

**EFFECT OF PIT LATRINES ON ENTERIC-PATHOGEN
AND PHYSICOCHEMICAL CONTAMINATION OF
GROUNDWATER IN TIGANIA WEST SUB-COUNTY,
MERU COUNTY, KENYA**

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**A Thesis Submitted in Partial fulfillment of the Requirements for Conferment of
Degree of Master of Science in Sanitation of Meru University of Science and
Technology**

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other institution

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DEDICATION

This study is devoted to my cherished parents. They have been a constant source of motivation and have provided me with the resilience to persist even when I considered abandoning my efforts. Their unwavering support in terms of morality, spirituality, emotions, and finances has been priceless. I am eternally grateful for their affection and direction.

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ABBREVIATIONS, ACRONYMS, AND SYMBOLS

ANOVA	Analysis of Variance
BEA	Bile Esculin Azide
BOD	Biological Oxygen Demand
CDC	Centers for Disease Control and Prevention
EMB	Eosin Methylene Blue
FSM	Faecal Sludge Management
GN	Gram-Negative
LIA	Lysine Iron Agar
MPN	Most Probable Number
NACOSTI	National Commission on Science Technology and Innovation
SDGs	Sustainable Development Goals
SPSS	Statistical Package for Social Sciences
TBX	Tryptone Bile X-glucuronide
TSI	Triple Sugar Iron
WHO	World Health Organization
XLD	Xylose Lysine Deoxycholate

ABSTRACT

Groundwater contamination is a global concern, posing risks to public health and ecosystems. In Kenya, particularly in rural areas like Tigania West Sub County, Meru County, groundwater contamination is a pressing issue due to use of pit latrines. The main objective of the study was to investigate the impact of pit latrines on enteric-pathogen and physicochemical contamination of groundwater in Tigania West Sub County, Meru County, Kenya. Data was collected using a questionnaires and laboratory analysis. The sample size for this research was 96 households, distributed among all the wards. Data was analysed using SPSS version 27. Descriptive statistics such as frequencies and percentages provided an overview of the data. Inferential statistics such as Analysis of Variance (ANOVA) and regression analysis were used to determine significant differences between groups and to identify relationships between variables. The study revealed significant associations between pit latrine proximity to groundwater sources and waterborne disease prevalence. The correlation coefficient ($r=0.456$) indicates a moderate positive relationship between the proximity of pit latrines and waterborne infections. Enteric pathogens (total coliforms, *E. coli*, Salmonella, Shigella, Staphylococcus aureus, Streptococcus faecalis and Helminths.) were detected in groundwater samples, indicating contamination. The correlation coefficient ($r = 0.076$) also indicated a very weak positive relationship between high enteric pathogen prevalence and waterborne infections. Physicochemical analysis revealed conformities with WHO guidelines. For example, in Mbeu ward, 93.33% of groundwater samples had optimal pH levels of 6.5-8.5. The BOD analysis indicated varying levels of organic pollution across different wards, with Athwana Ward showing the highest levels of contamination, followed by Kianjai and Nkomo Wards. Akithi and Mbeu Wards exhibited moderate levels of organic pollution. These findings highlighted the need for targeted interventions to reduce organic pollution in groundwater sources across the different wards. The study concluded that considerable proportion of pit latrines were found to be located in close proximity to water sources, below 30m, raising concerns about potential fecal contamination of groundwater. The study recommended construction and maintenance of pit latrines to ensure they are situated at safe distances from groundwater sources. The detection of pathogens such as total coliforms, *E. coli*, *Salmonella*, and *Shigella* indicates potential health risks associated with groundwater consumption, emphasizing the importance of regular water quality monitoring and treatment to ensure safe drinking water provision. The study recommended the construction and maintenance of pit latrines to ensure they are situated at safe distances from groundwater sources. Implement regulations and guidelines for the siting and construction of pit latrines to minimise the risk of fecal contamination of groundwater. Increased awareness and education among local communities on the importance of safe water practices, including proper sanitation and hygiene measures. Encourage the use of improved sanitation facilities and the adoption of water treatment methods to reduce the risk of waterborne diseases.

CHAPTER ONE: INTRODUCTION

1.1 Background Information

Globally, around 2.6 billion people lack sufficiently improved sanitation. Improved sanitation entails infrastructure and facilities that separate humans from getting into contact with their faecal waste. Some of these facilities for improving sanitation include water-based toilets that drain into sewers and pit latrines that can either be simple pit latrines or ventilated improved pit latrines (Adesakin *et al.*, 2020). Pit latrines remain the most common way of disposing off human excreta in low-income countries.

There is substantial evidence that improved sanitation plays a critical role in reducing infections that lead to diarrheal diseases, as well as lowering morbidity and mortality rates. Enhanced sanitation practices also significantly decrease the prevalence of soil-transmitted helminths (Kelly *et al.*, 2020). Despite this, open defecation remains a widespread practice, with nearly one billion people globally lacking access to proper sanitation facilities. Recognizing the urgency of this issue, the United Nations included improved sanitation and water management as key components of the Sustainable Development Goals (SDGs). Specifically, SDG 6 aims to "ensure availability and sustainable management of water and sanitation for all" by the year 2030. This goal emphasizes the importance of evidence-based policies and targeted interventions, particularly in underserved settlements and among marginalized populations, to achieve equitable access to safe sanitation.

Notably, appropriate sanitation services in a country are critical in improving dignity, economic stability, health, and protection of the local environment. With proper and safe sanitation, there is support for good health, and the local environment is improved and protected. When people access water in improved and reliable sources, the exercise takes

less time and minimal efforts (Kelly *et al.*, 2020). People can become productive in other ways.

Access to clean water means reduced disease burden hence improved economy because there will be less expenditure on health-related bills. Communities become empowered and remain more physically, mentally and economically productive. Children are at a reduced risk of water-related diseases, which increases their health and better school attendance, which has both short-term and long-term benefits to their lives (World Health Organization [WHO], 2011).

WHO has developed recommendations for ensuring the quality of drinking water to protect public health by reducing the risk of waterborne illness (WHO, 2021). These guidelines specify maximum acceptable levels of various contaminants in drinking water, including physical, chemical, and microbiological parameters such as pH, turbidity, nitrate, fluoride, total coliforms, and *E. coli* as shown in Tables 2.1 and 2.2.

