children and those with weakened immune systems. Therefore, there is a critical need for the development of inexpensive and effective disinfectants made from locally available materials like medicinal plants (Naranjo-Soledad *et al*., 2024; Yefanova *et al*., 2022) in developing countries to protect communities from waterborne diseases. According to Vahideh *et al*., (2020), nanotechnology is considered safe, environmentally friendly, and suitable for producing disinfectants on a large scale, particularly when using biological materials. While this technology is efficient in eliminating harmful bacteria from groundwater, reducing the incidence of waterborne diseases and deaths caused by contaminated water in communities without access to water treatment technologies, this technology have not fully exploited in developing countries like Tanzania.

This study focused on assessing the groundwater quality in Ifakara town in Morogoro Region, Southeastern Tanzania, by analyzing its physico-chemical properties, faecal indicator bacteria, and sources of faecal indicator bacteria. It aims to provide a comprehensive evaluation of the safety of the groundwater, which serves as the main water source for Ifakara town residents. Additionally, the study intends to synthesize silver bionanoparticles from extracts of *Cassia abbreviata*, a medicinal plant, using a cost-effective and environmentally friendly approach. These

bionanoparticles have the potential to be utilized as an affordable disinfectant to inactivate faecal bacteria in the groundwater, thereby potentially reducing the prevalence of waterborne diseases in the community.

1.2 Problem Statement

Ifakara town in the Morogoro region of Tanzania is experiencing rapid growth, with a total population of 290,424 people as reported by the National Bureau of Statistics (NBS) in 2022. The town has an annual population growth rate of 2.7% and a population density of 40.31 people per square kilometer (Ifakara Town Council Five-Year Strategic Plan, 2018/19-2022/23). The population increase in Ifakara town is driven by factors such as natural growth, migration, agriculture and improved social services, similar to other urban areas in Tanzania. The town's expanding population, combined with the growth of medium and small-scale industries, has led to a significant rise in the demand for drinking water (Kashaigili, 2010; Mushi *et al*., 2012). The current supply of 1,079 cubic meters per day falls short of the estimated demand of 4,900 cubic meters per day (Ifakara Town Council Five-Year Strategic Plan, 2018/19-2022/23).

The lack of a proper water distribution network in Ifakara has resulted in the proliferation of constructed deep and shallow wells, which are at risk of contamination by leaking pit latrines, overflowing septic tanks, sewage effluents, and surface runoff (Mushi *et al*., 2012). Additionally, the town's location in flood-prone areas leads to groundwater contamination by faecal bacteria and chemical pollutants during rainfall events, especially in shallow wells. Many residents in Ifakara town

rely on septic tanks and pit latrines for wastewater management, which can contaminate shallow wells during heavy rains. Therefore, it is crucial to assess the quality and safety of borewell water consumed in Ifakara town to protect public health from waterborne diseases. Furthermore, the construction of shallow and deep wells in Ifakara town often lacks proper safeguards against groundwater contamination, such as maintaining a safe distance from septic tanks and pit latrines and implementing contamination prevention measures. Additionally, these wells are neither fenced nor repaired, increasing the risk of contamination from animals and children.

Contaminated groundwater with faecal bacteria requires treatment before consumption to prevent waterborne diseases like dysentery, cholera, and typhoid. However, access to effective disinfectants for treating contaminated groundwater is limited in many fast-growing towns of developing countries like Ifakara, posing a significant health risk to the population.

1.3 Research Objectives

1.3.1 General Objective

To investigate the level of physico-chemical parameters and faecal indicator bacteria in the groundwater and develop cheap and efficient disinfectant for inactivation of faecal indicator bacteria in the contaminated groundwater in Ifakara town, Morogoro, Tanzania.

1.3.2 Specific Objectives

i. To measure the levels of physico-chemical parameters (temperature, pH, electrical conductivity, total suspended solids, arsenic, nitrate, ammonia, fluoride and phosphate) in the groundwater of Ifakara town.

ii. To assess the levels of faecal indicator bacteria (total coliforms, *Escherichia coli*, enterococci, coliphages) in groundwater and its correlation with the sanitary conditions of the groundwater catchment in Ifakara town.

iii. To determine the origin of faecal indicator bacteria (total coliform, *Escherichia coli*, enterococci, coliphages) in the groundwater of Ifakara town.

iv. To fabricate cheap and efficient nano-disinfectant for deactivating faecal indicator bacteria in contaminated water.

1.4 Hypotheses

i) The physico-chemical characteristics of the groundwater in Ifakara town are within the recommended WHO and TBS standards.

ii) The level of faecal indicator bacteria in the groundwater of Ifakara town is significantly correlated with the sanitation conditions of the groundwater catchment

iii) The origin of feacal pollution in the groundwater does not stem from predominant animal or human sources but rather from the baseline levels present in Ifakara town's soil.

iv) Nano-disinfectant synthesized from *Cassia abbreviata* plant has the potential to substantially diminish faecal indicator bacteria in contaminated water.

1.5 Significance of the Study

Effective management of groundwater quality requires accurate and current information on the microbiological and physico-chemical characteristics of the groundwater to make informed decisions. The increasing contamination of groundwater, which can lead to waterborne disease outbreaks, is a global concern that necessitates a better understanding of faecal indicator bacteria levels and chemical contaminants in groundwater. This knowledge is crucial for identifying natural and human-induced factors contributing to groundwater pollution and degradation of water quality. It is particularly valuable for designing groundwater management programs to safeguard water quality from pollution originating in the catchment area.

Understanding the microbiological properties of groundwater is essential for determining quantitative microbial risk assessment, predicting the prevalence of waterborne diseases in urban areas, and identifying wells requiring immediate water quality restoration. Moreover, analyzing the microbial and physico-chemical characteristics of groundwater is instrumental in formulating policies for effective groundwater monitoring, restoration, and treatment. By employing sensitive molecular techniques, this study identifies potential sources of faecal contamination in groundwater, enabling the development of legal measures against polluters and

community-based initiatives to protect groundwater resources sustainably. The data generated can also be used to evaluate the effectiveness of groundwater restoration efforts and prioritize interventions for polluted groundwater. This information is valuable for public health professionals, urban planners, policy makers, community health workers, and donors in devising evidence-based groundwater management strategies tailored to the specific needs of Ifakara town and its surroundings

CHAPTER TWO

LITERATURE REVIEW

2. Introduction

This chapter presents a literature review on the global status of groundwater. It covers source of groundwater, catchment condition of groundwater, physico-chemical characteristics, microbiological properties, sources of pollution, treatment option, and identifies research gaps.

2.1 Groundwater Source

Groundwater is freshwater from rain or snow that soaks into the soil and is stored in the tiny spaces between rocks and particles of soil. Neglecting the water masses stored in polar caps and in glaciers, 96% of all freshwater is thus found in aquifers, namely 10.5 million km^3 (Richts *et al*., 2011). It can stay underground for hundreds of thousands of years, or it can come to the surface and help fill rivers, streams, lakes, ponds, and wetlands (Mishra *et al*., 2023). Groundwater can also come to the surface as a spring or be pumped from a well (Lachassagne *et al*., 2023).

2.2 Groundwater Catchment Characteristics

Groundwater is deposited in saturated zones beneath the land surface (Schmidt *et al*., 2012). The upper surface of the saturated zone is called the water table according to the United States Geological Survey (2025). Despite common misconceptions that groundwater originates from underground rivers, it actually fills the pores and fractures in underground materials like sand, gravel, and rock, similar to how water saturates a sponge (Van *et al*., 2018; Yu *et al*., 2018). In situations where

groundwater naturally flows out of rock materials or can be extracted in significant quantities through pumping, these rock materials are referred to as aquifers. Groundwater typically moves at a slow pace, usually ranging from 7 - 60 centimeters (3-25 inches) per day within an aquifer (Hunter *et al*., 2002; Schmidt *et al*., 2012). As a result, groundwater resources need protection from chemical and microbiological pollutants due to their various uses such as drinking, irrigation, institutional, and industrial purposes. Therefore, effective management of the entire catchment area of the abstraction facility is necessary (WHO, 2016).

Adekunle *et al*. (2007) and Mushi *et al*. (2012) have found that the proximity of the pollution source to the groundwater abstraction facility affects groundwater quality, with a shorter distance posing a greater risk of pollution than a longer distance. Harwood *et al*. (2017) and Lapworth *et al*. (2017) found that an optimal distance for accessing high-quality groundwater is between 10 and 20 meters from potential sources of contamination like latrines, septic tanks, and dumpsites (Harwood *et al*., 2017). In contrast, Erick (2016) found that the 20 m distance between latrines and wells had common risks, while the concentration of faecal coliform was higher between 25 m and 46 m compared to 49 m away from the source of pollution. Loss of attachment area in the well handpump, cracks in the apron concrete, and a deficient drainage system also play a role in groundwater pollution (Erick, 2016).

Groundwater resource monitoring is essential to safeguard communities from consuming water with unknown physico-chemical and microbiological quality, which can result in waterborne diseases (Mushi *et al*., 2012). In developing

countries, groundwater monitoring is infrequent due to factors like limited awareness, poverty (Erick, 2016), inadequate laboratory infrastructure, and a shortage of skilled personnel. However, Mushi *et al*. (2012) introduced a simple method called the "Risk of Contamination Scoring" to assess groundwater catchment safety. This method has the potential to predict faecal indicator bacteria in groundwater, especially in developing countries. The tool establishes criteria as indicators of potential contamination risks, helping to forecast the microbiological quality of groundwater.

2.3 Physico-chemical Characteristics of the Groundwater

The chemical composition of groundwater is primarily determined by the geological nature of the soil (Rao *et al*., 2012; Sebastian *et al*., 2019). Generally, groundwater exhibits relatively stable temperature levels, low or negligible turbidity, except in karst areas. The mineral content of the groundwater also tends to remain consistent within the same geological environment. However, the physico-chemical properties of groundwater can undergo alterations due to anthropogenic contaminants (Kumar and Bhatt, 2020). The physical characteristics of groundwater encompass temperature, color, taste, and odor, while the chemical characteristics involve parameters such as electrical conductivity, pH, nutrients, heavy metals, organic materials and dissolved oxygen. Monitoring these parameters is essential for assessing groundwater quality and determining whether it complies with local (TBS, 2018) and international (WHO, 2016) water quality standards.

2.3.1 Temperature

Temperature plays a significant role in influencing water quality as it impacts the rate of chemical reactions in water, as well as factors like conductivity and solubility (Erick, 2013; Kale, 2016). Additionally, temperature can directly affect pH, with higher temperatures leading to lower pH values, and vice versa. For instance, temperature of +1 K is linked to a 4% decline in oxygen saturation and a pH drop of 0.02 due to the accumulation of carbon dioxide (Riedel, 2019). Microbial growth and activity in groundwater sources are strongly influenced by temperature (Kale, 2016). Furthermore, temperature determines the types of organisms that thrive in a particular water environment. For instance, in Africa, it has been reported that dug wells maintain a temperature range between 26.5 and 28.5 °C, which happens to be the preferred temperature range for certain bacteria to thrive (Ndububa and Idowu, 2015).

2.3.2 Electrical Conductivity

Electrical conductivity (EC) measures a substance's capacity to conduct an electric current, and in the case of water, it can conduct electricity due to the presence of ions (Erick, 2013). Electrical conductivity serves as an indicator of water pollution within a specific environment. Typically, drinking water should have EC of 300 μS/cm or less under normal conditions; EC value exceeding this threshold may signal water pollution (Erick, 2013). Furthermore, EC exhibits seasonal variations, with higher values during dry seasons compared to wet seasons. Additionally, it has been observed in many areas that during the dry season, there is a discrepancy in EC levels between the groundwater contaminated with leachate from dump sites and that

contaminated by waste from defecation sites, with groundwater contaminated with leachate from dumpsite having higher EC than that contaminated with waste from defecation sites (Gohain and Bordoloi, 2021).

2.3.3 pH

Water's pH serves as a determinant of the solubility and availability of biochemical components, such as nutrients (phosphorus, nitrogen, and carbon), as well as microorganisms (Kale, 2016). Typically, the pH of fresh water falls within the range of 6 to 9, although variations in pH, especially in groundwater sources, may be linked to factors such as pollution or the presence of minerals like Ca^{2+} and HCO_3^- due to limestone weathering in the catchment areas (Erick, 2013). It is worth noting that pH itself is reported to have no direct impact on human consumption (Erick, 2013; Kale, 2016).

2.3.4 Total Suspended Solids

Total suspended solids (TSS) are defined as waterborne particles exceeding 2 microns in size (Campbell, 2012). TSS comprises microbes, clay, gravel, sand, and silt, which contribute to water cloudiness. Globally, human anthropogenic activities are responsible for the presence of TSS in water sources (Campbell, 2012). The presence of TSS in groundwater diminishes groundwater quality and poses health risks when consumed. Colloidal clay is a common contaminant in groundwater, resulting in a cloudy appearance that adversely affects water quality. TSS is typically measured using a gravimetric method, as outlined by APHA (2005). The recommended concentration of TSS in water is 500 mg/L, as reported by the

Australian Drinking Water Guidelines (ADWG, 2002) and the World Health Organization (WHO, 2016).

2.3.5 Fluoride

Fluoride occurs naturally in most soils, rocks and in many water supplies (Erick, 2013). Fluoride is also sometimes present in industrial air pollution. It plays a great role in preventing dental decay and strengthening the teeth, but can be harmful at high concentrations. The maximum concentration in water allowed by WHO and Tanzania Bureau of Standard (TBS) is 1.5 mg/L. A high concentration of fluoride has been shown to cause bone and teeth disease (Erick, 2013).

2.3.6 Arsenic

Arsenic is a naturally occurring element found in the earth's crust and is present in various environmental sources such as soil, rocks, burning fossil fuels, drainage from old gold mines, and certain types of sheep dip (Yancheva *et al*., 2016). It is highly toxic in its inorganic form and can cause long-term harm. According to the Australian Drinking Water Guidelines (ADWG, 2002) and Erick (2013), at concentrations above 0.3 mg/L, arsenic can lead to acute and chronic toxic effects, including irreversible damage and cancer. The contamination of groundwater with inorganic arsenic is a widespread issue, with an estimated 140 million people in at least 70 countries consuming water containing arsenic levels above the WHO provisional guideline value (Ravenscroft *et al*., 2009). It is projected that between 94 and 220 million people who rely on groundwater for drinking water may be at risk of exposure to elevated arsenic concentrations (Podgorski *et al*., 2020). Therefore,

ensuring a safe water supply for drinking, food preparation, and irrigation of food crops is essential to prevent exposure to arsenic.

2.3.7 Ammonium ion

Ammonium ion (NH_4^+) is toxic to some fish and other aquatic organisms at concentrations below 1 mg/L in water according to Kale (2016). Human beings and higher animals are less sensitive to NH_4^+ in water, but long-term ingestion of water containing more than 1 mg/L NH_4^+ may damage internal organs (Erick, 2013). NH_4^+ may be present in groundwater as the results of the degradation of natural occurring organic matter also originates from fertilizers application, livestock waste and industrial effluents as reported by Erick (2013) and Kale (2016).

2.3.8 Nitrate

Nitrate (NO_3^-) is water-soluble and is made up of nitrogen and oxygen. It is formed when nitrogen from NH_4^+ or other sources combines with oxygen under assistance of *Nitrosomonas* and *Nitrobacter* organisms. Naturally, water contains less than 1 mg/L of NO_3^-, therefore it is not a major problem to public health. A higher than 50 mg/L levels of NO_3^- indicate that the water has been contaminated (Erick, 2013). Water is mainly contaminated with NO_3^- from intensive agriculture, poor managed septic tanks, sewage disposal, and industrial effluents. Nitrate is also naturally present in sources like igneous and volcanic rocks, as well as vegetables such as spinach, beets, and celery. Elevated levels of NO_3^- can have adverse effects on infants less than six months of age, potentially leading to a condition known as "blue-baby syndrome" or methemoglobinemia. This disease has the potential to cause brain damage or even result in fatality (WHO, 2016).

2.3.9 Phosphate

Phosphate (PO_4^{3-}) is naturally found in water bodies and rocks in very low concentrations less than 0.05 mg/L as reported by Kale (2016). High level of PO_4^{3-} in water bodies is an indication of pollution (Erick, 2013; Kale, 2016). Other sources of PO_4^{3-} which pollute groundwater include overlying soil, agricultural fertilizer, animal wastes, leaking septic system, as well as dissolution of minerals containing PO_4^{3-} in aquifer sediments (Fuhrer *et al*., 1999; Liu *et al*., 2019). Phosphate is harmless to human and animal health unless the concentration exceeds 0.3 mg/L (Kale 2016). Digestive problems are associated with uptake of PO_4^{3-} at concentration above the WHO standard (WHO, 2016).