Table 1. 1

WHO permissible limits for physicochemical water quality parameters

Parameter	Permissible Limit
pH	6.5-8.5
Colour	15 units
Odour	None
Turbidity	5 NTU
Biological Oxygen Demand (BOD)	5 mg/L

Source: (WHO, 2021)

Table 1. 2

WHO permissible limits for enteric pathogens and physicochemical parameters

Pathogen	WHO Permissible Limit
Total Coliforms	<10 CFU/100 mL
Faecal Coliforms	<2 CFU/100 mL
<i>E. coli</i>	<1 CFU/100 mL
<i>Salmonella</i>	<10 Salmonella/250 Ml

<i>Shigella</i>	<1 Shigella/100 mL
<i>Staphylococcus aureus</i>	<100 CFU/100 mL
<i>Streptococcus faecalis</i>	<100 CFU/100 mL
Helminthes	None

Source : (WHO, 2021)

Proper waste disposal of human excreta improves provision of safe water since there is little possibility of contaminating the environmental resources (Omarova *et al.*, 2018). Further, appropriate management of human wastes ensures significant control of the spread of diseases because transmission of water-related pathogens that cause sanitation-related diseases is kept under check. Fecal Sludge Management (FSM) programs encounter significant challenges that pose environmental and public health risks (Mariwah *et al.*, 2022). Without a well-designed post-fill-up management plan for pit latrines, fecal sludge finds its way into the waterways, insanitary landfills, and open drains.

Earlier studies have suggested that facilities' operational management remains unpredictable, posing economic and environmental sanitation constraints to the government, users, and property owners (Fischer *et al.*, 2019). Therefore, content leaching or stabilization should at least equal the sludge accumulation rates. Unfortunately, urbanization and technological advancements have made it more complicated to manage fecal sludge, and eventually, the pit latrines become full. Consequently, places where the water table is shallow are faced with pit latrine construction challenges and depth limitation (Othoo *et al.*, 2020). Some areas such as marshlands and riparian land, including those of perched water table are also known to present similar obstacles. In such areas, raised pit latrine technology is adopted and this has been widely adopted in areas that easily flood (Garcia-Becerra *et al.*, 2021).

One of the most important considerations when considering the construction process of a pit latrine is the spot where the construction has to take place. Garcia-Becerra *et al.*

(2021) suggest that The horizontal distance between the pit latrine and groundwater should be 30 meters to limit the microbial contamination. A pit latrine should be constructed downhill from any drinking water sources. The ground in the surrounding area may consist of a fissured rock and this would allow contaminants to easily move through the rocks and find their way into the groundwater. Therefore, it is suggested that pit latrines should be built around 33 yards from the water sources, but depending on the local geographical conditions, more distance may be recommended.

Paul *et al.* (2020) explains that the degree of pathogen removal tends to differ with the aquifer type, soil type, and other environmental factors such as humidity and temperature. Therefore, this research highlights that it is challenging to estimate the distance between pit and water source that can be regarded as safe. Although these safe distances have been suggested in different contexts, those building the pit latrines tend to ignore them in most instances. According to WHO recommendations, the distance between the groundwater level and the bottom of the pit should be at least two meters. Scholars such as Gokçekuş *et al.* (2020) argue that the distance between the bottom part of the pit latrine and the water table should be between 3 and 50 meters. Therefore, these researchers suggest that the water table is one of the most important parameters to consider when deciding on the spot to build a pit latrine.

There are two main categories of water sources which include protected (improved) and non-protected types. In improved water source, by nature of its construction is safe from external contamination by contaminants such as human excreta. Protected water sources are mainly covered to avoid entry of biological and chemical contaminants. If there is no protection, poor sanitation management is likely to affect the quality of water (Othoo *et al.*, 2020).

Additionally, insufficient protection also makes the water vulnerable to both chemical and biological contaminants from seepages and agricultural activities taking place in the area. Notably, poor sanitation practices increase the risk of infectious disease spread that include typhoid, cholera, and schistosomiasis.

Recent research by Saijuntha *et al.* (2021) suggests that 10% of individuals in developing countries are severely infected with intestinal worms that result from poor management of human excreta. In Kenya, it is estimated that around \$27 million is used annually on sanitation-related illnesses (Othoo *et al.*, 2020).

From 1990 to 2015, sanitation facilities in Kenya have increased and improved from 25% to 30 % (Gudda *et al.*, 2019). However, this is an extremely low type of change. Rural areas and urban areas all face different types of challenges. Peri-urban areas in Kenya are characterized by compromised groundwater because it has excess levels of chloride, nitrate, and microbial pathogens. There is a general concern that microbial and chemical contaminants can find their way into groundwater sources from pit latrines, which directly threatens human health. Early studies have indicated that groundwater quality has been progressively deteriorating in the past decade. Therefore, ensuring that the groundwater is protected from contamination or pollution from the pit latrines is critical. The findings of this study provided critical insights into the water quality situation in the Tigania West Sub County and inform the development of evidence-based policies and strategies to improve the availability and safety of drinking water in the area. Groundwater contamination is a pressing public health concern, particularly in areas where sanitation infrastructure is poorly regulated (Torondel *et al.*, 2016). Contaminants from pit latrines, including enteric pathogens, nitrates, and heavy metals, can infiltrate groundwater supplies, especially where the water table is shallow or geological conditions favor seepage. The physicochemical quality of groundwater is critical for

assessing its safety for consumption, as parameters such as pH, turbidity, total dissolved solids, nitrates, and coliform counts indicate the presence of pollutants (Nenninger *et al.*, 2023). WHO drinking water standards provide benchmarks for safe concentrations of these indicators. Deviation from these standards not only threatens human health but also undermines sustainable development goals, particularly SDG 6.1 and 6.3, which aim to ensure safe water access and reduce pollution through improved sanitation (Libby *et al.*, 2020).

In Tigania West Sub-County of Meru County, pit latrines remain the dominant sanitation method in both rural homesteads and institutions. However, the region is also highly dependent on shallow wells and boreholes for domestic water use due to limited piped water infrastructure (Orner *et al.*, 2018). These water sources are frequently located near pit latrines, especially in densely populated market centers and schools, increasing the risk of contamination. Despite the region's reliance on groundwater, systematic studies examining the spatial distribution of latrines relative to water points and associated health risks remain limited. Residents continue to experience outbreaks of waterborne diseases, raising concerns about potential groundwater pollution from fecal waste (Gwenzi *et al.*, 2023). A targeted understanding of local sanitation infrastructure and hydrogeological vulnerabilities is therefore essential in informing effective interventions (Kumwenda, 2019).

1.2 Statement of the Problem

Groundwater is a critical source of drinking water for many communities in rural areas of developing countries, but it is often at risk of contamination from various sources, including pit latrines (Moshi *et al.*, 2022). In Tigania West Sub County, Meru County, Kenya, pit latrines are commonly used for sanitation, but there is limited information on the potential impact of these latrines on the quality of groundwater sources used for

drinking and other domestic purposes (Omarova, *et al.*, 2018; Segun and Raimi, 2021). This knowledge gap is of concern, as exposure to contaminated water can result in various waterborne diseases and can also affect the physicochemical characteristics of groundwater (WHO, 2021).

To address this gap, this study aims to investigate the impact of pit latrines on enteric-pathogen and physicochemical contamination of groundwater in Tigania West Sub County, Meru County, Kenya. Specifically, the study aims to assess microbial characteristics of groundwater sources, compare the characteristics of sources located near and far from pit latrines, evaluate the compliance of the sources with WHO drinking water quality guidelines, and analyze the physicochemical characteristics of groundwater sources (WHO, 2017). The investigation focused on determining the presence of microbial indicators and physicochemical parameters in groundwater samples from areas with and without pit latrines in Tigania West Sub-County, Meru County, Kenya.

1.3 Research Questions

- i. What is the spatial relationship between pit latrine locations and groundwater sources and how does this relate to the prevalence of waterborne diseases in Tigania West Sub-County, Meru County, Kenya?
- ii. What are the physicochemical properties of groundwater in Tigania West Sub-County, and how do they compare with WHO drinking water quality guidelines?
- iii. How are enteric pathogens, groundwater physicochemical characteristics and pit latrine locations interrelated in contributing to the disease burden within Tigania West Sub-County, Meru County, Kenya?

1.4 Objectives

1.4.1 General Objective

To investigate the effect of pit latrines on enteric-pathogen and physicochemical contamination of groundwater in Tigania West Sub County in Meru County, Kenya.

1.4.2 Specific Objectives

- i. To assess the spatial relationship between pit latrine locations and groundwater sources, as well as the prevalence of waterborne diseases in Tigania West Sub County in Meru County, Kenya.
- ii. To determine the ground water physical chemicals properties as per the WHO drinking water guidance in Tigania West Sub-County in Meru County, Kenya.
- iii. To examine the relationship among enteric-pathogen, physicochemical properties and location of pit latrines with disease burden within Tigania West Sub-County in Meru County, Kenya.

1.5 Significance and Justification of the Study

1.5.1 Significance of the Study

The importance of this research stems from the potential implications of its findings in improving public health outcomes in Tigania West Sub County, Meru County, Kenya. The potential contamination of groundwater by enteric pathogens from pit latrines has substantial implications for public health. Diarrhoeal diseases, primarily caused by waterborne pathogens, remain a significant cause of morbidity and mortality, especially in developing countries. Moreover, the increasing physicochemical degradation of water sources further exacerbates the health risk.

However, the study's significance is not just confined to the health domain. Understanding the impact of pit latrines on groundwater contamination can inform policy and community initiatives aimed at improving sanitation infrastructure. The results may

stimulate action towards innovative, environmentally friendly, and sustainable sanitation solutions, thereby leading to healthier communities and a safer environment.

1.5.2 Justification of the Study

The justification of this study is deeply rooted in the correlation between water quality and public health, addressing a critical knowledge gap in the understanding of the extent and potential health impacts of enteric-pathogen contamination of groundwater from pit latrines in Tigania West Sub County, Meru County, Kenya.

The utilization of pit latrines is widespread in the region; however, there has been limited investigation into the potential for groundwater contamination and associated health risks. This study aims to provide crucial evidence necessary for assessing the situation, evaluating risks, and informing future interventions to improve sanitation infrastructure, in alignment with Sustainable Development Goal (SDG) 6, which seeks to ensure the availability and sustainable management of water and sanitation for all.

This study is further justified by the reliance of the region on groundwater as a primary source of water. Understanding the scope of contamination and its potential severity is a critical step towards devising solutions to mitigate it. This aligns with the aims of SDG 6 to "achieve access to adequate and equitable sanitation and hygiene for all and end open defecation."

Exploring the relationship between enteric pathogens, physicochemical characteristics, and waterborne diseases in the study can provide insights into factors influencing the prevalence of such diseases. These findings can inform targeted public health interventions and contribute towards ensuring the safe use of groundwater resources for domestic purposes, resonating with SDG 3's commitment to ensure healthy lives and promote well-being for all.

Finally, the engagement of local communities through questionnaires is an important aspect of this study. It is designed to raise awareness about water safety and encourage proactive improvement of sanitation practices. This action-oriented approach contributes not only to SDGs 3 and 6 but also to SDG 11's aspiration to make cities and human settlements inclusive, safe, resilient, and sustainable. Consequently, the study's justification extends beyond its scientific contributions, emphasizing its potential to support the realization of key Sustainable Development Goals and its anticipated positive social impact.

1.6 Study Limitations

This study faced several limitations. Firstly, due to resource constraints, the number of groundwater samples collected and tested may not fully represent all households in the region, potentially limiting the generalizability of the findings. Secondly, seasonal variations in rainfall and groundwater levels were not consistently monitored throughout the year, which may affect the consistency of the contamination levels observed. Thirdly, the reliance on self-reported cases of waterborne diseases from community health facilities may introduce reporting bias or underreporting, particularly in remote areas with limited access to healthcare services. Additionally, spatial mapping and GIS analyses depended on the accuracy of GPS coordinates and satellite imagery, which may have slight errors in positioning. Lastly, although this study sought to examine multiple variable physicochemical properties, pathogen presence, and latrine locations, the complex interplay of socio-economic and behavioral factors influencing sanitation practices was beyond the scope of this study.