2.4 Sources of Groundwater Pollution

Groundwater can be affected by pollutants that pose risks to human health (Mushi *et al*., 2012; Lapworth et al., 2017). These pollutants can originate from natural and/or anthropogenic sources (Akhtar *et al*., 2021). Natural sources of groundwater contamination, such as seawater, brackish water, surface waters with poor quality, and mineral deposits, can become significant sources of contamination when human activities disrupt the natural environmental balance (Li *et al*., 2021). Examples include the depletion of aquifers leading to saltwater intrusion, acid mine drainage from mineral exploitation, and leaching of hazardous chemicals due to excessive irrigation (Su *et al*., 2020). On the other hand, anthropogenic sources of groundwater contamination include industrial activities, mining operations, agriculture, and domestic activities, urban runoff, and radioactive contamination from nuclear power plants (Li *et al*., 2021). Industrial activities contaminate groundwater through various

pathways: chemical spills or leaks (e.g., from storage tanks, pipelines, or transportation) release hydrocarbons, solvents, or heavy metals (e.g., arsenic, chromium) that seep into soil and aquifers (Cheng, 2021); improper disposal of industrial waste (e.g., unlined landfills, illegal dumping) allows toxins like pesticides or pharmaceuticals to leach into groundwater (Appiah-Adjei *et al*., 2016); industrial effluent discharge (untreated or partially treated) introduces acids, cyanide, or synthetic organic compounds into surface water, which then infiltrates underground (Jain *et al*., 2021).

Mining activities exacerbate contamination through acid mine drainage, which consists of sulfuric acid and dissolved metals like lead and cadmium, along with leaks from tailings ponds (Galhardi *et al*., 2016). Similarly, oil and gas extraction presents the danger of fracking fluid migration, containing biocides and heavy metals, as well as brine spills (Dosari *et al*., 2024; Li *et al*., 2021). These contaminants can permeate porous aquifers, endure for extended periods due to slow degradation rates, and result in chronic health problems such as cancer and neurological damage. Consequently, stringent regulatory measures and remediation efforts are essential to mitigate these hazards.

Agricultural and domestic activities contaminate groundwater with excessive nutrients, chemicals, and pathogens. In agriculture, the use of too many fertilizers (nitrates, phosphates) and pesticides/herbicides leads to the leaching of these substances into aquifers through irrigation or rainfall, resulting in harmful nitrate levels (associated with methemoglobinemia) and cancer-causing pesticide residues

(Kumar *et al*., 2021). Livestock waste (such as manure lagoons and grazing runoff) introduces pathogens (such as E. coli and Salmonella) as well as antibiotics and hormones (Li *et al*., 2023), while salinization from irrigation causes salt concentrations to increase in aquifers (Said *et al*., 2022). Domestic pollution arises from malfunctioning septic systems that release untreated sewage (containing pathogens, nitrates, and pharmaceuticals) (Sridhar *et al*., 2024) and improper disposal of household chemicals (such as cleaners, motor oil), paints, or pharmaceuticals into the soil, which then seep into the groundwater (Khalil *et al*., 2022).

Urban runoff contributes to groundwater pollution by carrying pollutants from impermeable surfaces such as roads, rooftops, and parking lots into aquifers through infiltration or stormwater systems (Jafarzadeh *et al*., 2024). Common contaminants include road salts (chlorides), heavy metals (lead, zinc, copper) from vehicle brake dust and tire wear, hydrocarbons (oil, grease), lawn chemicals (fertilizers, pesticides), and pathogens from pet waste (Singh *et al*., 2022). When it rains, these pollutants mix with runoff and enter groundwater without being filtered by natural soil processes due to the limited permeability of urban areas. Unfiltered stormwater can flow into groundwater through storm drains connected to recharge basins or seep directly through cracked pavements. Over time, the accumulation of pollutants like nitrates, chlorides, and harmful organic compounds can degrade water quality, endangering groundwater resource by causing issues such as salinization, and exposure to carcinogens.

Groundwater contamination can have negative effects on human health, the environment, and economic development. Therefore, regular monitoring of groundwater is essential to protect public health and preserve its quality.

2.5 Microbial Quality of Groundwater

Groundwater remains a major source of water for drinking purpose especially in Sub-Saharan Africa. The groundwater is extracted via constructed shallow wells or deep wells. These infrustructures are either privately or publicly owned (Howard *et al*., 2003; Lapworth *et al*., 2017). Shallow wells are highly prone to pollution (Mkude, 2015; Kilungo *et al*., 2018) as compared to boreholes. Boreholes are trusted to be stable in terms of microbiological quality due to their confined aquifers which play a great role in filtration of microbial contaminants. This sets them apart from dug and shallow wells (Mkude, 2015). Kilungo *et al*., (2018) determined faecal pollution on the deep wells (20-30m), hand dug open wells (<5 m) and closed dug wells (>5 m) in Kilombero District and indicated that total coliforms (2×10^3 cfu/100 mL) on deep wells were less compared to closed dug wells (3×10^3 cfu/100 mL) and open dug wells (4×10^4 cfu/100 mL). High faecal contamination in shallow wells is due to poor protection from pollution sources (Kilungo *et al*., 2018). It is worth noting that protected deep wells are sometimes polluted by on-site sanitation facilities when there is a hydrologic connection between deep aquifers and younger geologic layers on the surface (Parker *et al*., 2010; Lapworth *et al*., 2017). This is more evident in developing countries and especially in peri-urban areas of sub-Saharan Africa where onsite sanitation systems are predominant (Lapworth *et al*.,

2017). The presence of on-site sanitation systems renders groundwater sources vulnerable to faecal pollution.

2.6 Faecal Indicator Bacteria in Groundwater

Faecal indicator bacteria originate from human and animal faecal matter (King, 2016). These faecal indicator bacteria are normal inhabitants of the gastrointestinal tract of humans and warm-blooded animals (Lapworth *et al*., 2017). Faecal indicator bacteria are released in the environment through faeces of human or animals as well as sewage effluents (King, 2016). These feacal indicator bacteria tend to perish under unfavorable conditions. When feacal indicator bacteria are detected in groundwater, signify a high likelihood of the presence of microbial pathogens as well (Howard *et al*., 2003). This indication assists water quality managers in using feacal indicator bacteria as a proxy for identifying pathogens, rather than having to identify each individual pathogen responsible for waterborne diseases (Howard *et al*., 2003; Myers *et al*., 2014). This method is known for being easily testable, precise, and cost-effective (Myers *et al*., 2014; King, 2016). However, it is important to note that the feacal indicator bacteria offer a foundational estimate of waterborne pathogens, not a direct confirmation of their presence (Myers *et al*., 2014; King, 2016). Despite its significance, feacal indicator bacteria do not provide clues regarding the source of feacal pollution, as these indicators originate from multiple sources (Lamendella, 2007; Kirs *et al*., 2011). Furthermore, the determination of faecal pollution in tropical regions requires careful selection of faecal indicator bacteria in order to yield accurate information (Goshu *et al*., 2021).

2.7 Microbial Source Tracking in Groundwater

Microbial source tracking (MST) refers to a group of methods intended to discriminate between human and non-human source of faecal contamination. Some MST methods are designed to differentiate between faecal contaminations originating from individual animal species (Griffith *et al*., 2003; EPA, 2011). MST method plays a great role on generating information to help management of water resources (Griffith, *et al*., 2003). Another reason behind the development of this method was to identify the specific source of faecal contamination in water (Field, 2008; Harwood, *et al*., 2011; Schriewer *et al*., 2015; Domingo *et al*., 2023). Also, MST is used in the determination of faecal bacteria abundance in the host, clonal diversity, temporal stability and geographic continuity (Domingo, 2023; Bradshaw *et al*., 2016).

However, the type of faeces to be identified by MST depends upon some characteristics of faeces in relation to host species specificity (Field, 2008; Bradshaw *et al*., 2016). Regardless of the variety of MST methods, only a few have been given rigorous testing due to their efficiency and effectiveness (Field, 2008; Bradshaw *et al*., 2016). One of the MST method is library dependent, involves isolates identification of bacteria grown from various faecal sources and water sample and comparing them to a library of bacteria strain from known faecal sources. A library independent method identifies faecal sources based on known host-specific characteristics of the bacteria without the need of the bacteria library (Ahmed *et al*., 2007).

Despite the usefulness of MST methods, some challenges should be taken into consideration for obtaining better results. These are intrinsic method performance such as sensitivity and specificity of the sample, method performance in the field and knowledge of the ecology of the organisms, the persistence of the markers in the environment, correlation with faecal indicator bacteria (FIB) and pathogens in water (Harwood *et al*., 2011).

2.8 Nanotechnology for Water Treatment

Nanotechnology utilizes physico-chemical particles in the size range of 1 to 100 nm (Kunduru *et al*., 2017). Nanoparticles are highly efficient, versatile, and multifunctional, offering cost-effective solutions for water and wastewater treatment (Kunduru *et al*., 2017; Henry *et al*., 2019). Silver nanoparticles, which are commonly used in the size <100 nm, are a prime example of nanoparticles. These nanoparticles consist of metallic silver (Ag^0) core functionalized with organic or inorganic coatings to prevent aggregation and improve stability (Gul *et al*., 2025). Silver nanoparticles are synthesized in various shapes including spheres, rods, triangles, or wires. Some metallic nanomaterials can be harmful (Egbuna *et al*., 2021; Soares *et al*., 2021). The toxicity of metallic nanoparticles is primarily determined by their properties and their ability to release reactive oxygen species (Egbuna *et al*., 2021). Toxic nanoparticles release reactive oxygen species, causing damage to cell membranes and leading to cell apoptosis (Nie *et al*., 2023). Therefore, it is essential for nanomaterials used in water treatment to be environmentally friendly, non-toxic, and safe for human health (Kunduru *et al*., 2017). The synthesis of silver nanoparticles using aqueous extracts from medicinal plants is considered environmentally friendly, cost-effective, and

readily available (Sreeprasad *et al*., 2013; Henry *et al*., 2019). However, there is limited information on the use of nanoparticles derived from medicinal plants in water treatment (Henry *et al*., 2019). Further research in this area is needed to explore the potential of this promising and non-toxic technology for improving drinking water treatment.

2.9 Research Gap

Despite the high groundwater usage and frequent outbreaks of waterborne diseases in Ifakara town, regular monitoring of groundwater quality, as recommended by the WHO (2016), is not conducted. The available data on groundwater quality in Ifakara town is limited to initial measurements taken right after the construction of water abstraction facilities to ensure the safety of the water supply for public use. This lack of comprehensive and frequent monitoring hampers effective management and restoration of groundwater quality, as well as the development of appropriate policies for safeguarding groundwater resources in Ifakara town. Previous studies in Ifakara town have primarily focused on faecal pollution levels in relation to well designs (Kilungo *et al*., 2018) without considering geological and physico-chemical characteristics of groundwater or identifying sources of faecal indicator bacteria, making it challenging to address water quality issues and combat waterborne diseases. Moreover, these studies did not assess the sanitary conditions of water abstraction facilities and their surroundings, which are crucial for understanding contamination risks and implementing effective management strategies.

The current prevalence of waterborne diseases in Ifakara town indicates the potential presence of microbial pathogens in groundwater, exacerbated by the lack of affordable disinfection methods. Therefore, an integrated study is needed to evaluate groundwater pollution, identify pollution sources, assess the link between groundwater quality and sanitation, and develop cost-effective disinfection solutions to improve water quality and public health.

CHAPTER THREE

METHODOLOGY

3.1 Study Area

Ifakara town lies between longitudes 08°15’0’’S and 07°5’0’’S and latitudes 36°30’0’’E and 36°55’0’’E (Figure 3.1) at 270 m above sea level, within the Kilombero catchment, covering an area of 537 km^2, and representing a typical lowland flood plain wetland. The population within this town is approximately 290,424 and annual increase of 2.7% (NBS, 2022) and people usually live in a compound with either one or more houses.

Despite being occupied with savannah grassland with natural grass fields, evergreen banks of the Kilombero River and cultivated land (Nindi *et al*., 2014), the area is regarded as the trading centre for Kilombero, Ulanga, Malinyi and Mahenge districts. Economic activities are centred on agricultural trade, farming and provision of higher education, especially in the medical fields (Msofe *et al*., 2019). As a consequence, Ifakara town is undergoing rapid changes in its built environment and social structure, with people from more than 70 ethnic groups currently residing in the town, 60% of whom are in-migrants to the area (Geubbels *et al*., 2015).

According to Msofe *et al*., (2019), the climate in Ifakara town varies between the highlands and lowlands and between the dry and wet seasons. In Ifakara the temperature has an annual mean of 24 ^{o}C. Wet season average rainfall is 198 mm/month compared to the dry season average of 21 mm/month. Increasing temperatures and changing precipitation patterns are expected to cause increased evapotranspiration, reduced runoff and reduced groundwater recharge.

Information regarding geology of the study area is only available from the geological map by the Geological Survey of Tanganyika (1962). With respect to this map, the metamorphic hard rocks building the mountain fringe are represented by neoproterozoic migmatitic quartzo-feldspathic gneisses and pyroxene granulites. In the extreme northwestern section of the study area, garnet-bearing biotite gneisses are present. These metamorphic rocks are geologically isolated from the sedimentary basin by a normal fault system that formed during rifting. Two major faults traverse the study site in a west-east orientation, creating a tilted block faulting structure with a northern tilt (Whittingham, 1960). .

According to the Burghof *et al*., (2017), the sedimentary basin's fill can be categorized into two types: non-alluvial sediments, which extend along the mountain fringe and the tilted block situated between the two major faults, and alluvial sediments found in the south. In the central and western portions of the basin, the non-alluvial sediments primarily consist of pale, locally ferruginous sands. In contrast, the eastern part of the basin and the depressions along the mountain fringe feature red-brown and sandy earth overlays on the hard rocks (Whittingham, J. K. (1960). Along the southern fault, there are isolated occurrences of ferruginized cemented sands and gravels. These non-alluvial sediments date back to the Neogene era and once covered the entirety of the Kilombero Valley but have been eroded extensively over time. The Quaternary alluvial sediments, which underlie the entire study site, are described as alluvial sands, silts, and clays without further differentiation (Figure 3.2).

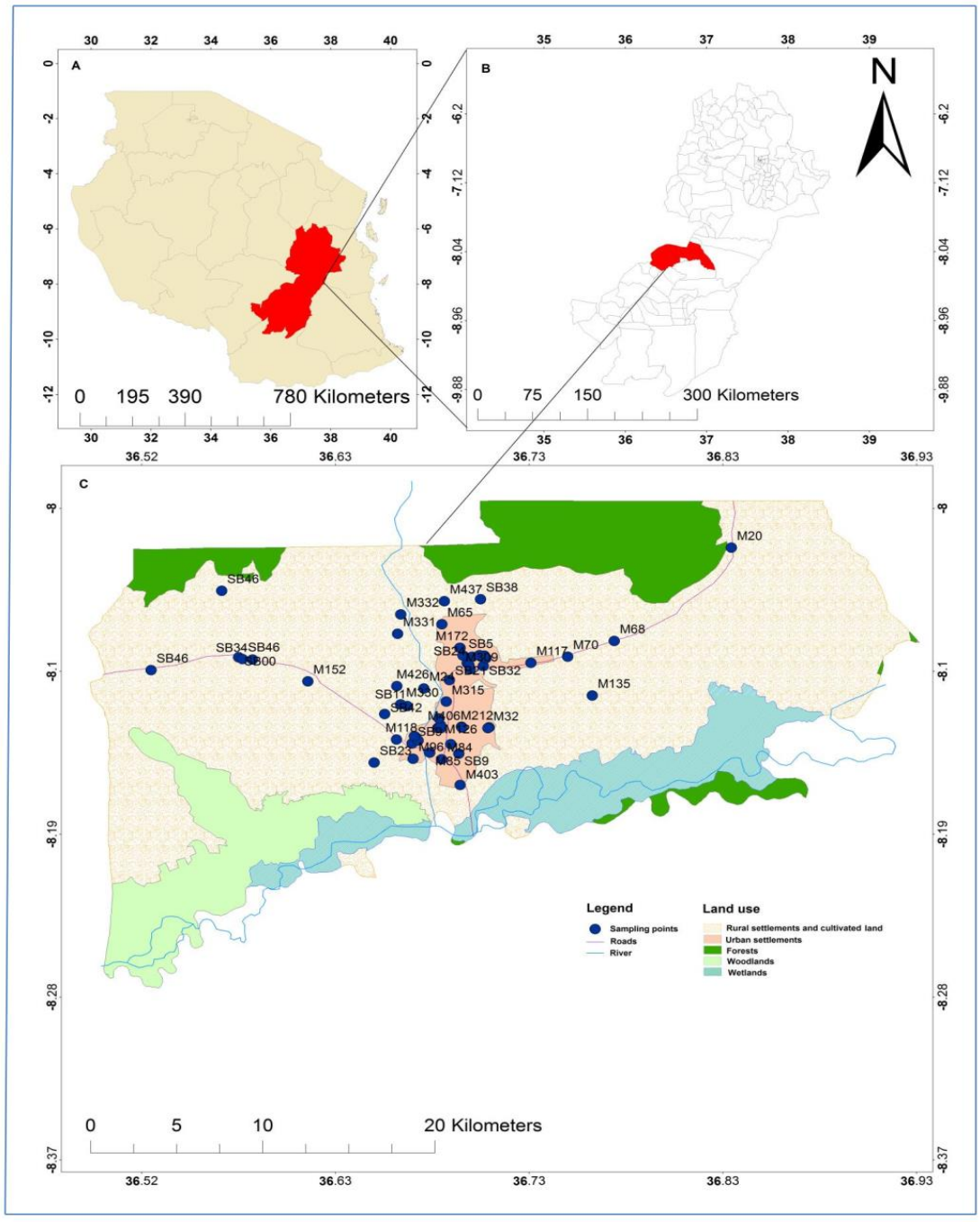

Figure 3.1: A Map of Ifakara Town Showing the Sampling Sites in Relation to the Land Use Characteristics