CHAPTER TWO: LITERATURE REVIEW

2.0 Introduction

This study explores literature review on the relationship between pit latrines and groundwater contamination with a focus on five key areas: the impact of pit latrines on groundwater, enteric pathogens associated with groundwater, physicochemical water quality parameters, health challenges related to fecal contamination of groundwater, and intervention measures.

2.1 Overview of Impact of pit Latrines on Groundwater

Groundwater is a critical source of drinking water for many communities in rural areas of developing countries. Still, it is often at risk of contamination from various sources, including pit latrines (Moshi *et al.*, 2022). Pit latrines are commonly used for sanitation in low-income areas where access to centralized sewer systems is limited (Simiyu *et al.*, 2020). However, improper management and maintenance of pit latrines can lead to groundwater contamination, which serves as a significant drinking water supply in most developing countries (Nadimpalli *et al.*, 2020).

One potential source of groundwater contamination is pit latrines, which can release enteric pathogens into the subsurface. These pathogens can contaminate groundwater sources, leading to waterborne diseases such as cholera, typhoid, and dysentery (Masindi and Foteinis, 2021). Studies in Tanzania, Zimbabwe, Ethiopia, and Kenya have found that groundwater in areas with pit latrines is often contaminated with fecal coliforms and other bacteria (Masindi and Foteinis, 2021; Gebrewahd *et al.*, 2020; Opere *et al.*, 2021; Zerbo *et al.*, 2020; Sila, 2019; Titcomb *et al.*, 2021).

In addition to microbial contamination, pit latrines can also affect the physicochemical characteristics of groundwater. For example, leachate from pit latrines can alter the pH of groundwater and increase its turbidity and color. These changes can affect the

solubility and availability of nutrients and minerals in the water, as well as the toxicity of certain substances. High levels of Biological Oxygen Demand (BOD) in groundwater can also indicate high levels of organic pollution from pit latrines (Salaudeen *et al.*, 2018).

In light of these findings, it is important to carefully consider the location of pit latrines in relation to groundwater sources when constructing pit latrines. Studies have suggested safe distances between pit latrines and groundwater sources to limit microbial contamination. For example, Back *et al.* (2018) suggest that the horizontal distance between a pit latrine and groundwater should be at least 30 meters, while Gokçekuş *et al.* (2020) argue that the distance between the bottom of a pit latrine and the water table should be between 3 and 50 meters. However, these distances may vary depending on local geographical conditions and other environmental factors such as soil type, humidity, and temperature (Usman and Aliyu, 2020).

2.2 Enteric Pathogens Associated with Groundwater

Enteric pathogens, a diverse group of microorganisms, represent a significant concern in public health due to their potential to induce illness upon ingestion through contaminated food or water sources. These pathogens encompass various microbial species, each with distinct characteristics and health implications. Among the notable enteric pathogens are total Coliforms, faecal Coliforms, *Escherichia coli* (*E. coli*), *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus*, *Streptococcus faecalis*, and helminthes (Varghese *et al.*, 2024).

2.2.1 Assessment of total coliforms in groundwater

Total coliforms serve as indicators of the sanitary condition of water and possible presence of pathogens (Amoah *et al.*, 2020). They are found in the environment, including soil and vegetation, and are also present in the gut of warm-blooded animals.

Being ubiquitous in nature, they can easily contaminate water sources. Their presence, therefore, does not necessarily indicate recent fecal contamination but could suggest a potential conduit for enteric pathogens.

Detection of coliforms is the primary step in assessing the microbiological quality of drinking water (Amoah *et al.*, 2020). Their presence may indicate failures in water treatment processes, breaches in the distribution system, or fecal contamination. However, since total coliforms themselves are not necessarily harmful, the U.S. Environmental Protection Agency revised its drinking water regulations to require assessment for *E. coli*, a more direct indicator of fecal contamination. Despite this, total coliform testing remains a valuable preliminary screening tool in many contexts globally.

2.2.2 Fecal coliforms associated with groundwater contamination

Fecal coliforms, such as *E. coli*, are a subset of the total coliform group and are more specifically associated with fecal material from warm-blooded animals. The presence of fecal coliforms in water is a more direct indication of fecal contamination than the presence of total coliforms. Fecal coliforms are capable of growth at higher temperatures, which aligns more closely with the internal temperature of warm-blooded animals and less with the environmental conditions. Therefore, they provide a more accurate measure of fecal contamination and potential presence of enteric pathogens in water (WHO, n.d.). Testing for fecal coliforms has long been a standard method for assessing the microbiological quality of water. Their presence is an indication that other disease-causing pathogens may also be present, leading to a higher risk of waterborne diseases. Despite recent shifts towards more specific indicators like *E. coli*, fecal coliform testing remains an important component of comprehensive water quality monitoring (Osiero *et al.*, 2019).

2.2.3 *Escherichia coli* (*E. coli*)

E. coli is a member of the total coliform group and is commonly found in the intestines of humans and other warm-blooded animals. The majority of *E. coli* strains are harmless and even play a vital role in a healthy human intestinal tract. However, certain strains can cause illnesses such as diarrhea, urinary tract infections, and pneumonia (Centers for Disease Control and Prevention [CDC], 2019).

Most importantly, *E. coli* is a more specific indicator of fecal contamination than total coliforms, as it is primarily found in the gut and feces of warm-blooded animals. As such, the presence of *E. coli* in water is a strong indication of recent fecal contamination, which may carry other bacteria, viruses, and parasites causing disease. Therefore, *E. coli* testing is recommended as a more accurate method of assessing the sanitary quality of drinking water (Arias and Murray, 2020).

2.2.4 *Salmonella* role in groundwater

Salmonella is a group of bacteria that can cause illnesses such as typhoid fever, paratyphoid fever, and food poisoning. The bacteria are usually transmitted to humans through contaminated food or water, particularly in areas with poor sanitation (CDC, 2020).

Salmonella can be found in the gut of both humans and animals, and hence, its presence in water indicates fecal contamination. Despite improvements in sanitation, *Salmonella* continues to be a significant cause of morbidity and mortality globally. It is essential to test for the presence of this bacteria in drinking water, particularly in areas with poor sanitation, as it can lead to severe health consequences (Hassan *et al.*, 2022).

2.2.5 Shigella role in groundwater contamination

Shigella is another group of bacteria that causes shigellosis, a diarrheal disease. Shigella is usually found in water polluted with human feces, making it a significant concern in areas with poor sanitation and crowded living conditions (CDC, 2019).

Shigellosis is highly contagious and can spread through direct contact with the bacteria in the stool. For this reason, it is often found in water after it has been contaminated with the feces of an infected person. Therefore, testing for Shigella in drinking water is crucial to prevent the spread of the disease, especially in areas with poor sanitation (Thompson *et al.*, 2021).

2.2.6 Staphylococcus aureus

Staphylococcus aureus is a bacterium that can cause various illnesses, from minor skin infections to life-threatening diseases like pneumonia, endocarditis, and sepsis. Although *S. aureus* is typically associated with hospital-acquired infections, it can also be found in the environment, including water (CDC, 2019).

S. aureus can be resistant to a range of antibiotics, including methicillin (MRSA), complicating treatment when infections occur. Although it's not typically considered an indicator organism for water quality like *E. coli* or other coliforms, its presence in water still poses a significant public health concern, particularly where immune-compromised individuals may be exposed (Foster *et al.*, 2022).

2.2.7 Streptococcus faecalis

Streptococcus faecalis, now known as *Enterococcus faecalis*, is a common bacterium found in the gut of humans and other mammals. It can cause a variety of infections, including urinary tract infections, endocarditis, and wound infections (CDC, 2019).

E. faecalis is a robust organism that can survive in environments with a wide range of temperatures and pH levels, making it a valuable indicator of water quality. Its presence

in water signifies fecal contamination and a potential risk of other pathogenic organisms. *E. faecalis* is also known for its resistance to many antibiotics, making infections difficult to treat (Khan *et al.*, 2019).

2.2.8 Helminths

According to the CDC (2020), helminths, or parasitic worms, are a diverse group of organisms that can infect humans, causing various diseases. The three main types of helminths that infect humans are flatworms (trematodes), roundworms (nematodes), and tapeworms (cestodes). Helminths are typically transmitted through contact with contaminated soil (soil-transmitted helminths) or contaminated water (water-based helminths).

Infected individuals excrete helminth eggs in their feces, which can contaminate soil or water if not properly managed. As such, the presence of helminths in water is a strong indicator of fecal contamination and a significant public health concern, particularly in areas with poor sanitation or where water treatment is inadequate. Helminth infections can lead to malnutrition, anemia, and impaired physical and cognitive development, making them a major contributor to the global burden of disease (Hotez *et al.*, 2022).

2.3 Physicochemical Water Quality Parameters

Physicochemical water quality parameters refer to the physical and chemical characteristics of water that can affect its quality and suitability for various uses, including pH, color, odor, turbidity, and Biological Oxygen Demand (BOD). By analyzing these parameters, we hope to provide valuable insights into the potential impact of pit latrines on the physicochemical contamination of groundwater sources and ensure the safety and health of the local population.

2.3.1 pH and quality of water

The pH scale quantifies the acidic or basic properties of water. It ranges from 0 (strongly acidic) to 14 (strongly alkaline), with a pH of 7 being neutral (WHO, n.d.). Changes in the pH levels of groundwater could significantly impact the solubility of minerals and nutrients, potentially altering the water's taste, corrosiveness, and toxicity (WHO, 2021).

In the context of groundwater contamination, pH levels play an essential role in determining the survival and proliferation of pathogens. For instance, some pathogens thrive in acidic conditions, while others prefer more basic environments (Liu *et al.*, 2023). Furthermore, pH can affect the solubility of heavy metals, with certain metals becoming more soluble and thus, potentially more hazardous, at lower pH levels (Sawyer *et al.*, 2022). These factors make pH an important indicator of potential groundwater contamination.

2.3.2 Colour

The color of water can provide initial information about its quality. Different substances, like dissolved organic matter, minerals, and algae, may give water its color (WHO, 2021). Excessive color in water can indicate the presence of contaminants and detract from its aesthetic quality, thereby affecting its acceptance for drinking and other household uses (Edokpayi *et al.*, 2022).

Furthermore, water discoloration can suggest issues with water treatment processes or signal contamination from industrial waste, agricultural runoff, or domestic sewage (Parker & Sommer, 2011). Therefore, monitoring water color plays a key role in early detection of contamination and appropriate water treatment.

2.3.3 Odour

Odour is an essential sensory aspect of water quality evaluation. Different substances such as dissolved gases, organic matter, and chemicals could contribute to water's odor

(WHO, 2021). A noticeable odor in water may indicate contamination, possibly affecting its acceptability for consumption (Yang *et al.*, 2022).

Moreover, certain smells, like a sulfurous or "rotten egg" smell, could indicate the presence of hydrogen sulfide, which is often associated with sewage contamination. Conversely, a musty or earthy smell may suggest the presence of certain types of algae or bacteria (Lee *et al.*, 2022). Hence, odor detection serves as a vital step in water quality assessment and helps detect contamination early.

2.3.4 Turbidity

Turbidity in water refers to its cloudiness or haziness, often due to suspended particles (WHO, 2021). High turbidity can indicate the presence of sediment, organic matter, or pollutants, which could potentially harbor pathogens. Not only does turbidity affect the aesthetic quality of water, but it also hampers disinfection processes, since pathogens can hide behind suspended particles, making them more resistant to disinfectants (Kumar and Puri, 2022).

Furthermore, high turbidity levels may increase water temperature by absorbing more heat, which can influence the type and number of microorganisms in the water. Consequently, turbidity is an essential parameter in maintaining the quality and safety of water supplies (Huang *et al.*, 2024).

2.3.5 Biological oxygen demand (BOD)

BOD is a critical measure of organic pollution in water bodies. It signifies the amount of oxygen consumed by microorganisms in decomposing organic matter (WHO, 2021). High BOD values can indicate substantial organic pollution, potentially contributing to hypoxic or anoxic conditions detrimental to aquatic life (Smith *et al.*, 2023).

Moreover, elevated BOD levels can imply the presence of fecal pollution, as human waste is rich in organic material that increases BOD (Rossi *et al.*, 2023). Thus, BOD

serves as an indirect measure of potential fecal contamination, which is especially relevant when evaluating the impact of pit latrines on groundwater quality.

2.4 Health Challenges Related to Fecal Contamination of Groundwater

Pit latrines are commonly used in many parts of the world as a means of sanitation, particularly in areas without access to centralized sewer systems (Zhou *et al.*, 2022).