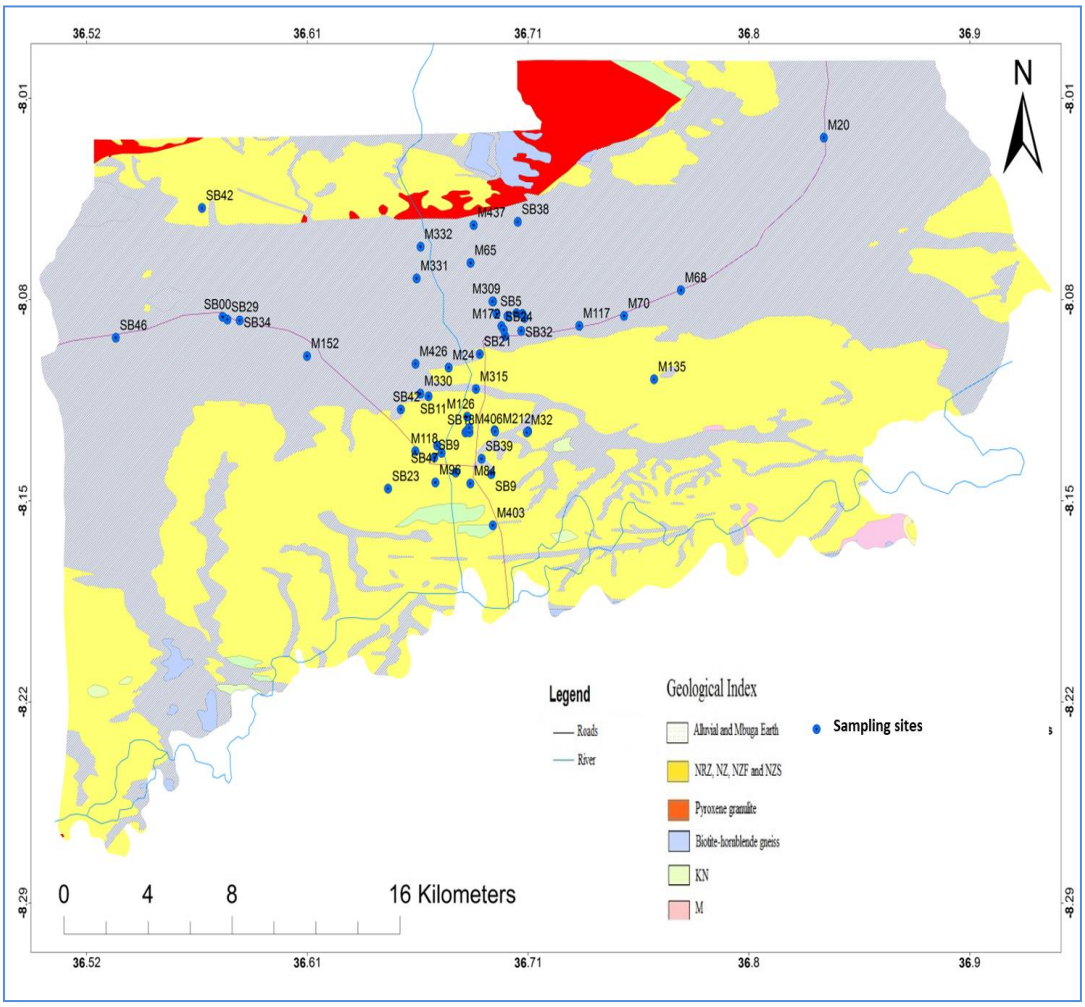

Figure 3.2: A Map of Ifakara Town Showing the Location of Sampling Sites in Relation to the Geological Characteristics (Whitigham, 1960)

3.2 Research Design

In order to gain a better understanding of groundwater pollution in Ifakara town, the selection of sampling sites took into account the design of groundwater abstraction facilities, land use patterns, and geological characteristics of the area. The study area featured various types of groundwater abstraction facilities, including open shallow wells, deep motorised boreholes, and handpump borewells. Open shallow wells and deep motorised boreholes were present in limited locations within the study area and

did not reflect the diverse land uses and geological characteristics of Ifakara town. Therefore, these types of groundwater abstraction facilities were not included in this study. On the other hand, hand-pump bore wells were widely distributed across different land uses and geological formations of the study area with similar design. By omitting the non-functional and unaccessible hand-pump bore wells, a total of 54 functional hand-pump bore wells with similar designs, located in various land uses and geological formation, were selected for this study (Figure 3.1; 3.2; 3.3). It is worth noting that previous studies reported that different designs of groundwater abstraction facilities have distinct levels of faecal indicator bacteria (Kilungo *et al*., 2018). Therefore, this variation was minimised by selecting groundwater abstraction facilities of similar design. The latitude and longitude coordinates of each sampling site were recorded, and data on the sanitary conditions of each hand-pump bore well and its immediate surroundings were collected using a standardized questionnaire developed by WHO (2018) and utilized by groundwater users worldwide to assess the risk of groundwater contamination.

To minimize the impact of rainfall on groundwater quality (Howard *et al*., 2003), on-site measurements and water sampling for laboratory analysis were conducted during the period of no rainfall (June and July). For microbiological analysis, a grab sample was taken from each of the 54 selected hand-pump bore wells by using sterile wide-mouthed polyethylene bottles. It is worth noting that hand pumping of the groundwater was done three times before water sampling to avoid any contamination from hand-pumping device. All samples were stored at 4 ^{o}C and transported to the laboratory in Ifakara town for analysis within 6 hours of sampling.

Figure 3.3: A plate showing the representative of a functional hand-pump bore-well in Ifakara town selected for water sampling

3.3 Data Collection

3.3.1 In Situ Measurements of Temperature, Electrical conductivity and pH

Measurements of temperature, electrical conductivity (EC) and pH were performed *in situ* with potentiometric probe meter (Wagtech^{TM}, Germany). Probes were calibrated by using standard buffer with pH of 4, 7 and 10 at 25°C before sampling and calibration was verified upon returning from the field.

3.3.2 Water sampling for physico-chemical analysis

For analysis of physico-chemical parameters in the laboratory, three samples were taken from each of the 54 selected sampling sites, resulting in a total of 162 water samples. The collected water samples were stored in a cool box with ice packs and

transported to the laboratory of the Department of Molecular Biology and Biotechnology at the University of Dar es Salaam for analysis of physic-chemical parameters.

3.3.3 Laboratory Measurements of Physico-chemical Parameters

Total suspended solids (TSS) were analyzed using the gravimetric method (2540D) as specified by the American Public Health Association (APHA, 2017). Chemical parameters (nitrate, phosphate, ammonium ion, arsenic, fluoride) were measured according to standard methods for water and wastewater analysis established by the American Water Works Association (AWWA) and the United States Environmental Protection Agency (US-EPA). Nitrate levels were determined using an ultraviolet-visible spectrophotometer (HACH DR/2010). Fluoride was measured using the Ion-Selective Electrode Method, while phosphate levels were determined using a colorimetric method with a UV/Vis spectrophotometer at a wavelength of 880 nm. Ammonia levels were determined using the Ammonia-Selective Electrode Method. For arsenic analysis, a test tube containing 16.0 mL of groundwater sample was acidified with 2.0 mL of concentrated hydrochloric acid, followed by the addition of 2.0 mL of a pre-reducing solution (potassium iodide). The mixture was thoroughly mixed and allowed to stand for 1-2 hours at room temperature before analysis using Esel Atomic Absorption Spectrometry SBW/1200. It is important to mention that arsenic was part of this study because it is classified as a Group 1 carcinogen along with beryllium, cadmium, chromium, and nickel (Zhu and Costa, 2020). Among these substances, arsenic is a major contributor to cancer in millions of people globally (Kumar *et al*., 2021).

3.3.4 Measurement of Faecal Indicator Bacteria in the Laboratory

Faecal indicator bacteria were analyzed from the collected water samples after internal quality control measures in order to monitor contamination during collection, transportation and processing of samples. In this study, WG5 strain of *Escherichia coli* and sterile water were used as positive and negative control, respectively. Growth of colonies on negative control reflected failure of the sterilization process and samples analysed were considered invalid. Besides, laboratory blanks and field blanks were tested for the possibility of contamination during sample collection and transportation. In this study coliphages, *enterococci*, *Escherichia coli* and total coliforms were determined in each of the 54 water samples collected from 54 hand-pump bore well using the standard procedures described in detail by Mushi *et al*., (2012).

3.3.5 Determination of *Escherichia coli* and Total Coliform in Groundwater

The load of *E. coli* and Total coliform in the collected water samples were determined by using membrane filtration method (Harwood, *et al*., 2011). Vacuum hand pump filtration was used to filter 100 mL of water sample through filter paper with 0.45 µm pore size and 47 mm diameter (Wagtech^{TM}, Germany). The filter was transferred to a sterile plate containing Chromocult Coliform Agar and incubated at 37 °C for 24 hours. After incubation, the presence of blue colonies signifies *E. coli* while pink/purple colonies were regarded as Total Coliforms (Figure 3.4). All *E. coli* and Total Coliform colonies were counted and reported as CFU/100 mL (EPA, 2017).

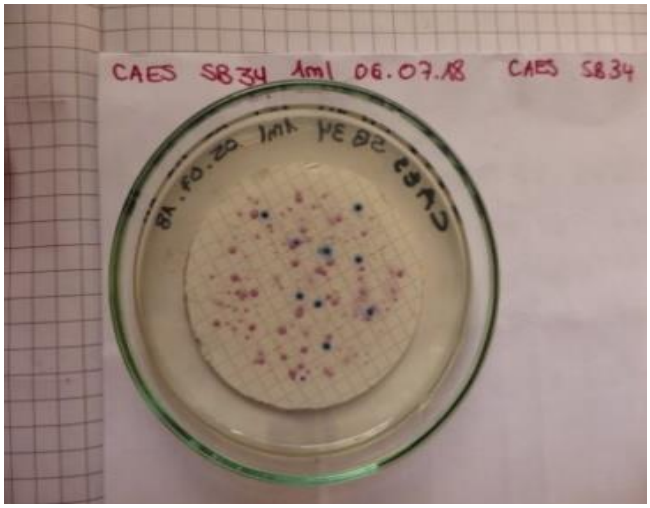

Figure 3.4: A plate showing *E. coli* (blue colonies) and total coliform (pink/purple) detected in the groundwater of the investigated hand-pump bore wells

3.3.6 Determination of *Enterococci* in the Groundwater

Enterococci were analyzed by using membrane filtration method as described by Harwood *et al*., (2011). 100 mL of the water sample was passed through a filter membrane (Wagtech Company) of spore size and diameter of 0.45 micrometre and 47 mm, respectively. The filter was placed in a sterile petri-dish containing sterile *Enterococci* Agar. The plates were incubated at 37 ^{o}C for 24 hours. After incubation, growth of pink colonies indicated the presence of *Enterococci*. For confirmation, membrane filters with pink colonies were placed on bile asculine agar plate and incubated at 37 ^{o}C degrees for 1 to 2 hours and dark brown or black complex colonies indicated the presence of *Enterococci* (Figure 3.5). After confirmation, the colonies grown on the bile asculine agar were counted manually and the concentration was reported as colony forming units per millilitre (cfu/100 mL).

Figure 3.5: Plate showing dark brown colonies of *Enterococci* detected in the water samples of investigated hand-pump bore wells

3.3.7 Determination of Coliphages in the Groundwater Samples

The procedure involved incubating the *E. coli* in nutrient broth at room temperature in a shaker for a period of 3 to 4 hours. The presence of turbidity in the broth indicated the growth of *E. coli*. Subsequently, 100 mL of the sample was blended with 10 mL of *E. coli* culture and 110 mL of modified Shorten Agar, and this mixture was poured into plates where it was allowed to solidify. Once solidified, the plates were placed in an incubator at 37 degrees Celsius for duration of 24 hours. Coliphages, which had developed as a clear zone on plates containing Modified Shorten Agar (Figure 3.6), were manually counted and their concentration was expressed as plaque-forming units per 100 mL (PFU/100 mL) (Harwood *et al*., 2011).

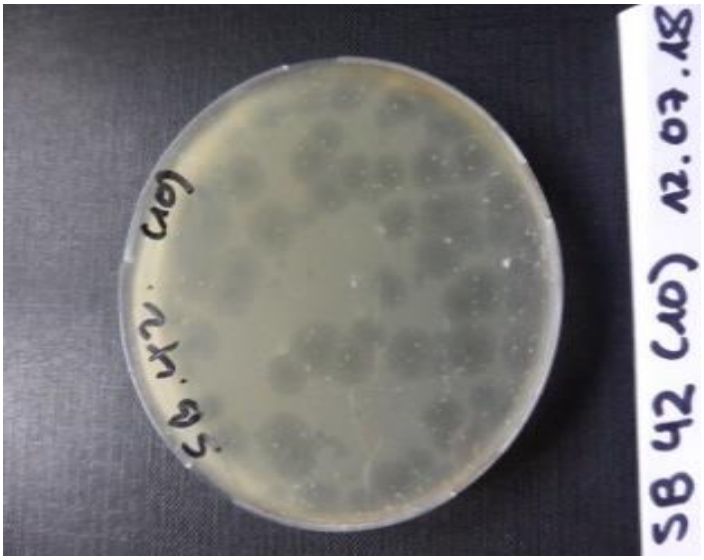

Figure 3.6: Plate showing clear zone of Coliphages detected in the groundwater samples of the investigated hand-pump bore wells

3.3.8 Determination of Sanitary Characteristics of the Hand-pump Bore wells

As part of the comprehensive risk-based assessments to improve drinking water quality, WHO recommended sanitary inspections of groundwater abstraction facility to support the maintenance and operation of the water source and provide clear guidance and remediation proceedings on the improving and protection of groundwater (Mushi *et al*., 2012; Pantaleo, 2019). Therefore, in this study, a cross-sectional sanitary risk inspection of hand-pump bore wells were assessed based on the standardized protocol defined by WHO (2016). This assessment involved an on-site sanitary inspection of each of the 54 selected hand-pump bore wells and its surrounding environment using a standardized sanitary inspection protocol to identify actual and potential risk of contamination. This protocol contains eleven standardized optional questions designated for assessing sanitary condition of each hand-pump bore well. For each "yes" answer (risk observed) to the question in the protocol, a score of one point was awarded and for each "no" answer (no risk

observed) to the question in the protocol, zero score was awarded. Total score was obtained by summing all scores obtained for “yes” which provided the overall assessment of the sanitary profile of each hand-pump bore well. A higher score is believed to represent a greater risk that groundwater is contaminated (Pantaleo 2019). The total sanitary risk score (%) was calculated by dividing sum of all the questions with a “yes” answer with total number of questions multiplied by 100.

3.3.9 Microbial Source Tracking in Groundwater of Ifakara Town

The microbial source tracking (MST) method was used to determine the source(s) of faecal indicator bacteria (Reischer *et al.,* 2006: Haugland *et al.,* 2010: Harwood, *et al*., 2011) detected in the groundwater of Ifakara Town. MST was applied in water samples contaminated with *E. coli*, Total coliform or *Enterococci*. About 200 mL of groundwater sample was filtered using a polycarbonate filters with a pore size of 0.2 µm. These filters were placed into Eppendorf tube and kept at 4 ^{o}C before being shipped to Bonn University, Germany for DNA extraction and real time qPCR determination. Two human faecal samples, two piglets and two calf anal smears were used as positive control. Extraction of DNA from polycarbonate filters and the control samples were done by Aquadien DNA extraction and purification kit (BioRad PLC USA) following the manufacturer’s instructions. DNA concentration was measured using the AQ-07 Nucleic Acid Meter (AmpliQuant, USA), by diluting the sample 1:1 with Aquadien Elution Buffer (BioRad, USA) and adding 10 µL to the micro-well plate. Quantification of DNA took place at 260 nm as programed for dsDNA by the manufacturer.