However, improper management and maintenance of pit latrines can lead to groundwater contamination, which serves as a significant drinking water supply in most developing countries (Nadimpalli *et al.*, 2020). Inadequate sanitation facilities, including pit latrines, have been identified as a major cause of illnesses transmitted through contaminated water sources, such as cholera, typhoid fever, and hepatitis A (Prüss-Üstün *et al.*, 2019).

When groundwater is contaminated with feces, enteric pathogens such as total coliforms, *E. coli*, Salmonella, Shigella, Staphylococcus aureus, Streptococcus faecalis, and Helminths may be present. These pathogens can cause various waterborne diseases and pose a significant risk to public health. Several studies have examined the relationship between enteric pathogens isolated from groundwater and the disease burden within communities. For example, a study by Omondi (2021) in Kenya found that faecal contamination of groundwater was associated with an increased risk of diarrhoea. Another study by Manetu and Karanja (2021) in Kenya found that faecal contamination of groundwater was linked to an increased risk of cholera.

In addition to microbial contamination, fecal contamination of groundwater can also affect its physicochemical characteristics. For example, leachate from pit latrines can alter the pH of groundwater and increase its turbidity and color. These changes can affect the solubility and availability of nutrients and minerals in the water, as well as the toxicity of certain substances. High levels of BOD in groundwater can also indicate high levels of organic pollution from pit latrines (Suleiman, 2019).

By achieving objectives iii and iv of this study - relating isolated enteric pathogens and physicochemical characteristics with disease burden within the community of Tigania West Sub County in Meru County - we aim to provide valuable insights into the potential health risks associated with using groundwater contaminated by pit latrines. This information can inform public health interventions and water treatment strategies.

2.5 Intervention Measures

There are a number of different projects that have been offered as potential solutions in order to decrease the detrimental impact that pit latrines have on the quality of groundwater. These interventions include the utilization of lined pit latrines, composting toilets (Saxena and Den, 2022), urine-diverting toilets (Stopelli et al., 2021), septic tanks (Banu et al., 2021), and decentralized wastewater treatment systems (Zhou et al., 2022). These are only few of the interventions that fall under this category. The employment of these measures is anticipated to avoid the leaching of faecal matter and other contaminants into groundwater. This is something that is predicted to be prevented. Nevertheless, their efficiency and practicability are contingent upon their being employed and maintained in the appropriate manner. This is the case.

The installation of efficient management strategies for pit latrines is of the utmost necessity in order to reduce the quantity of groundwater contamination. This is in addition to the measures that have already been taken. A few examples of these practices include the following: lining the pit with materials that are impermeable (Gray, 2020; Prommachote et al., 2022), desludging the pit on a regular basis (Burt et al., 2019), and disposing of the sludge in an acceptable manner (Ezenwaji and Ezenweani, 2019; Ogbeifu et al., 2019). These are just a few examples. In spite of the fact that these activities have been suggested to be effective in reducing groundwater contamination brought about by the utilization of pit latrines, there is a requirement for additional

research to evaluate the effectiveness of these practices in a variety of distinct environments and circumstances.

2.6 Summary of Literature and Identified Gaps

The reviewed literature demonstrates that pit latrines contribute significantly to groundwater contamination through leaching of enteric pathogens and alteration of physicochemical parameters such as pH, turbidity, color, and Biological Oxygen Demand. Previous studies conducted in Tanzania, Zimbabwe, Ethiopia, and parts of Kenya consistently highlight the risk of groundwater contamination and associated health challenges such as cholera, typhoid, and diarrhea. Intervention measures, including lined pits and alternative sanitation technologies, have been proposed, but their uptake and effectiveness vary depending on local environmental and socio-economic contexts.

Despite these insights, several knowledge gaps remain. First, most existing studies focus on generalized contamination risks without adequately integrating the spatial relationship between pit latrine locations, groundwater sources, and community health outcomes, particularly in rural sub-counties such as Tigania West. Second, while enteric pathogens and physicochemical water parameters have been studied independently, few studies have examined the combined influence of microbial and physicochemical contamination in relation to WHO drinking water standards. Third, there is limited empirical evidence linking the specific hydrogeological context of Tigania West to the burden of waterborne diseases associated with pit latrine use. This study therefore seeks to address these gaps by investigating the influence of distances of pit latrines on enteric pathogens and physicochemical contamination of groundwater sources in Tigania West Sub-County within Meru County, Kenya.

CHAPTER THREE: METHODOLOGY

3.1 Introduction

This section describes the methodology used in the study as guided by the objectives. It covers the study area, the study design and population, the sampling procedure, sample size determination, analysis, data interpretation, and ethical considerations.