3.3.10 Real Time Quantitative Polymerase Chain Reaction

Quantitative polymerase chain reaction (qPCR) was monitored on a Light Cycler 480 II (Roche, CH). The reaction mixture consisted of 12.5 µL Maxima Probe qPCR Master Mix (2x) with separate ROX (Thermo Fischer, USA), 0.3 µM forward and reverse primers targeting faecal specific *Bacteroides* species, 0.2 µM Taq-Man probe <500 ng DNA and water to a final volume of 25 µL. Primers and probes used in this approach are described in Table 3.1. The thermal cycler conditions were as follows: 95°C for 10 min, followed by 45 cycles of 95 °C for 15 s and 60 °C for 60 s. After the final amplification a melting curve analysis was performed, with the following thermal conditions: 95 °C for 15 s, 60 °C for 30 s and 95 °C for 15 s. In order to check the performance of the faecal markers, a qPCR with the positive controls was carried out first. All possible cross-reactions were also tested by pairing each marker with each positive control. For the analysis of samples with a cycle threshold (Ct), values <40 obtained by using qPCR software were considered positive (Harwood, *et al*., 2011).

Table 3. 1: Faecal markers used for the detection of faecal specific bacteroides species in groundwater samples.

Primer's	5'-3'
BacR-P LNA	FAM-CTTCCGAAAGGGAGATT-NFQ-MGB
Pig2Bac-P LNA	FAM-TCCAGGGATAGCC-NFQ-MGB
HF183-P	FAM-CTGAGAGGAAGGTCCCCCACATTGGA-TAN-MRA
BacR-F	GCGTATCCAACCTTCCCG
BacR-R	CATCCCCATCCGTTACCG
PiG2Bac-F	GCATGAATTTAGCTTGCTAAATTTGAT
PiG2Bac-R	ACCTCATACGGTATTAATCCGC
HF183-F	ATCATGAGTTCACATGTCCG
HF183-R	CGTAGGAGTTTGGACCGTGT

3.3.11 Fabrication of Nano-Disinfectant for Groundwater Treatment

3.3.11.1 Collection of Plant Materials and Preparation of Aqueous Extracts

Cassia abbreviata is a small-to-medium-sized branched tree belonging to the family Fabaceae. It is widely spread in the tropics, especially in Southeastern Africa, with a long history in traditional medicine for the treatment of numerous conditions, such as headaches, diarrhea, constipation, some skin diseases, malaria, syphilis, pneumonia, stomach troubles, uterine pains, and gonorrhea (Zheng *et al*., 2021). The secondary bioactive metabolites present in *C. abbreviata* plant such as alkaloids, flavonoids, tannins, phenols, coumarins and steroids (Raman Ibrahim *et al*., 2022) make this plant suitable for reducing metallic ions to stable nanoparticles that have strong antimicrobial activity.

The fresh leaves (CA-FL), stem bark (CA-SB) and root bark (CA-RB) of *C. abbreviata* plant (Figure 3.7) were collected from Dar es Salaam Region (6° 48′ S, 39° 17′ E) in Tanzania after taxonomic identification by an expert from the Department of Botany, University of Dar es Salaam (UDSM), Dar es Salaam, Tanzania. The voucher specimen (No. AAM 01) was deposited in the Herbarium of the Department of Botany, UDSM. Samples were washed thoroughly with sterile distilled water and dried under shade on clean drying tables at room temperature. The dried materials were cut into small pieces of approximately 1-2 cm^2.

Figure 3.7: *Cassia abbreviata* plant from which root, stem bark and leaf samples were obtained for the preparation of aqueous extracts

To prepare the aqueous extract, 100 g of the respective sample was combined with 100 mL of sterile distilled water in a clean, oven-dried 300 mL Erlenmeyer flask. The mixture was heated for 30 minutes at a temperature of 60 °C. It is important to highlight that this boiling process mirrors the traditional method used by healers for preparing extracts before treating ailments. After boiling, the extract was filtered using no.1 filter paper and stored at 4 °C until further analysis.

3.3.11.2 Biosynthesis of Silver Bionanoparticles

Aqueous extract (2 mL) of *C. abbreviata* was added dropwise into 10 mL of 0.0025 M $AgNO_3$ (Merck, Darmstadt, Germany) under magnetic stirring condition. The resultant mixture was kept at a room temperature until deep brown color was observed. This change in color preliminarily indicated the formation of colloidal silver bionanoparticles (SBNs) as there was no color change observed in the silver

nitrate solution or aqueous extracts after incubating them under condition similar to that of the colloidal SBNs.

3.3.11.3 Characterization of Silver Bionanoparticles

To confirm the formation of colloidal suspension of biosynthesized SBNs, optical density values of the colloidal solution were determined. This analysis was conducted using a SPECTROstar Nano microplate reader (BMG Labtech GmbH, Allmendgruen, Ortnberg, Germany) over a wavelength range of 290 to 790 nm within a 10-minute time frame, following the manufacturer's protocol. For the assessment of the surface morphology of phytosynthesized SBNs, Atomic Force Microscopy (AFM) was employed following the procedure described by Masalu *et al*., (2020). In brief, a Scanning tip AFM (NSC36, Mikro-Masch, Poland) was utilized with a nominal spring constant of 1.0 nN nm-1 and a resonance frequency of 90 KHz. Approximately 10 µL of the SBNs complex (0.4 mg/mL) was applied to freshly cleaned negatively charged mica, and the solution was allowed to dry on a sterile glass plate. Atomic force microscopy imaging was conducted under dry conditions in non-contact mode, with a scanning area of 5.0 µm.

3.3.11.4 Testing Silver bionanoparticles for Antimicrobial Activity

Silver bionanoparticles antibacterial activity was determined using well diffusion method as per published protocols (Divya *et al*., 2019). Briefly, suspensions of reference pathogenic bacteria including *Escherichia coli* ATCC 25922 (*E. coli*), *Pseudomonas aeruginosa* ATCC 27853 (*P. aeruginosa*), *Staphylococcus aureus* ATCC 25923 (*S. aureus*) and *Enterococcus faecalis* ATCC 51299 (*E. faecalis*)

adjusted to 0.5 McFarland standard were separately prepared in screw caps test tubes using sterile phosphate-buffered saline (Gunti *et al*., 2019). A volume of one milliliter from the standard suspension (approximately 10^6 cfu/mL) of each reference bacterial strain was evenly spread onto separate Muller Hinton agar (MHA) plates using a sterile glass rod spreader. Subsequently, 6 mm diameter wells were created on 4 mm thick Muller Hinton agar plates containing a reference bacterial lawn using a sterile cork borer. Into these wells, 50 µg/mL of SBNs was carefully pipetted. As a standard control, gentamycin (16 µg/mL) was loaded, while silver nitrate solution (16 µg/mL) and *C. abbreviata* extracts (500 µg/mL) were loaded separately as controls. The plates were then incubated at 37°C for 24 hours to facilitate bacterial growth, following a one-hour period at room temperature to allow for the diffusion of SBNs into the agar. After incubation, the clear zones of inhibition were measured and recorded in millimeters. This experiment was repeated three times to obtain the average size of inhibitory zone.

3.3.11.5 Minimum Inhibitory Concentration of Silver Bionanoparticles

The minimum inhibitory concentration (MIC) of SBNs was performed as described by Masalu *et al*., (2020) using sterile 96-well microtiter plate and standard broth microdilution methods. In brief, different concentrations of SBNs and 10 µL of reference bacterial culture of 0.5 McFarland standards were added to the wells of 96-well microtiter plates and final volume was adjusted to 100 µL with Muller Hinton broth. The plates were incubated at 37 °C for 24 hours and optical density at 600 nm was assessed using the spectrophotometer. The lowest concentration of SBNs that inhibits the growth of bacteria was taken as the MIC value.

3.3.11.6 Testing Disinfection Efficacy of Silver Bionanoparticles

Water disinfection efficacy of SBNs was tested by separately adding 0, 0.58, 1.17, 2.34, 4.69, 9.37 mg/mL of SBNs to individual samples (n = 3) of water contained a mean planktonic coliform bacteria of $1.51 \times 10^5 \pm 5 \times 10^3$ cfu/100 mL that was collected from Ngerengere river (coordinates 6.8S, 37.6E) in Morogoro, Tanzania. It should be noted that the experiment utilized river water due to its high faecal coliform bacteria levels, ideal for measuring SBNs' reduction of colony forming units. Groundwater was unsuitable for this experiment due to its low faecal coliform bacteria levels. The mixture was constantly shaken for one hour at room temperature. One hundred microliter (100 µL) of the mixture was spread in triplicate onto m-Endo agar (DIFCO, Michigan, U. S. A.) plates. Plates were then inverted and incubated at 37 °C for 24 hours. Simultaneously, control plates with m-Endo agar were spread with 100 µL of river water and incubated in the condition similar to that of the plates inoculated with a mixture of SBNs and river water. After incubation, colonies of coliform bacteria were counted and expressed as colony forming units per 100 mL (cfu/100 mL). The data were used to determine the ability of SBNs in reducing the number of coliform bacteria in the investigated river water. On the contrary, to determine time kill, SBNs concentrations of 0.58, 1.17, 2.34, 4.69, 9.37 mg/mL were separately added into the river water samples contaminated by coliform bacteria ($1.51\times10^5 \pm 5\times10^3$ cfu/100 mL) and agitated at 150 rpm. One hundred microliter from each of the treated water samples was separately spread on m-Endo agar plates at time 0.1, 0.2, 0.5, 1, 2 and 3 h. The number of colonies on the m-ENDO agar plates was quantified in cfu/100 mL after incubation at 37°C for 24 hours. The

experiment was carried out in triplicates and the resultant data were used to construct time-kill curves of coliform bacteria in the contaminated river water.

3.4 Data Analysis

The collected data set were loaded into Microsoft Excel version 2010, and inferential statistics including mean, median, minimum, maximum, standard deviation, skewness and kurtosis were calculated for both physicochemical and microbiological parameters. Spearman correlation matrices for both sets of parameters were generated using Primer-E software. A clustering dendrogram of physicochemical parameters was created using complete linkage coupled with log(x+1) transformed Eucldean distance. Additionally, the correlation between the sanitary conditions of hand-pump bore wells and faecal indicator bacteria dataset was quantified using a weighted Spearman correlation test. The physicochemical and microbiological parameters of water quality were compared to the WHO (2011) and TBS (2018) standards for drinking water to generate information regarding the status of the groundwater quality in Ifakara town.

Triplicate values of zone of inhibition, MIC and number of coliform bacteria were presented as Means ± SD. Infographics of zone of inhibition, inactivation of faecal indicator bacteria by silver bionanoparticles were performed in Microsoft Excel version 2010. SPSS software version 16.0 (SPSS, Inc. Chicago, IL, USA) was used to determine the significant levels in bactericidal activity between biosynthesized SBNs and aqueous extracts of *C. abbreviata* employing Mann-Whitney U test. A *P* value of <0.05 was considered as significant.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Level of Physico-chemical Parameters in the Groundwater

The groundwater samples collected from handpump borewells in Ifakara town were analyzed for 9 physico-chemical parameters, including pH, temperature, EC, TSS, fluoride, arsenic, NH_4^+, NO_3^-, and PO_4^{3-}. The basic descriptive statistics of these physicochemical properties and the maximum admissible levels based on local and international drinking water quality standards are summarized in Table 4.1. The mean temperature of the groundwater was 27.5 ± 1.5 °C, while the pH was 6.7 ± 0.4. TSS, fluoride, and arsenic had mean values of 3.5 ± 3.6, 0.26 ± 0.18, and 0.007 ± 0.019 mg/L, respectively. NH_4^+, NO_3^-, and PO_4^{3-} were also detected in the investigated groundwater, with recorded mean values of 0.03 ± 0.05, 1.5 ± 4.6, and 0.04 ± 0.1 mg/L, respectively (Table 4.2). Except temperature and pH, the remaining parameters showed high variations among sampling sites but not between replicate samples, reflecting geological differences existing in the studied environment (Figure 3.2). Excluding temperature, which had no permissible value according to WHO and TBS, the mean values of the other analyzed physico-chemical parameters were within the range of WHO and TBS permissible levels for drinking water (Table 4.2), indicating good quality in 100% of the analyzed samples from 54 handpump borewells. EC classifies the investigated groundwater as freshwater (EC <1500 mg/mL), reflecting low degrees of mineralization related to water-rock interaction and indicating little water transit within the aquifer (Marrugo Negrete *et al*., 2024). These findings are similar to those of previous studies conducted in the Dar es Salaam (Mdoe and Buchweishaija, 2014) and Tanga (Hadija and Chove, 2020)

regions but differ significantly from those reported in the studies conducted in the northeastern part of Tanzania with respect to fluoride levels (Pantaleo *et al*., 2018; Makoba and Muzuka, 2019; Malago *et al*., 2017). This difference may be explained by the geological differences between the two regions, as the northeastern part of Tanzania is dominated by basalt and volcanic materials rich in fluoride (Chacha *et al*., 2018), whereas the geology of Ifakara town is characterized by rocks like neoproterozoic migmatitic quartzo-feldspathic gneisses and pyroxene granulites, which contain low levels of harmful chemical substances like fluoride. Similarly, the observed low level of nitrate in this study differs from that reported in Manyara, Arusha, Babati, and Dodoma (Pantaleo *et al*., 2018 and 2019; Elisante and Mzuka, 2017). The elevated nitrate reported in these studies was attributed to pollution from septic tanks, animals, human waste, and agricultural activities (Agarwal *et al*., 2019; Lwimbo *et al*., 2019; Hamad *et al*., 2021). These polluting sources had no effect in Ifakara groundwater probably due to properly constructed handpump borewells and ability of the underlying geology in filtering the athropogenic pollutants. On the other hand, phosphate detected in the Ifakara groundwater showed a contrasting result with the study conducted by Kisaka (2018) in Dodoma where the phosphate concentration of the water from the borewells were higher than that of Ifakara town. These contrasting findings can be explained by differences in environmental conditions existing among locations in which the previous and present works were conducted (Kisaka, 2018; Chacha *et al*. 2018).

Table 4.1: Levels of Physico-Chemical Variables of the Investigated Groundwater in Relation to the WHO and TBS Standards

Variables	Mean ± SD	WHO standard	TBS standard
Temperature (°C)	27.5±1.5	-	-
pH	6.7±0.4	6.5-8.5	5.5-9.5
Conductivity (*µS/cm*)	326.6±149	-	2500
TSS (mg/L)	3.5±3.6	>45.0	
Fluoride (mg/L)	0.26±0.18	>1.5	>1.5
Arsenic (mg/L)	0.007±0.019	>0.05	0.01
Ammonia (mg/L)	0.03±0.05	>1.5	0.50
Nitrate (mg/L)	1.5±4.6	45.0	45.0
Phosphate (mg/L)	0.04±0.1	>0.3	2.2

The Spearman statistical test was used to quantify the correlation between the analyzed physicochemical parameters in groundwater samples collected from 54 handpump borewells, and the correlation coefficients are presented in Table 4.2. A strong positive correlation was observed between electrical conductivity and pH (r=0.65, $P<0.05$), while moderate positive correlations were found between electrical conductivity and nitrate (r=0.47, $P<0.05$), and electrical conductivity and phosphate (r=0.48, $P<0.05$). Additionally, moderate significant correlations were identified between arsenic and temperature (r=0.37, $P<0.05$), arsenic and TSS (r=0.28, $P<0.05$), arsenic and nitrate (r=0.37, $P<0.05$), nitrate and ammonia (r=0.33, $P<0.05$), phosphate and ammonia (r=0.43, $P<0.05$), as well as phosphate and nitrate (r=0.35, $P<0.05$). The remaining Spearman's correlation coefficients were weak and not significant (Table 4.2). The presence of strong, moderate, and weak correlations among the analyzed physicochemical parameters suggests that these parameters have different mechanisms of release (chemical weathering and hydrolysis), which may vary due to the diverse geological settings in Ifakara town. Furthermore, the varied patterns of correlation coefficients among the physicochemical variables indicate that

these parameters may originate from distinct geological settings given the significant geological variations occurring in the studied area. Therefore, geological processes in Ifakara town likely play a key role in determining the physicochemical properties of groundwater, consistent with previous research findings (Ojecunle *et al*. 2020; Mushi *et al*. 2012).

Table 4.2: Spearman Correlation Coefficient Matrix for Physico-Chemical Parameters Determined in Water Samples of Studied Handpump Borewells. TSS, Total Suspended Solids. Significant (*P*<0.05) Spearman Correlation Coefficients are Bolded

	Temperature	pH	Conductivity	TSS	Fluoride	Arsenic	Ammonia	Nitrate
pH	0.03							
Conductivity	0.01	**0.65**						
TSS	0.12	-0.13	-0.17					
Fluoride	0.07	0.01	-0.06	0.09				
Arsenic	**0.37**	0.13	0.15	**0.28**	0.17			
Ammonia	0.08	-0.13	-0.13	-0.06	0.02	0.11		
Nitrate	0.05	-0.08	**0.47**	0.25	0.22	**0.37**	**0.33**	
Phosphate	0.14	0.18	**0.48**	-0.05	-0.01	0.03	**0.43**	**0.35**

Figure 4.1 illustrates two clusters identified from the dendrogram containing 9 physico-chemical variables. Cluster 1 consists of NO_3^-, pH, PO_4^{3-}, arsenic,

ammonia, TSS, and fluoride, while cluster 2 includes electrical conductivity and temperature. These distinct groupings suggest that two factors impact the physicochemical quality of groundwater in the study area. The clustering of fluoride, NO_3^-, pH, PO_4^{3-}, arsenic, and ammonia with TSS in cluster 1 indicates that these substances are released from suspended solids present in the groundwater. The grouping of conductivity with temperature suggests that temperature accelerates the dissolution process of minerals in groundwater, leading to the addition of ions in groundwater, which subsequently affects the electrical conductivity of groundwater. These findings are consistent with those reported by Acero *et al*., (2015).

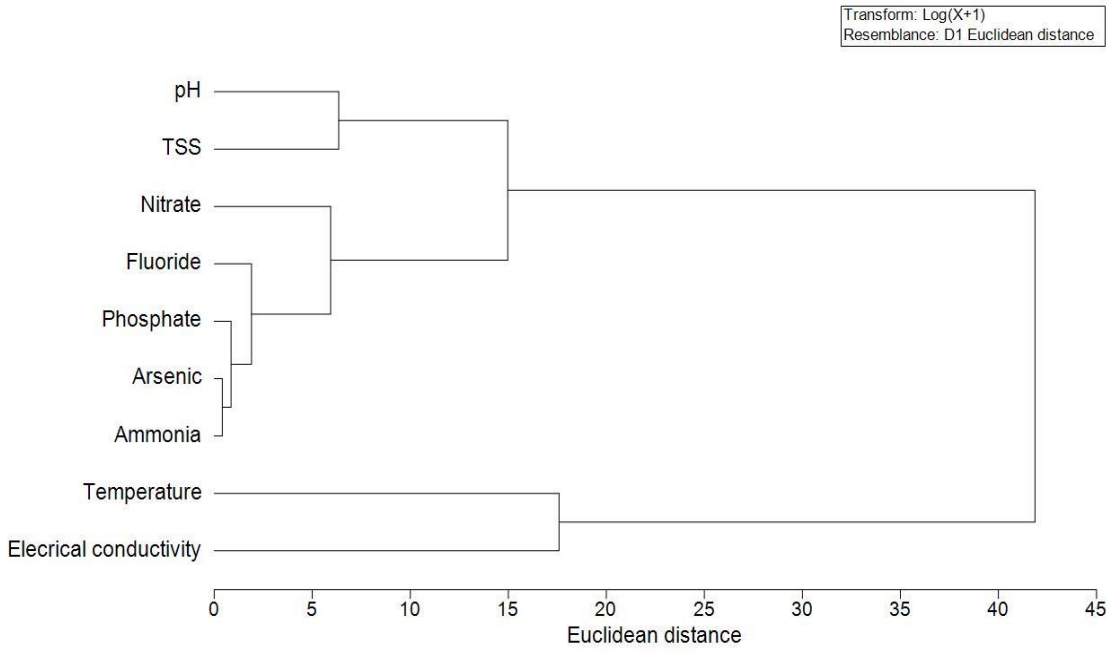

Figure 4.1: Classfication of Physico-Chemical Parameters Using Cluster Analysis. The Displayed Dendogram were Constructed Using Complete Linkage Clustering Coupled with log (X+1) Euclidean Distance Generated from the Physico-Chemical Data Set

4.2 Faecal Indicator Bacteria Retrieved from Groundwater in Ifakara Town

Total coliform was found in 78% of the sampling points, while *Escherichia coli* was only present in 40% of the sampling points. Similarly, enterococci were detected in 48% of the sampling points. It is worth noting that coliphages were identified in only 13 out of 54 total sampling points, and among those 13 sampling points, coliphages were found in 10 of them. Interestingly, 16% of the sampled sites (54) did not show any presence of the tested faecal indicator bacteria during the study period (Table 4.3), indicating that these faecal indicator bacteria are reliable for monitoring water quality in rapidly growing towns like Ifakara. Among the wells where faecal indicator bacteria were detected (84%), total coliform levels ranged from 0 to 48,000 cfu/100 mL with an average of 1,500 cfu/100 mL, while *Escherichia coli* concentrations ranged from 0 to 18,000 cfu/100 mL with an average of 350 cfu/100 mL. *Enterococci* and coliphage levels ranged from 0 to 800 cfu/100 mL and 0 to 600 pfu/100 mL with average values of 40 cfu/100 mL and 180 pfu/100 mL, respectively (Table 4.3).

The presence of faecal indicator bacteria in the hand pump borewells being investigated indicates that the water from this source does not meet the WHO standards for drinking water, which require water designated for drinking to have 0 cfu/100 mL of faecal indicator bacteria. Moreover, the presence of these bacteria in the groundwater suggests that this water may contain pathogenic bacteria that could lead to waterborne diseases, as there is a strong correlation between faecal indicator bacteria and pathogenic microorganisms in contaminated water (Ferguson *et al*., 2012). People in the area are consuming this untreated water, assuming it is free from

contamination, putting them at a high risk of contracting waterborne illnesses. Cases of typhoid fever (D'Acremont *et al*., 2014) and cholera outbreaks (Acosta *et al*., 2001) have been frequently reported in Ifakara town, emphasizing the need for water quality managers in the area to implement effective measures to improve groundwater quality and educate the community on the importance of treating groundwater before consumption to prevent waterborne disease outbreaks.

Table 4.3: Occurrence and Concentration of Faecal Indicator Bacteria in the Groundwater of Ifakara town. TC, Total Coliforms; sd, Standard Deviation.

Statistics	Faecal indicator bacteria (cfu/100 mL or pfu/100 mL)			
	TC (n=54)	*Escherichia coli* (n=54)	Enterococcus (n=54)	Coliphages (n=13)
% of wells with cfu/100 mL	76% (41/54)	31% (17/54)	(48%) 26/54	(77%)10/13
% of wells without cfu/100 mL	24% (13/54)	69% (37/54)	(52%) 28/54	(23%) 3/13
Minimum	0	0	0	0
Maximum	48000	18000	800	607
Mean	1500	350	40	180
Median	23	0	0	120
SD	± 6900	± 2500	± 135	± 206
Skewness	6	7	4	1
Kurtosis	39	53	23	0.05

The Spearman statistical test revealed a significant positive correlation between *Escherichia coli* and total coliforms (r=0.64, $P<0.001$), as well as between *Escherichia coli* and *Enterococcus* (r=0.50, $P<0.05$). Moreover, a significant positive correlation was observed between enterococcus and total coliforms (r=0.50, $P<0.05$).

These findings suggest that the faecal indicator bacteria in the groundwater of Ifakara town likely have a common source. However, there was no significant correlation (r=0.05-0.22, *P*>0.05) between faecal indicator bacteria and the physico-chemical parameters of water quality, indicating different sources of contamination. This conclusion aligns with the results of Mushi *et al*. (2012), despite variations in study design.

Table 4.4: Relationship Between the Sanitary Characteristics of Handpump Borewells and Faecal Indicator Bacteria. TC, Total Coliforms

Sanitary characteristic[ǂ]	Correlation coefficients[†]		
	TC	*E. coli*	*Enterococci*
1. Loose hand pump at the point of attachment	**0.50***	**0.60**	**0.50**
2. Cracked concrete floor	0.44	**0.55**	0.42
3. Inadequate inspection cover	**0.54**	**0.58**	**0.50**
4. Inadequately sealed points of the well wall	0.22	0.37	0.32
5. Headwall absent or inadequate	0.41	**0.55**	0.45
6. Poor drainage causing stagnant water in the wall area	0.36	0.42	0.44
7. Fence absence or faulty	0.37	**0.50**	0.45
8. Latrine, septic tank or sewer line within 30 meters of the well	0.34	0.45	0.38
9. Latrine, septic tank or sewer line on higher ground within 30 m of the well	0.32	0.32	0.37
10. Signs of other sources of pollution within 10 m of the well (animals or human faeces)	0.02	0.22	0.21
11. Open/uncapped well or borewell within 100 m of the well	0.01	0.31	0.22

[†]Weighted Spearman correlation coefficients
[ǂ] Sanitary characteristics of handpump borewells described in WHO (2018)
* Underlined values mean that the correlation is statistically significant at *P*<0.05

In the 54 wells investigated, 11 sanitary characteristics were observed. The most common risk factors identified around the surveyed wells were cracked concrete floors (84%), absence or faulty fences (97%), and the presence of animal and solid wastes within 10 meters of the well (100%). Other risk factors observed had frequencies ranging from 2.2 to 13%. A weighted Spearman statistical test was used to determine the association between these sanitary characteristics and faecal indicator bacteria in the groundwater of Ifakara town. Five sanitary characteristics, including the presence of a loose hand pump, cracked concrete floors, inadequate inspection covers, absence or inadequate headwalls, and absence or faulty fences, were significantly associated with the presence of faecal indicator bacteria in the groundwater (Table 4.5). Further studies are needed to understand how these sanitary characteristics contribute to groundwater contamination. One possible explanation is that faecal indicator bacteria are introduced onto the concrete floor of the hand pump borewell by human or animal contact (Figure 4.2), entering the groundwater through cracks in the concrete floor, loose or broken hand pumps, or unsealed points in the well wall due to poor drainage systems (Figure 4.2). Interestingly, septic tank/pit latrines and animal waste near some wells did not impact the detected faecal indicator bacteria, indicating they were not a significant source of contamination. This was supported by the very low levels of physico-chemical parameters especially electrical conductivity, nitrate and phosphate, in the groundwater of these borewells. The study findings align with previous research on the impact of sanitary characteristics on groundwater quality (Mushi *et al*., 2012; Howard *et al*., 2003) and suggest that poor operation and maintenance of handpump borewells in Ifakara town significantly contribute to faecal contamination of groundwater. Understanding the

sanitary characteristics of hand pump borewells is crucial for identifying pollution sources, implementing effective groundwater quality restoration measures, and developing appropriate policies to ensure safe groundwater supply for public health protection against waterborne diseases.

Figure 4.2: Images Showing Sanitary Characteristics of the Representative of the Investigated Handpump Borewells in Ifakara Town

Land use was also examined as a separate risk factor for groundwater contamination in this study. Through mapping of the Ifakara town using the geographic information system (GIS) revealed distribution of the handpump wells in two main land use types: cultivated land and urban environment (Figure 3.1). Both land uses had handpump borewells with varying levels of faecal indicator bacteria (0 cfu/mL and

>0 cfu/mL) (Figure 4.3). There was no significant difference (P>0.05) in faecal indicator bacteria between the two land uses, indicating that land use did not impact the microbiological pollution of the handpump borewells. This finding contradicts those of the previous studies (Wachira *et al*., 2024) which focused on open shallow wells that are more susceptible to contamination compared to the handpump borewells. The lack of correlation between faecal indicator bacteria and land uses suggests that land use may not be a critical factor to consider when restoring groundwater quality in Ifakara town.

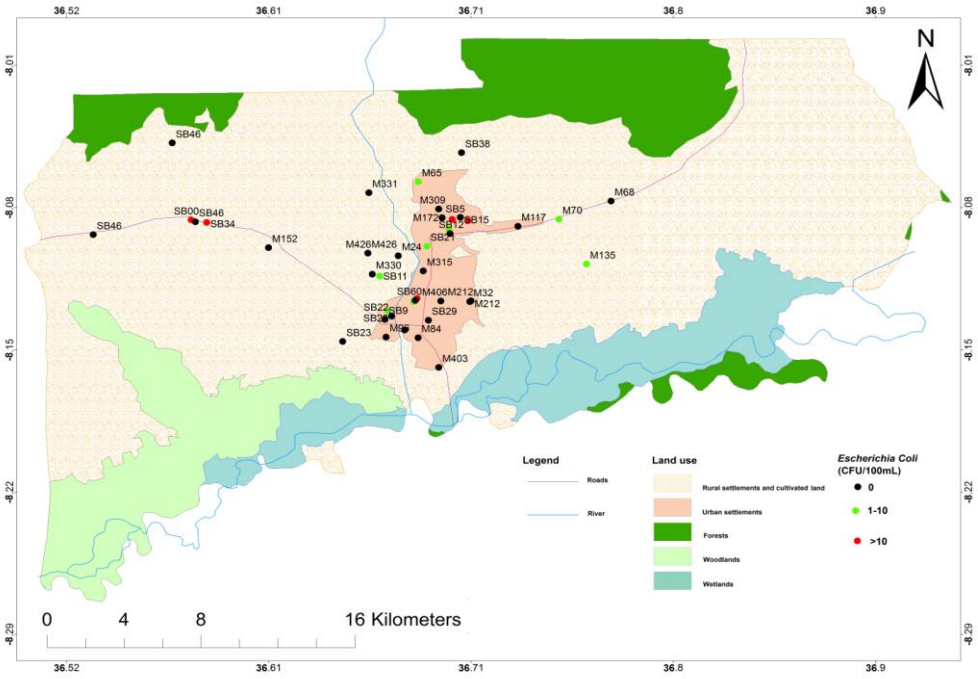

Figure 4.3: Distribution of *Escherichia Coli* Concentration Based on WHO Guidelines in Ifakara Town

4.3 Tracking the Source of Faecal Indicator Bacteria in the Groundwater

In this study, faecal indicator bacteria were found in 25 out of 54 handpump borewells that were examined. Subsequently, water samples from these 25 borewells

were filtered, and DNA was extracted from the filters using the procedure outlined in the methodology section. The DNA concentrations in the water samples from the 25 borewells ranged from 2.92 to 22.83 µg/mL, which were sufficient for conducting quantitative polymerase chain reaction (qPCR) analysis.

4.3.1 Quantitative Polymerase Chain Reaction Positive Controls

The study found that the human faecal marker (HF183) was present in all human faeces and in the faeces of calf 2, but not in piglet faeces. The ruminant faecal marker (BacR) was not detected in any of the samples, indicating its specificity for adult ruminants that feed on plant matter. The pig marker (Pig2Bac) was only detected in the piglet faecal samples. These findings confirm that HF183 and Pig2Bac are specific markers for human/calf and pig faeces, respectively, while BacR is specific to adult ruminant faeces (Reischer *et al*., 2006; Haugland *et al*., 2010; Harwood *et al*., 2011). The cycle threshold (Ct) values displayed in Table 4.5 support these conclusions.

Table 4.5: Ct values of Human, Calf and Piglet Faeces Tested Using HF183, BacR and Pig2Bac Faecal Markers, Respectively at a Noise Band of 3,4551 and a Cycle Range of 40

Positive control	Faecal marker HF183	Faecal marker BacR	Faecal marker Pig2Bac
Human 1	25.58	Not detected	Not detected
Human 2	18.42	Not detected	Not detected
Calf 1	Not detected	Not detected	Not detected
Calf 2	35.56	Not detected	Not detected
Piglet 1	Not detected	Not detected	35.1
Piglet 2	Not detected	Not detected	35.19

4.3.2 Human, Ruminant and Pig Faecal Markers in the Groundwater

Microbial source tracking (MST) technique was used to identify the source of faecal indicator bacteria in groundwater, specifically determining if they originated from human, ruminant, or pig feces. The study results (refer to Appendix 4) showed that none of the water samples tested positive for human, pig, or ruminant faecal markers. This indicates that the faecal indicator bacteria in the groundwater likely came from sources other than humans, pigs, and ruminants (Harwood *et al*., 2013). While it was expected to find positive MST results in samples containing total coliforms, *Escherichia coli*, and *enterococci*, it is important to note that these bacteria can also be present in plants and the feces of various animals, including cold-blooded and warm-blooded species other than pigs, humans, and ruminants. Additionally, *Escherichia coli* and total coliforms can thrive in tropical soil environments (Desmarais *et al*., 2002; Stewart *et al*., 2013; Rochelle-Newall *et al*., 2015). The absence of positive MST results could also be due to low concentrations of faecal markers below the detection limit or the limited number of samples analyzed.

4.4 Inactivation of Faecal Indicator Bacteria in the Water

Following the occurrence of faecal indicator bacteria in the groundwater, the primary source of drinking water in Ifakara town, it is likely that pathogenic bacteria are also present in the groundwater. This is because there is a strong correlation between the presence of faecal indicator bacteria and pathogenic bacteria in the feacally contaminated water. To address this issue, this study presents the results for the synthesis, characterization, and antibacterial properties of silver bionanoparticles.