3.2 Study Area

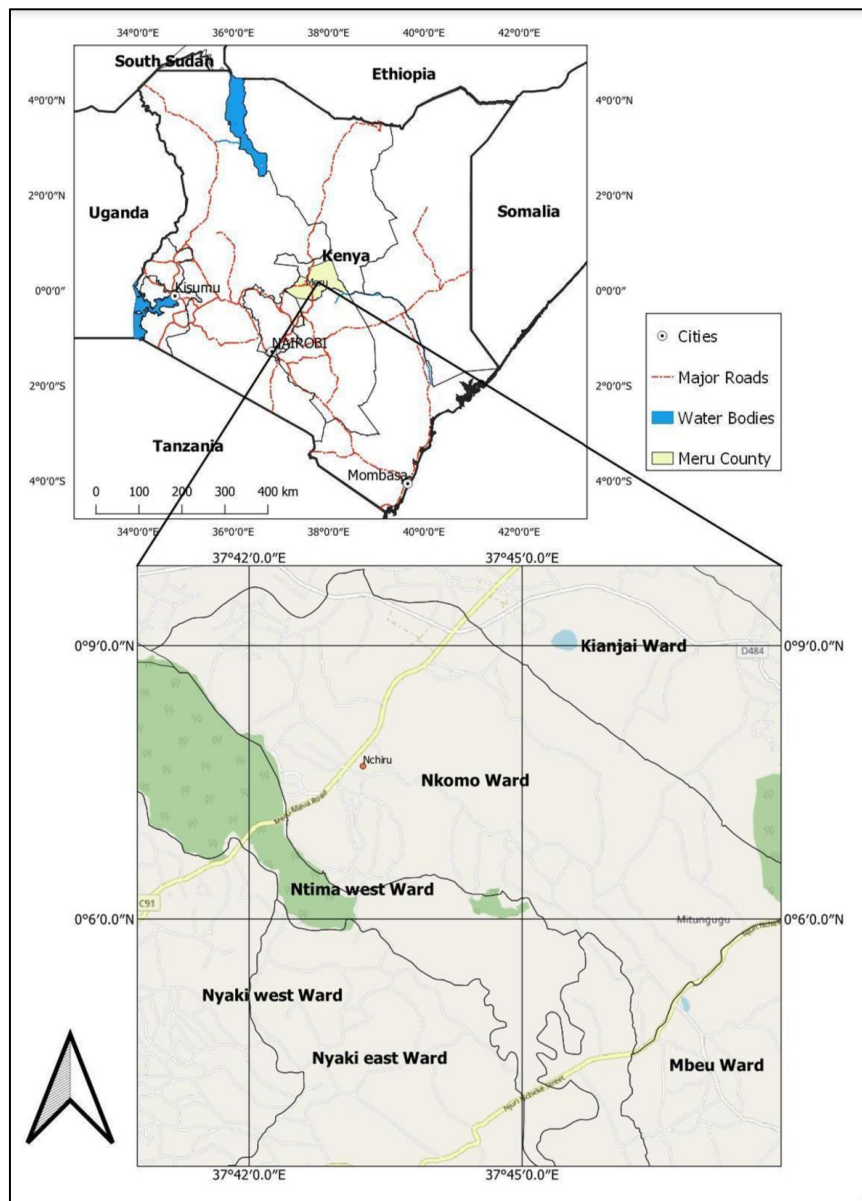
This research was carried out in Tigania West Sub-County, Meru County, in Kenya (Figure 3.1). The study employed a cross-sectional design, where data was collected from the targeted population at a single point in time and analyzed in response to the study questions. This design is appropriate for assessing the prevalence of enteric pathogens in groundwater sources and their relationship with disease burden within the community.

Data was collected using a combination of methods, including a structured questionnaire to gather information from local residents on their knowledge, attitudes, and practices regarding pit latrines and groundwater hygiene, as well as laboratory tests to analyze water samples for the presence of enteric pathogens and physicochemical parameters.

The questionnaire was administered by trained research assistants who visited households in the study area to collect information. Water samples were collected from groundwater sources near pit latrines (<30 meters) and at a safe distance (>30 meters) from pit latrines using sterile techniques. The collected data was analyzed using descriptive and inferential statistics to address the research objectives and draw conclusions about the impact of pit latrines on enteric-pathogen contamination of groundwater in Tigania West Sub County, Meru County, Kenya.

Figure 3. 1

Map of Tigania West Sub- County, Meru County, Kenya



3.3 Research Design

This study employed a cross-sectional analytical design to investigate the effect of pit latrines on enteric-pathogen and physicochemical contamination of groundwater in Tigania West Sub-County, Meru County, Kenya. The design facilitated simultaneous data collection from the target population at a single point in time, enabling the assessment of contamination prevalence and its association with pit latrine proximity and community practices (Wang & Cheng, 2020).

3.4 Sampling Technique

The study employed a convenient sampling technique as explained by Stratton (2021), where samples were collected from groundwater sources (wells and boreholes) near pit latrines (<30 meters) and at a safe distance (>30 meters) from pit latrines. The sampling collection area was identified using Google Maps to pinpoint the exact location of each sample collection point. The owners of the wells and boreholes were required to give informed consent to allow samples to be collected from their groundwater sources.

Water samples were collected using a sterile 500 mL glass bottle, which was aseptically opened to avoid contamination. The bottles were sterilized by autoclaving at 121°C for 15 minutes, and the mouth was flamed using an alcohol cotton swab to ensure sterility. Water from wells were collected using a sterile bucket and aseptically poured into the collection bottle. In contrast, water from boreholes was collected directly from the tap after flaming the nozzle. Three water samples (triplicates) were collected from each source to ensure the accuracy and reliability of the results.

It is important to note that the distance between the pit latrine and groundwater sources can vary depending on local geographical conditions, such as the type of aquifer, soil type, and environmental factors such as humidity and temperature (Usman and Aliyu, 2020). Therefore, it can be challenging to estimate a safe distance that is universally applicable. However, following WHO recommendations and suggestions from researchers such as Gokçekuş *et al.* (2020), a distance of at least two meters between the groundwater level and the bottom of the pit, and between 3 and 50 meters between the bottom of the latrine and the level of groundwater is generally considered safe.

3.5 Sample Size

The sample size for this research was 96 households, distributed among all the wards in Tigania West sub-county. The wards include Athwana, Akithii, Kianjai, Nkomo, and

Mbeu. The sample size was calculated using adjusted Fisher's formula for calculating sample size for a proportion (Fischer *et al.*, 2019), with a desired precision of 0.1 (margin of error), a desired confidence level of 95%, and an estimated population proportion of 50%.

Using this formula, the sample size for this research can be computed in the following manner:

$$n = Z^2 * p * (1 - p) / d^2 \quad (1)$$

where:

n = the sample size

Z = the Z-score for the desired confidence level (1.96 for 95% confidence)

p = the estimated proportion of the attribute present in the population (50% in this case)

d = the desired precision (0.1 in this case)

Plugging in these values, we get:

$$n = 1.96^2 * 0.5 * (1 - 0.5) / 0.1^2$$

$$n = 96.04$$

$$n \approx 96$$

Therefore, based on our desired precision, confidence level, and estimated population proportion, we have calculated that a sample size of approximately 96 households were sufficient for this study.

The sample size of 96 households was distributed among the five wards in Tigania West sub-county to ensure that the study sample is representative of the population of interest. The specific number of samples that were collected from each ward (Table 3.1) were determined based on the population size of the study area according to the 2019 census data from the Kenya National Bureau of Statistics (KNBS).