4.4.1 Biosynthesis of Silver Bionanoparticles (SBNs)

The dark brown color of SBNs (Figure 4.4) observed in this study is similar to that observed elsewhere when extracts of leaf, stem and root from plant species other than *C. abbreviata* were mixed with silver nitrate solution (Sigamoney *et al*., 2016). The formation of a dark brown solution is therefore an evidence of phyto-reduction of Ag^{+} by *C. abbreviata* metabolites to form SBNs. This observation correlated with the previous report (Sigamoney *et al*., 2016) which showed that phytochemicals present in the plant extracts exhibit strong capping and robust reducing agents needed for the formation of nanoparticles. In addition, upon subjecting SBNs to UV-vis spectrophotometer at room temperature, surface plasmon resonance of silver was formed at intensive maximum absorption in the visible regions of 481 (CA-RB), 468 (CA-FL) and 490 nm (CA-SB) (Fig. 4.5). Despite the fact that these absorption peaks were within the range (400 - 500 nm) exhibited by SBNs (Beyene *et al*., 2017), the observed difference in absorbance values reflected the varying size of SBNs among CA-FL, CA-SB and CA-RB samples. SBNs synthesized from CA-SB had larger sizes than those from CA-FL and CA-RB extracts (Figure 4.6). Further, we observed a single SRP band for each sample (Figure 4.5), indicating that synthesized SBNs displayed typical characteristic of nanosized particles (average size of 38 nm) with spheroidal shape as supported by AFM results (Figure 4.6). Additionally, synthesized SBNs were stable at room temperature for more than 90 hours (see appendix 10).

Figure 4.4: Set up Showing the Reaction of Silver Nitrate Solution With Aqueous Extracts of Leaf, Stem Bark and Root Bark Of *C. Abbreviata* Forming Siver Bionanoparticles

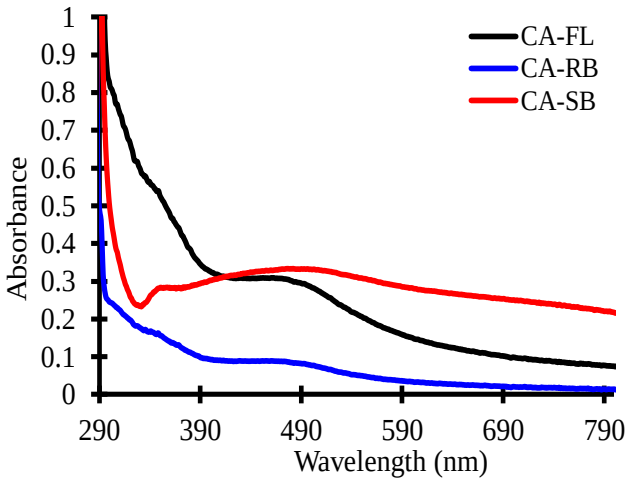

Figure 4.5: The UV-Visible Spectra of Newly Synthesized SBNs from Leaf Extract (CA-FL), Stem Bark Extracts (CA-SB) and Root Bark Extracts (CA-RB)

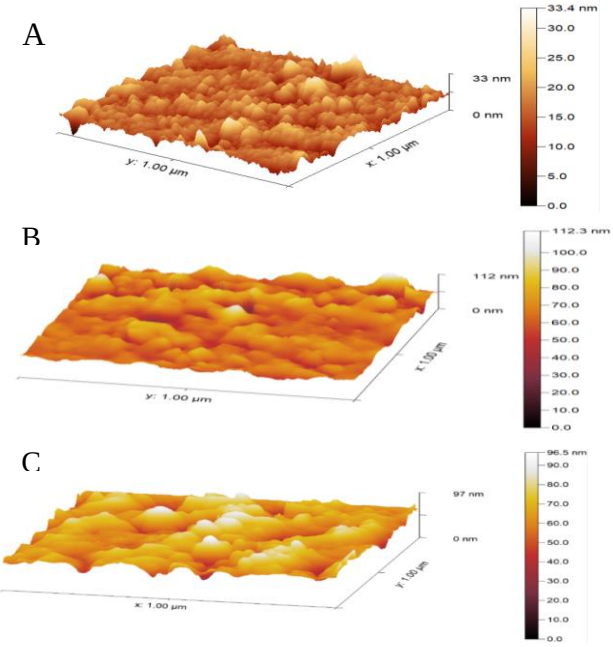

Figure 4.6: A Three-Dimensional AFM Images Showing SBNs Synthesized from Aqueous Extract of Leaf (A), Stem Bark (B) and Root Bark (C)

4.4.2 Bactericidal Activity of Synthesized Bionanoparticles

The aqueous extracts of CA-FL, CA-SB and CA-RB showed inhibition zones

ranging from 7 to 18 mm (Figure. 4.7), indicative of the bactericidal activity, supporting the traditional use of *C. abbreviata* as the herbal plant. Gram positive bacteria were highly susceptible to aqueous extracts of CA-FL, CA-SB and CA-RB as compared to gram-negative bacteria. This finding allowed synthesis of SBNs using economic, one-step and ecofriendly approach.

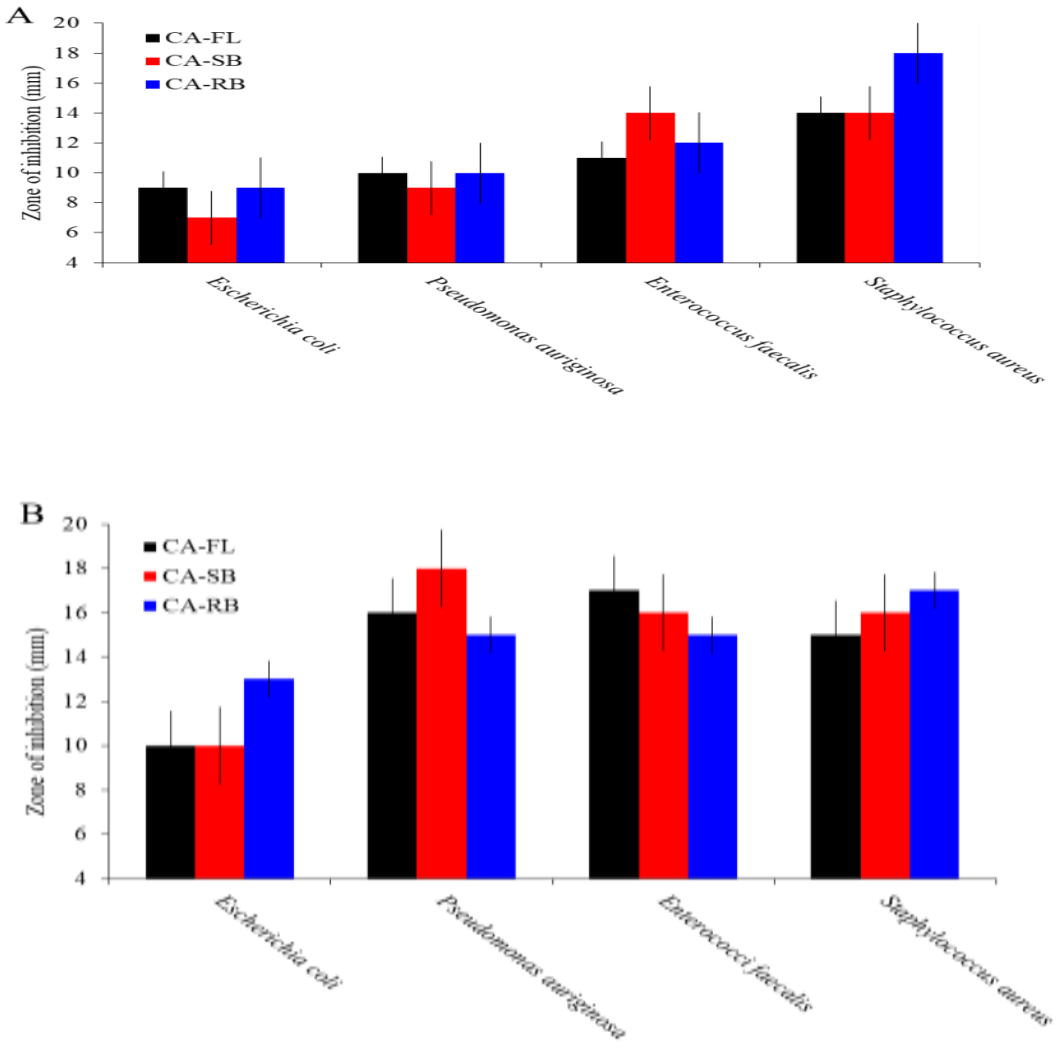

Figure 4.7: Mean Zone of Inhibition for Extracts (A) and Silver Bionanoparticles (B) Prepared from CA-FL, CA-SB, CA-RB Against *E. coli*, *P. aeruginosa*, *S. aureus* and *E. Faecalis*. Error bars Indicate Standard Deviations of the mean. CA-FL = *C. Abbreviata* Fresh Leaves; CA-SB = *C. Abbreviata* stem bark; CA-RB = *C.*

Synthesized SBNs showed significantly higher bactericidal activity compared to the aqueous extracts ($P<0.05$; Figure 4.7) due to the presence of silver in the SBNs complex. This observation is similar to the findings reported by Chandrasekharan *et al*., (2022) though different plant species other than *C. abbreviata* were applied. Except for *E. coli*, the remaining reference pathogenic bacteria were substantially susceptible to the SBNs irrespective of the plant part used in the preparation of SBNs. In essence, several studies have reported the bactericidal activity of silver nanoparticles against *E. coli, P. aeruginosa, E. faecalis* and *S. aureus* (Ghaffari-Moghaddam and Hadi-Dabanlou, 2014; Saravanan *et al*., 2018; Sigamoney *et al*., 2016). In these studies, it was observed that SBNs can attach to the cell wall of bacteria, penetrate it and cause membrane damage leading to leakage of cellular contents and death, or SBNs may release silver ions which interfere with sulfhydryl groups of proteins and enzymes.

The MIC was determined to further evaluate the reference bacterial strains' susceptibility to the newly synthesized SBNs and aqueous extract of *C. abbreviata* (Table 4.6). The MIC values of SBNs and the aqueous extract of *C. abbreviata* plant differ remarkably and support the data from the zone of inhibition experiment. The SBNs exhibited the best antimicrobial properties on all studied pathogenic bacterial strains, with the MIC values ranging from <0.026 to 1.69 µg/mL. In contrast, the corresponding values for the aqueous extract of *C. abbreviata* ranged between 0.36 and 14.5 µg/mL. The elevated inhibitory activity of the greenly synthesized SBNs was possibly associated with their relatively large surface area, which makes them more available for the surface interactions with bacteria cells and small size of the

SBNs that allow them to easily cross the bacterial cell wall and inhibit the growth of the cells.

Table 4.6: MIC of Extracts and SBNs for Both Gram Positive and Gram-Negative Bacterial Species

Bacteria	MIC (µg /mL)					
	Aqueous extracts			SBNs		
	CA-FL	CA-SB	CA-RB	CA-FL	CA-SB	CA-RB
E. coli	14.5	11.5	9.5	1.69	<0.034	<0.03
P. aeruginosa	ND	ND	ND	ND	ND	ND
E. faecalis	7.25	1.09	2.37	< 0.026	< 0.034	< 0.030
S. aureus	14.5	0.36	1.19	1.69	< 0.034	< 0.030

ND = not determined

4.4.3 The Effectiveness of SBNs in Killing Bacteria

The killing time activity of faecal coliform bacteria in the contaminated water is shown in Figure 4.8. The bactericidal activity of SBNs is effective against the model organism and the reduction in number of cfu/100 mL was 3 log units. The biocidal endpoint of CA-FL associated SBNs for faecal coliform bacteria was reached after 1 hour of incubation at room temperature irrespective of the concentration of SBNs applied (Figure 4.8). In contrast, endpoint of SBNs associated with CA-SB and CA-RB for faecal coliform bacteria was reached after 4 hours of incubation irrespective of the concentrations of SBNs used. No significant differences in disinfection efficacy (P>0.05) were found among the tested sources of SBNs. This indicated that SBNs synthesized from *C. abbreviata* plant parts can be used for reducing bacteria such as faecal coliform bacteria in the environmental water before being used for consumption.

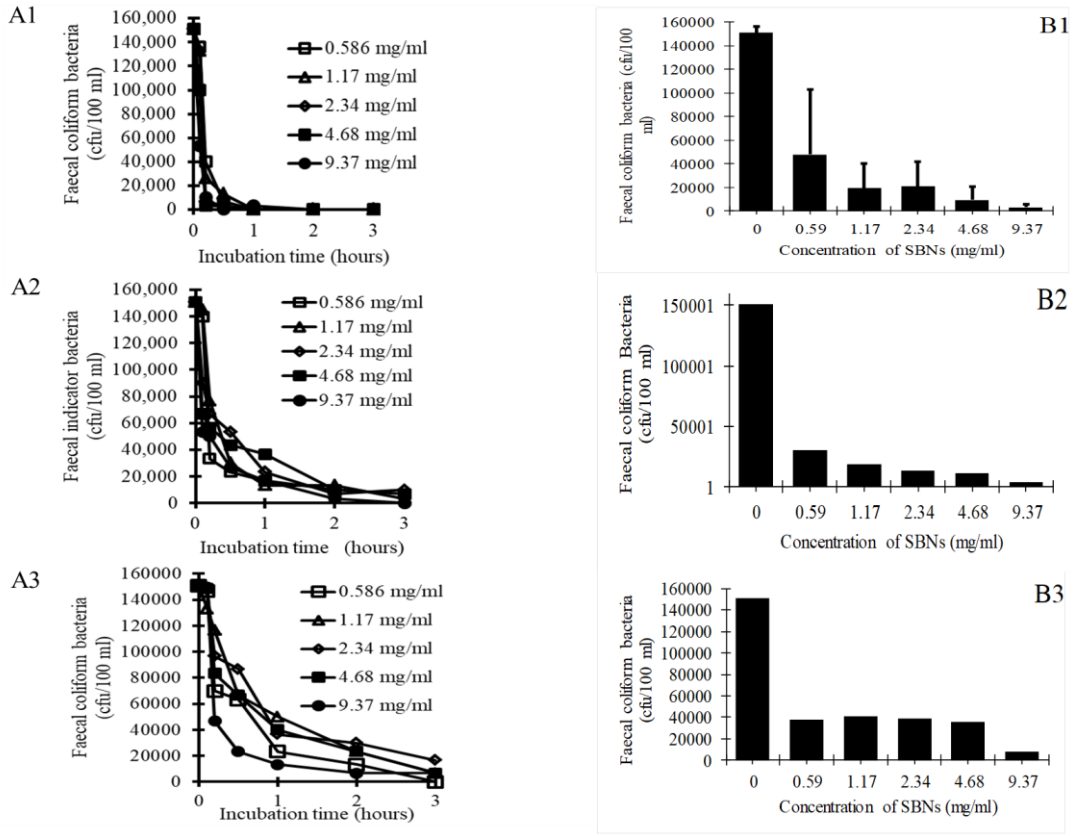

Figure 4.8: Time kill plots (A1, A2, A3) and disinfection efficacy (B1, B2, B3) following the treatment of faecal coliform bacteria (n = 3) with different concentrations of SBNs made from fresh leaves(A1 and B1), stem bark (A2 and B2), and root bark (A3 and B3) extracts

4.4.4 SBNs Substantially Reduced Bacterial Load in the River Water

Bactericidal activity of SBNs was determined against faecal coliform bacteria that are used as an indicator of water quality (Cabral *et al*., 2006) and treatment efficiency (Elmund *et al*., 1999). Water samples treated with SBNs had significantly reduced concentrations (98.8 - 99.9%) of faecal coliform bacteria (P<0.05) compared to the control (river water without SBNs) (0.08%). There were no detectable faecal coliform bacteria at concentrations > 9.37 mg/L of SBNs synthesized from aqueous extract of either CA-LF, CA-SB or CA-RB (Figure 4.8), suggesting that SBNs

synthesized from any of the *C. abbreviata* organs can be used as disinfectant. Further, SBNs achieved 3-log reduction of faecal coliform bacteria in the river water, suggesting that they may be used as an effective disinfectant. Although, this is the first report regarding the application of SBNs synthesized from *C. abbreviata* extract in water disinfection, SBNs from other biomaterials have been previously demonstrated for drinking water treatment with inactivation efficacy consistent to that reported herein although different experimental designs were employed (Dankovich and Gray, 2011; Haider *et al*., 2016; Kallman *et al*., 2011; Mecha and Pillay, 2014). As a result, SBNs is a promising effective water disinfectant, particularly in tropical developing countries where point-of-use water disinfection is common. The biosynthesis of SBNs is environmentally friendly, cost-effective, and non-toxic, using plant materials readily available in Tanzania as reducing agents. This approach reduces the need for expensive imported chemicals and lowers costs significantly. The biosynthesis process is quick and uses affordable equipment, making it accessible to low-income countries. While this study did not assess the recoverability of SBNs from treated river water, it is likely that they can be recovered through centrifugation or sedimentation for reuse. Embedding newly synthesized SBNs in a matrix that can release appropriate levels of the bactericide in finished water while allowing for reuse is important. Additionally, the potential human health risks and ecological impacts of these SBNs are unknown compared to chlorine disinfectants, highlighting the need for further research to ensure their safety to human and environmental health.

4.5 Limitation of this Study

This study utilized standardized laboratory procedures and field-based standardized questionnaires to collect data from Ifakara town, including households, secondary schools, primary schools, and church-owned hand-pump bore wells. Microbial source tracking was conducted on 25 out of 54 samples without triplicate analysis due to budget constraints. Other faecal markers besides human, ruminant, and pig faeces were not included in the study due to limited budget. To validate the negative MST results, it is recommended to repeat the molecular technique using primers efficiently validated using samples of the study area. The primers used in this study were developed in a different geographical context and have not being necessitating validated in the study area.

Sanitary inspections were carried out only once without considering the temporal dynamics of anthropogenic activities and potential errors from surveyors. Some sampling points were inaccessible during the study due to remote locations and lack of infrastructure. Details such as depths, age, and management of hand-pump bore wells were not recorded during the survey due to lack of access to this information.

Additionally, due to resource constraints, the synthesized silver nanoparticles were not separated into different size fractions to test their efficacy in inactivating faecal indicator bacteria in water.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATION

This Chapter provides a summary of the key findings derived from the study and presents the conclusions that can be drawn from these findings. Additionally, it identifies areas requiring further research and attention.

5.1 Conclusions

The groundwater quality status of Ifakara town, as revealed by a recent study, highlights distinct insights from physico-chemical parameters and faecal indicator bacteria. While all hand-pump borewells examined met the World Health Organization (WHO) and Tanzania Bureau of Standards (TBS) guidelines for physico-chemical parameters, 25 out of 54 surveyed wells (46%) exceeded permissible limits for faecal indicator bacteria. The physico-chemical parameters, showing correlation patterns linked to local geology rather than human activity, suggest natural influences dominate groundwater composition. In contrast, faecal contamination exhibited a strong positive correlation with poor sanitary conditions around borewells. Microbial source tracking indicated that detected bacteria likely originated not from humans, pigs, or ruminants but potentially from domestic animals such as chickens, ducks, dogs, and cats observed near sampling sites. To address contamination, silver-based nanoparticles (SBNs) synthesized from *C. abbreviata* plant parts demonstrated promising bactericidal efficacy, effectively inhibiting both gram-negative (*E. coli*, *P. aeruginosa*) and gram-positive (*S. aureus*, *E. faecalis*) bacteria, with inhibition zones of 10–18 mm. Remarkably, SBNs achieved 99.9% removal of faecal bacteria from contaminated water, with efficacy

dependent on nanoparticle concentration and exposure time rather than the plant parts used in synthesis. These findings position SBNs as a viable, scalable water disinfection solution, particularly for low-income regions vulnerable to waterborne disease outbreaks due to inadequate sanitation infrastructure.

5.2 Recommendations

- Community-based maintenance and protection of handpump borewells should be implemented in Ifakara town to ensure that groundwater remains free from faecal contamination.
- Communities in Ifakara town should be guided on the appropriate locations for installing handpump borewells to prevent contamination from sources such as pit latrines, surface runoff, livestock, agricultural runoff, sewer lines, and septic tanks.
- Borewell owners in Ifakara town should receive education on the importance of the standardized sanitary inspection questionnaire created by WHO and how to interpret the results. This will enhance the management and protection of handpump borewells.
- Future research should consider analyzing a larger sample size and using additional faecal markers for a wider range of cold-blooded and warm-blooded animals, beyond humans, pigs, and ruminants, to ensure robust results and draw reliable conclusions about the sources of faecal indicator bacteria in Ifakara town's groundwater.

- Further research on faecal indicator bacteria other than coliform bacteria is necessary to develop sustainable SBNs for treating various microbial pollutants in groundwater effectively.
- The effectiveness of SBNs against biofilm in groundwater should also be investigated experimentally.

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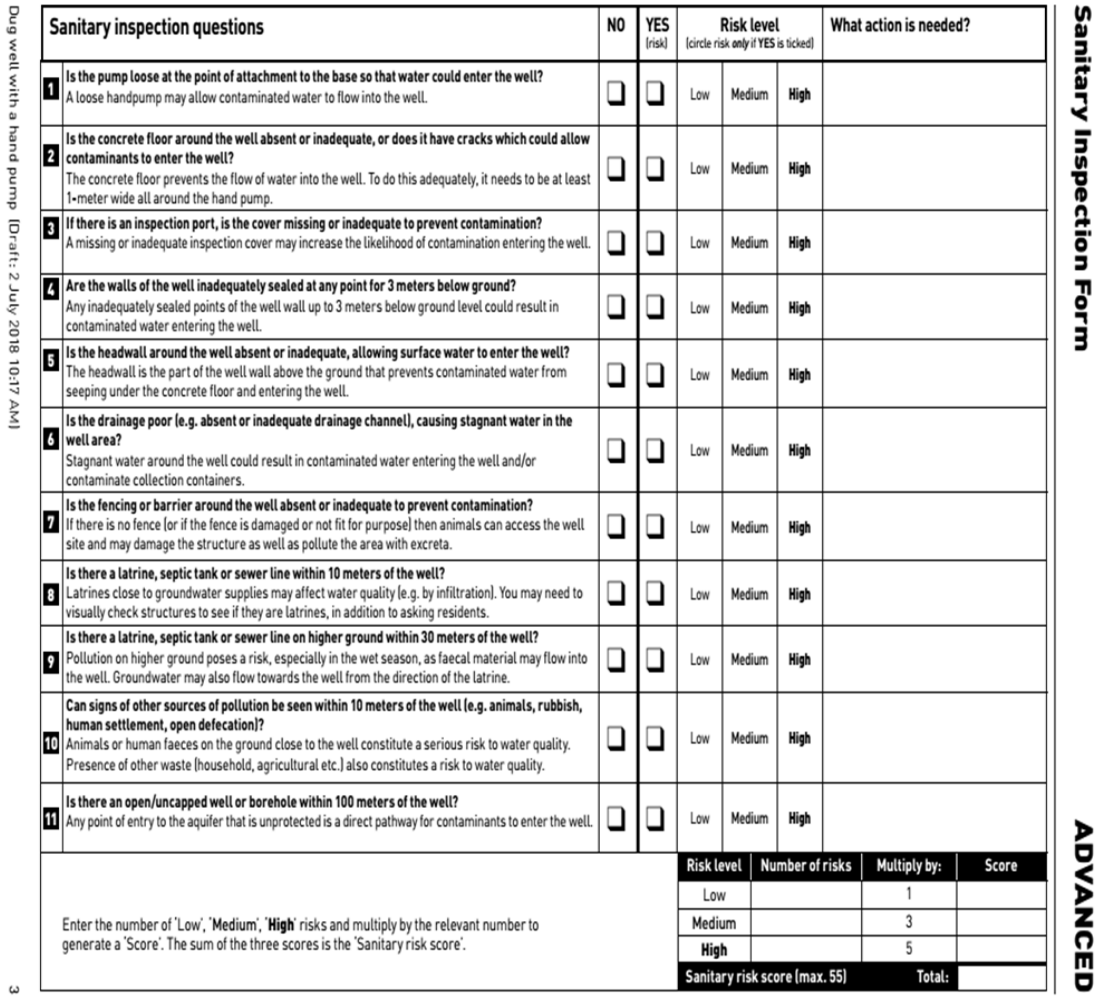
APPENDICES

Appendix 1: Sanitary inspection form describe by WHO (2018)



Dug well with a hand pump (Draft: 2 July 2018 10:17 AM)

Sanitary Inspection Form

ADVANCED

	Sanitary inspection questions	NO	YES (risk)	Risk level (circle risk only if YES is ticked)			What action is needed?
1	**Is the pump loose at the point of attachment to the base so that water could enter the well?** A loose handpump may allow contaminated water to flow into the well.	❑	❑	Low	Medium	**High**	
2	**Is the concrete floor around the well absent or inadequate, or does it have cracks which could allow contaminants to enter the well?** The concrete floor prevents the flow of water into the well. To do this adequately, it needs to be at least 1-meter wide all around the hand pump.	❑	❑	Low	Medium	**High**	
3	**If there is an inspection port, is the cover missing or inadequate to prevent contamination?** A missing or inadequate inspection cover may increase the likelihood of contamination entering the well.	❑	❑	Low	Medium	**High**	
4	**Are the walls of the well inadequately sealed at any point for 3 meters below ground?** Any inadequately sealed points of the well wall up to 3 meters below ground level could result in contaminated water entering the well.	❑	❑	Low	Medium	**High**	
5	**Is the headwall around the well absent or inadequate, allowing surface water to enter the well?** The headwall is the part of the well wall above the ground that prevents contaminated water from seeping under the concrete floor and entering the well.	❑	❑	Low	Medium	**High**	
6	**Is the drainage poor (e.g. absent or inadequate drainage channel), causing stagnant water in the well area?** Stagnant water around the well could result in contaminated water entering the well and/or contaminate collection containers.	❑	❑	Low	Medium	**High**	
7	**Is the fencing or barrier around the well absent or inadequate to prevent contamination?** If there is no fence (or if the fence is damaged or not fit for purpose) then animals can access the well site and may damage the structure as well as pollute the area with excreta.	❑	❑	Low	Medium	**High**	
8	**Is there a latrine, septic tank or sewer line within 10 meters of the well?** Latrines close to groundwater supplies may affect water quality (e.g. by infiltration). You may need to visually check structures to see if they are latrines, in addition to asking residents.	❑	❑	Low	Medium	**High**	
9	**Is there a latrine, septic tank or sewer line on higher ground within 30 meters of the well?** Pollution on higher ground poses a risk, especially in the wet season, as faecal material may flow into the well. Groundwater may also flow towards the well from the direction of the latrine.	❑	❑	Low	Medium	**High**	
10	**Can signs of other sources of pollution be seen within 10 meters of the well (e.g. animals, rubbish, human settlement, open defecation)?** Animals or human faeces on the ground close to the well constitute a serious risk to water quality. Presence of other waste (household, agricultural etc.) also constitutes a risk to water quality.	❑	❑	Low	Medium	**High**	
11	**Is there an open/uncapped well or borehole within 100 meters of the well?** Any point of entry to the aquifer that is unprotected is a direct pathway for contaminants to enter the well.	❑	❑	Low	Medium	**High**	

Enter the number of 'Low', '**Medium**', '**High**' risks and multiply by the relevant number to generate a 'Score'. The sum of the three scores is the 'Sanitary risk score'.

Risk level	Number of risks	Multiply by:	Score
Low		1	
Medium		3	
High		5	
Sanitary risk score (max. 55)		**Total:**	

3

Appendix 2: Geographical coordinates of the surveyed well-handpumps

SAMPLING SITE	**GPS COORDINATES**	
	South(-)	East(+)
IFAKARA(MAENDELEO)	08.10173	036.68554
IHANGA(KIHOGOSI)	08.05101	036.56650
IDETE(MISONO BARIDI)	08.09606	036.52958
IHANGA(KIHOGOSI)	08.08976	036.57733
KIKWAWILA (ISOMBA)	08.08847	036.74731
SIGINALI (NDULULU)	08.02655	036.83287
LUNGONGOLE B	08.07955	036.77169
KIKWAWILA	08.09194	036.72815
MAOTANGA(MIKOROSHINI)	08.13548	036.65791
VIWANJA 60(JONGO)	08.12348	036.68012
KIUNGANI	08.14291	036.67521
LIPANGALALA(MKUYA)	08.14671	036.68146
LIPANGALALA(LIAMI)	08.16127	036.69115
MAGOA	08.14637	036.66649
KIBAONI(MJI MPYA)	08.07005	036.68157
MACHIBI(MAKERO CHINI)	08.07546	036.65844
MACHIBI	08.06445	036.66013
MICHENGA(MICHENGA C)	08.10516	036.65800
KININGINA(UMOJA)	08.10647	036.67217
KIBAONI(KILOLELO)	08.05693	036.68289

KIBAONI(MILOLA)	08.08346	036.69111
KIBAONI(MILOLA)	08.08346	036.69091
MBASA(KATI)	08.11051	036.76018
MHOLA(MHOLA)	08.12868	036.69210
MCHENGA(MCHENGA C1)	08.11549	036.66005
MAENDELEO(SHUNGU)	08.11392	036.68390
KATINDIUGA(PRI SCHOOL)	08.12858	036.70620
KATINDIUGA(PRI SCHOOL)	08.12899	036.70565
IHANGA	08.10243	036.61155
MHOLA(MHOLA)	08.12829	036.69179
NDUNA	08.13812	036.68526
JONGO	08.12898	036.67938
JONGO	08.12828	036.68011
JONGO	08.12896	036.68109
JONGO(FPCT)	08.12740	036.68095
KIKWAWILA(KAPOLO)	08.09336	036.69578
KAPOLO(PRIMARY SCHOOL)	08.09374	036.70318
KIKWAWILA(KAPOLO)	08.08925	036.70464
KAPOLO(SHULE)	08.08782	036.70373
KIKWAWILA(KAPOLO)	08.08754	036.70176
KIKWAWILA(KAPOLO)	08.08852	036.69739
KAPOLO(PAROKIA KIBAONI)	08.09198	036.69482
KIKWAWILA(KAPOLO)	08.09549	036.69642

KIBAONI(MILOLA)	08.08782	036.69267
KILOLELO(KILOLELO)	08.05580	036.70178
KININGINA	08.11650	036.66356
LUMEMO(WALIALA)	08.13353	036.66725
LUMEMO	08.13768	036.66601
MLABANI	08.14349	036.69046
YANGA(KIHOGOSI)	08.09010	036.58166
IHANGA(KIHOGOSI)	08.08891	036.57539
MICHENGA B	08.12095	036.65170
MAUTANGA	08.14853	036.64618
LUMEMO(KWALIALA)	08.13611	036.66910

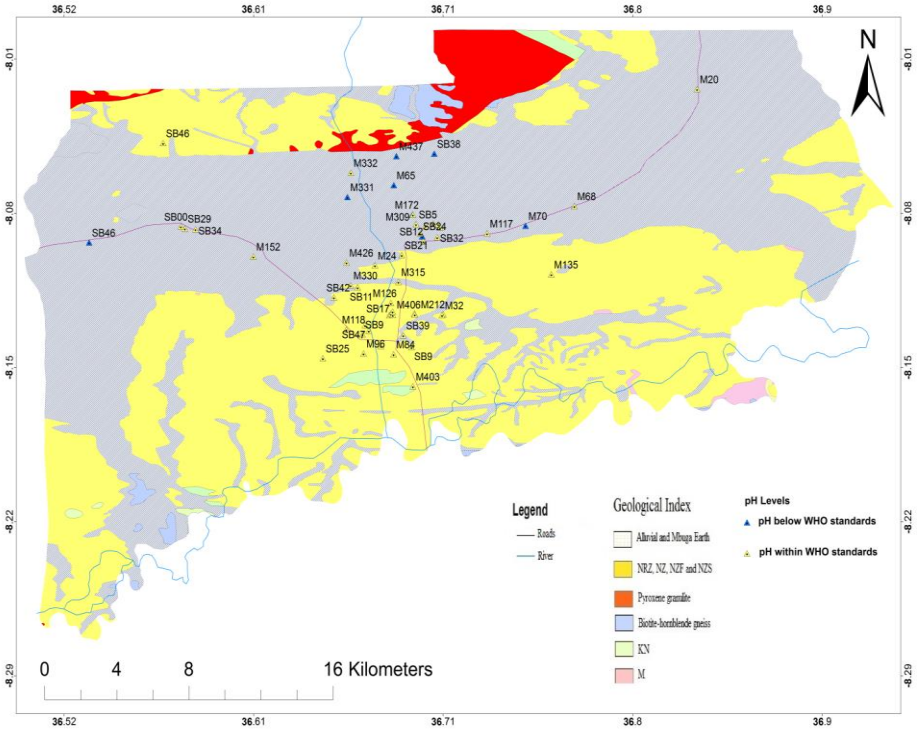
Appendix 3: Well-handpumps selected for the MST investigations, DNA concentrations used for qPCR assay and the results obtained for Pig2Bac, BacR and Hf183 markers. X showed that neither Pig2 Bac, BacR nor HF183 faecal markers were detected by qPCR.

Selected well-handpumps	**Total DNA obtained [µg/ml]**	**DNA (in 25 µL) used for qPCR assay [ng/ml]**	**Pig2Bac detected**	**HF183 detected**	**BacR**
M20	9.07	181.48	Not detected	Not detected	Not detected
M24	14.49	289.80	Not detected	Not detected	Not detected
M32	2.92	116.96	Not detected	Not detected	Not detected

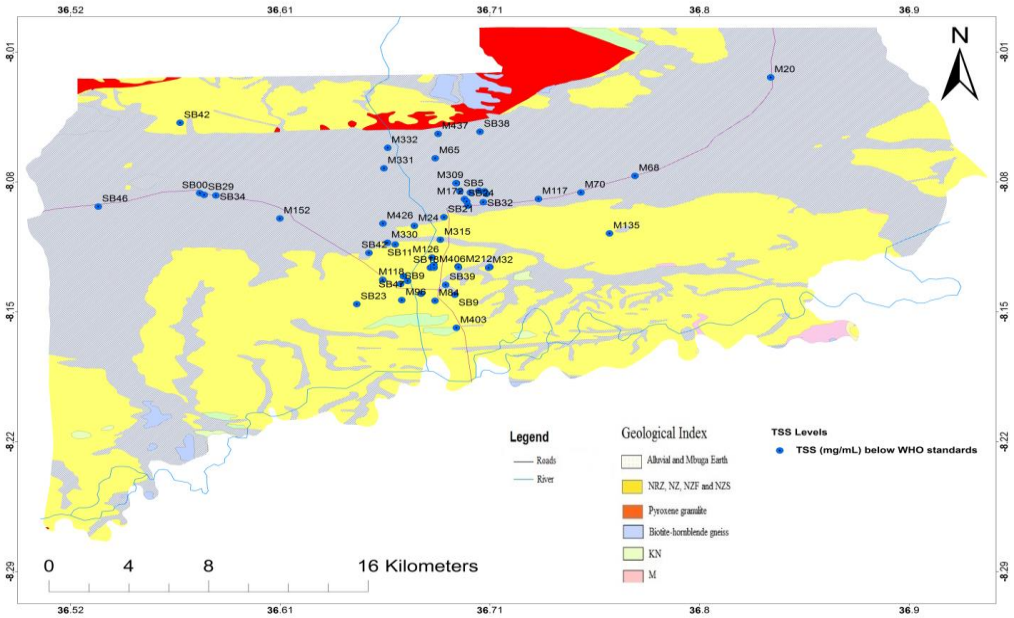
M65	9.67	193.44	Not detected	Not detected	Not detected
M68	16.51	330.16	Not detected	Not detected	Not detected
M70	10.54	210.72	Not detected	Not detected	Not detected
M84	21.08	421.60	Not detected	Not detected	Not detected
M85	10.55	211.04	Not detected	Not detected	Not detected
M96	10.75	214.96	Not detected	Not detected	Not detected
M117	6.37	127.40	Not detected	Not detected	Not detected
M118	3.81	152.40	Not detected	Not detected	Not detected
M126	6.98	139.60	Not detected	Not detected	Not detected
M135	18.57	371.44	Not detected	Not detected	Not detected
M152	3.16	126.48	Not detected	Not detected	Not detected
M172	15.33	306.64	Not detected	Not detected	Not detected
M212	9.55	191.00	Not detected	Not detected	Not detected
M309	6.45	128.96	Not detected	Not detected	Not detected
M315	13.38	267.60	Not detected	Not detected	Not detected
M330	11.00	219.92	Not	Not detected	Not detected

			detected		
M331	5.28	105.68	Not detected	Not detected	Not detected
M332	12.57	251.40	Not detected	Not detected	Not detected
M403	12.46	249.28	Not detected	Not detected	Not detected
M406	22.83	456.68	Not detected	Not detected	Not detected
M426	5.68	113.52	Not detected	Not detected	Not detected
M437	8.81	176.20	Not detected	Not detected	Not detected

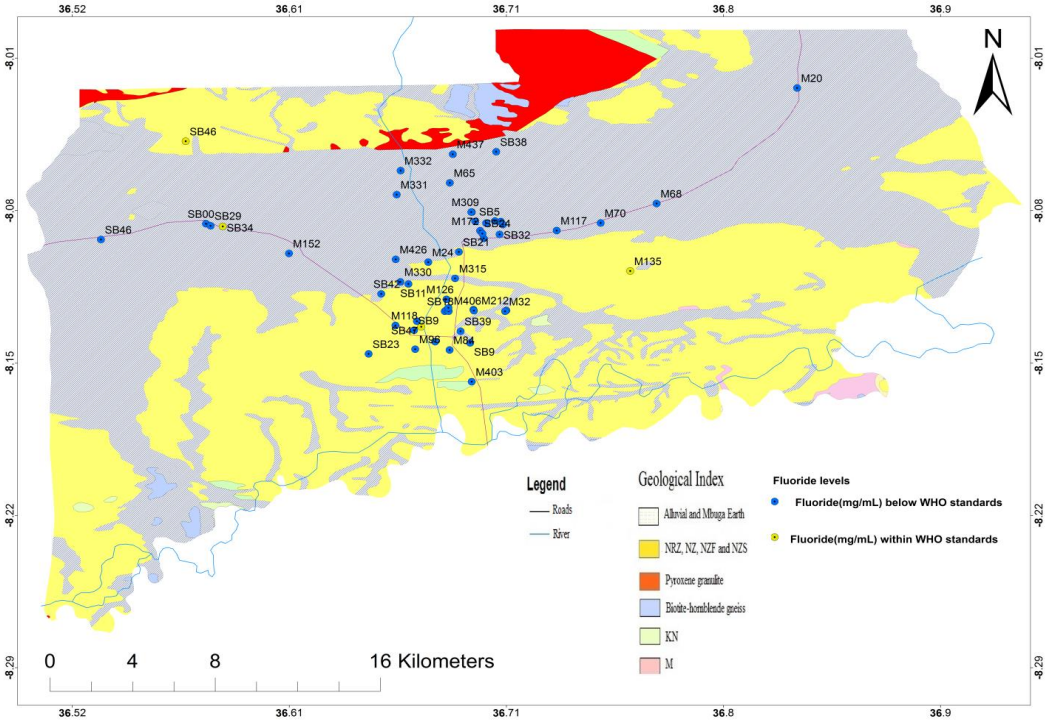
Appendix 4: pH Values Compared to WHO Guidelines



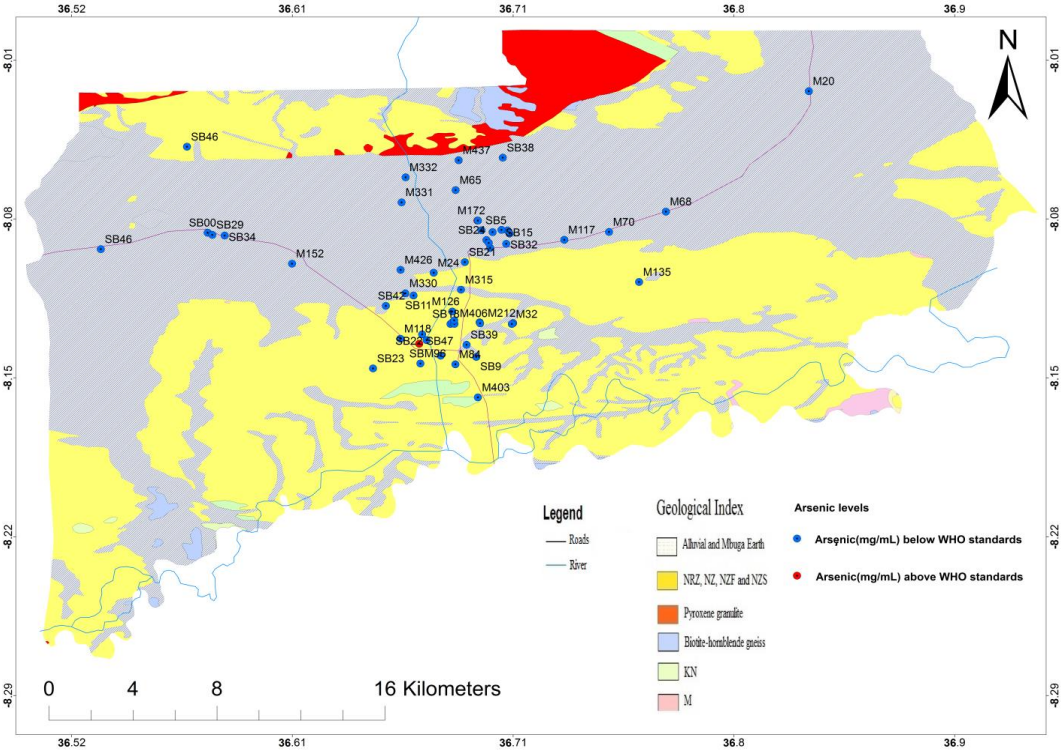
Appendix 5: Total Suspended Solids Concetrations in Relation to WHO Guidelines



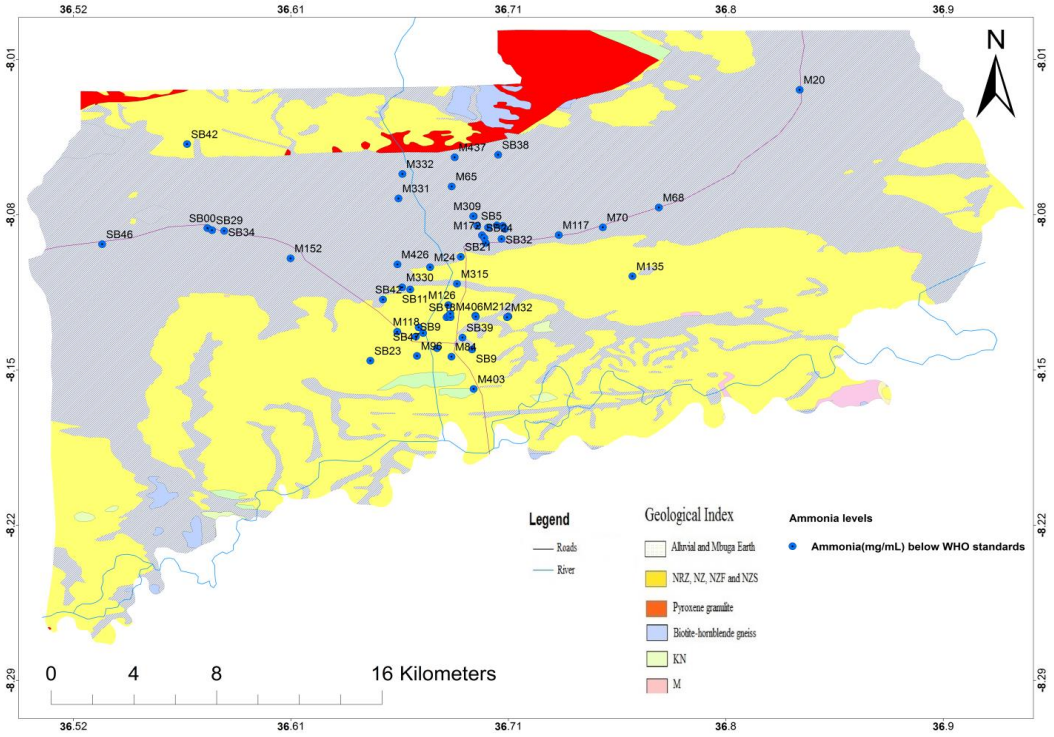
Appendix 6: Fluoride Conentrations in Relation to WHO Guidelines



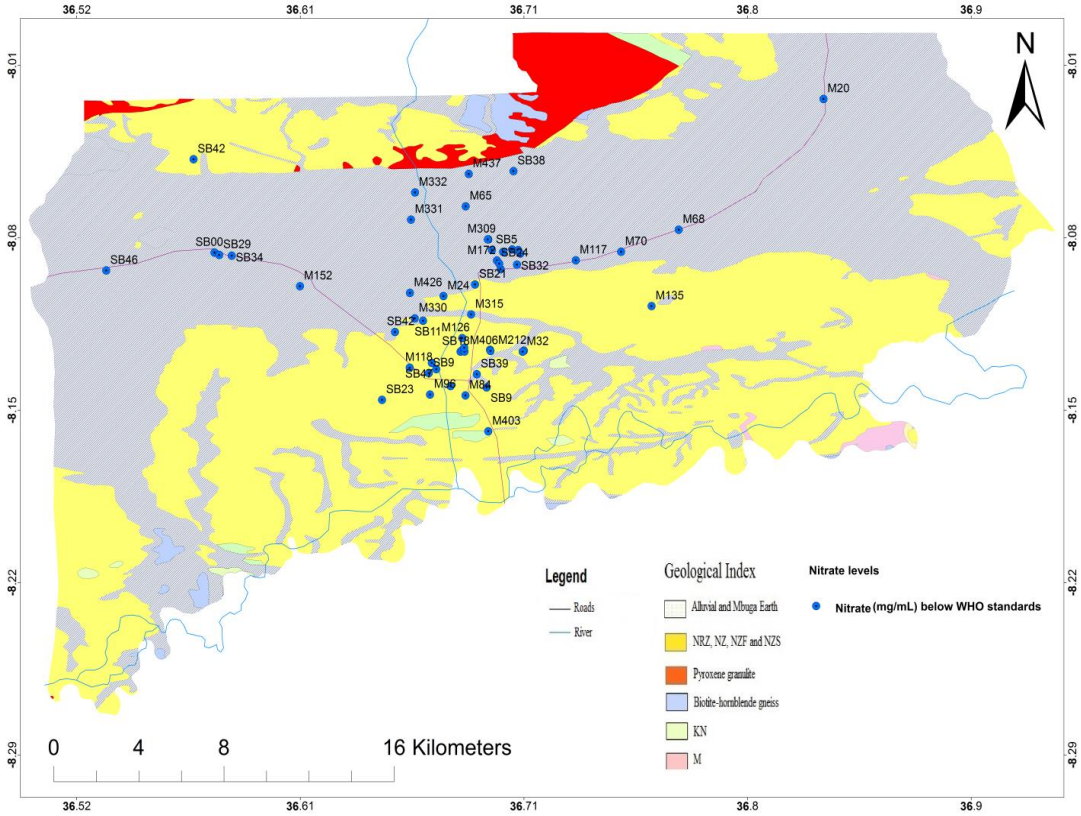
Appendix 7: Arsenic Concetrations in Relation to WHO Guidelines



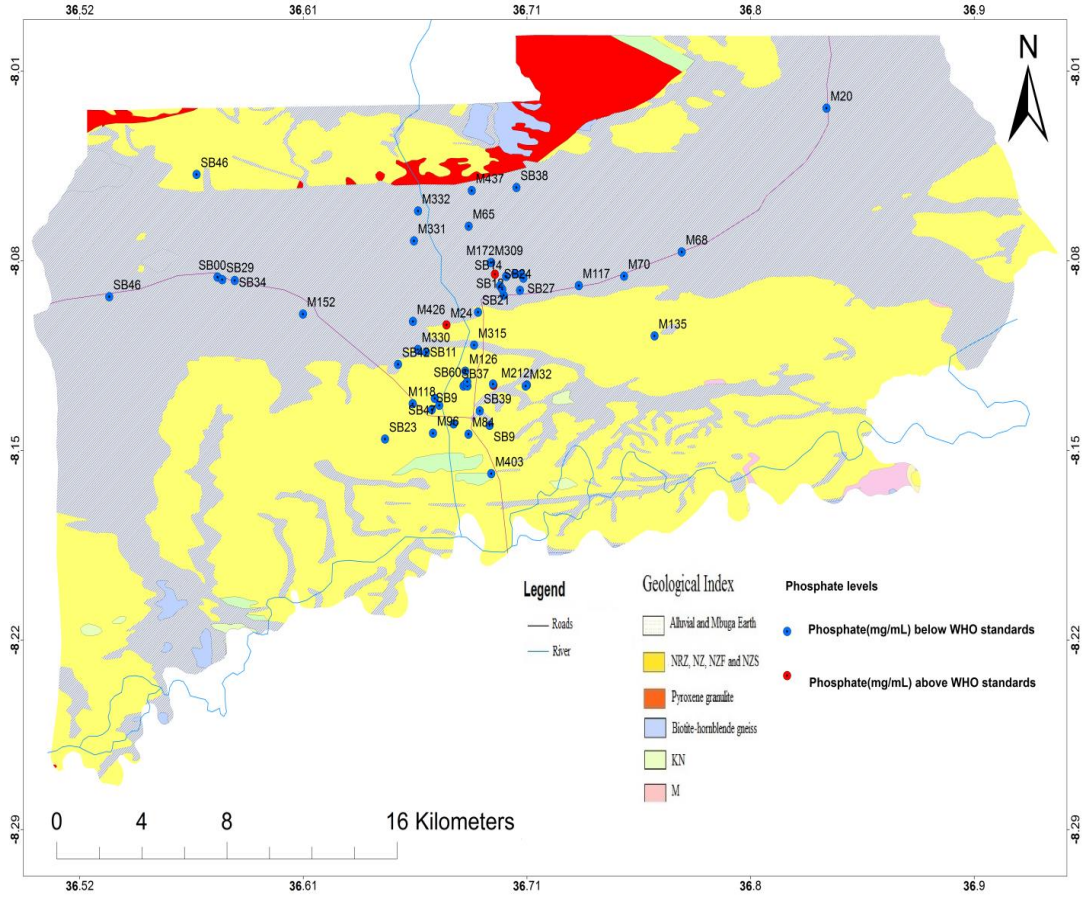
Appendix 8: Ammonia concentration in relation to WHO guidelines



Appendix 9: Nitrate Concetrations Compared to WHO Guidelines



Appendix 10: Phosphate Concetrations Compared to WHO Guidelines



Appendix 10: Stability of silver nanoparticles with respect to time.

Serial no.	Duration of incubation	Colour of silver bionanoparticles suspension remain the same irrespective of duration of incubation	Absorbance (nm) of silver bionanoparticles were stable and within the range for silver nanoparticles irrespective of the duration of incubation
1	30 Minute		481

2	**90 Minutes**		**482**
4	**120 Minutes**		**482**