Table 3. 1*Distribution of Sample Households across Different Wards in Tigania West Sub-County*

Ward	Population	Proportion of Total Population	No. of Sample
Athwana	18,127	$(18,127 / 135,980) * 100 = 13.33\%$	$(13.33 / 100) * 96 = 13$
Akithii	35,646	$(35,646 / 135,980) * 100 = 26.21\%$	$(26.21 / 100) * 96 = 25$
Kianjai	36,874	$(36,874 / 135,980) * 100 = 27.12\%$	$(27.12 / 100) * 96 = 26$
Nkomo	24,361	$(24,361 / 135,980) * 100 = 17.92\%$	$(17.92 / 100) * 96 = 17$
Mbeu	20,972	$(20,972 / 135,980) * 100 = 15.42\%$	$(15.42 / 100) * 96 = 15$
Total	135,980		

Kenya National Bureau of Statistics, ([KNBS], 2019)

The calculations have been made to determine the proportion of the total population in each area, with values rounded to three decimal places. These proportions have been adjusted to provide whole numbers, resulting in the number of sampled households per ward.

3.6 Eligibility Criteria

The eligibility criteria for this study defined the characteristics that households must have in order to be included in the study. These criteria was used to ensure that the study sample is representative of the population of interest and that the data collected was relevant to the research objectives.

3.6.1 Inclusion Criteria

The study included 96 households with boreholes in Tigania West Sub County, Meru County who consented to participate in the study.

3.6.2 Exclusion Criteria

All households who did not consent to participate in the study were excluded. This complied with the Helsinki Declaration adopted by the World Medical Association in 1964 (Shrestha and Dunn, 2019). People who had not resided in the study area for more than six months and individuals of unsound mind were excluded from the study.

3.7 Data collection

Data was collected from respondents about their pit latrines and boreholes using a structured questionnaire manually (*See Appendix 3*). The questionnaire was designed to capture details on the participant's socio-demographic profile and their practices related to pit latrines and groundwater hygiene. The focus was on gathering information about the existence of a pit latrine in the participant's household, its construction oversight, its proximity to groundwater sources, and the measures implemented to maintain the safety of these water sources. The role of the pit latrine in groundwater hygiene, as well as the sanitation practices followed in its construction and use, was also probed. The questionnaire was administered by trained research assistants who obtained informed consent from participants (*See Appendix 4*).

3.7.1 Water samples collection

Water samples were collected to determine microbial contamination. The water samples were drawn from groundwater sources (wells and boreholes) near pit latrines (<30 meters) and at a safe distance (>30 meters) from pit latrines, as suggested by Garcia-Becerra *et al.* (2021). A sterile 500 mL glass bottle was used, where the bottle was aseptically opened. The bottles were sterilized by autoclaving at 121°C for 15 minutes. The mouth of the bottles was flamed using alcohol cotton swab to ensure the mouth is sterile in order to avoid contamination. Water from wells was collected by sterile bucket and aseptically poured into a collection bottle laid on the flat surface. Water from bore hole was collected from the tap. The nozzle of the tap was flamed first then water was collected to into the sterile bottle. Three water samples (triplicates) were collected from each source.

It is important to note that the distance between the pit latrine and groundwater sources varied depending on local geographical conditions, such as the type of aquifer, soil type,

and environmental factors such as humidity and temperature (Usman and Aliyu, 2020). Therefore, it was challenging to estimate a safe distance that is universally applicable. However, following WHO recommendations and suggestions from researchers such as Gokçekuş *et al.* (2020), a distance of at least two meters between the groundwater level and the bottom of the pit, and between 3 and 50 meters between the bottom of the latrine and the level of groundwater was generally considered safe.

3.7.2 Water transportation

Immediately after collection, the water samples were transported to the laboratory in a cooler with ice packs to prevent any change in temperature that could affect the viability of the bacteria (Ogbeifun *et al.*, 2019).

3.8 Materials

In order to conduct the proposed research, a variety of materials were required. These materials include reagents, equipment, and instruments that were used to collect and analyse water samples. The analyses were conducted in laboratories at Meru University, including the chemistry laboratory and the microbiology laboratory, which were equipped to perform the necessary tests.

The necessary tests that were performed using these materials included microbiological tests to isolate and identify enteric pathogens in water samples (such as total coliforms, *E. coli*, *Salmonella*, *Shigella*, *Staphylococcus aureus*, *Streptococcus faecalis*, and Helminths), as well as physicochemical analysis of water samples to measure parameters such as pH, colour, odour, turbidity, and BOD.

3.8.1 Reagents

Reagents are chemical substances that are used to perform tests on water samples. Some common reagents that may be required for this research include pH buffers, colourimetric reagents, microbiological media, lactose broth, MacConkey broth,

Tryptone Bile X-glucuronide (TBX) agar, Kovac's reagent, selenite cystine broth, Xylose Lysine Deoxycholate (XLD) agar, Triple Sugar Iron (TSI) agar, Lysine Iron Agar (LIA), Baird-Parker agar, Bile Esculin Azide (BEA) agar, and Gram-Negative (GN) broth.

3.8.2 Equipment and instruments

Equipment and instruments are tools that are used to collect and analyse water samples. Some common equipment and instruments that may be required for this research include sampling bottles, thermometers, pH meters, turbidimeters, spectrophotometers, BOD bottles, colorimeters, odor wheels, membrane filters, and microscopes.

3.9 Isolation and Identification of Enteric Pathogens

Microbiological tests were conducted to isolate and identify enteric pathogens in water samples. These tests involved the use of various culture media and biochemical assays to detect the presence of specific pathogens, such as total coliforms, *E. coli*, Salmonella, Shigella, Staphylococcus aureus, Streptococcus faecalis and Helminths.

The isolation and identification of enteric pathogens in water samples is an important step in assessing water's microbial quality and identifying potential contamination sources. These tests can provide valuable information on the presence and abundance of specific pathogens in water samples, which can be used to inform public health interventions and water treatment strategies.

3.9.1 Total coliforms

Analysis was done as described by Bekuretsion *et al.* (2018). The Most Probable Number (MPN) method was used to ascertain total coliforms in water samples. This method involves a series of presumptive, confirmed, and completed tests to determine the quantity of coliform bacteria in a water sample.

Before water is inoculated, it was completely mixed through the inversion of the bottles several times. The cap of the bottles was opened and flamed then water samples inoculated in MacConkey broth. The water was then be incubated at 44 °C for 24 hours. After one day the samples were observed for color change.

Table 3. 2

Analysis Set Up for Total Coliforms

Sample Type	No. of Bottles	mL of Broth	Strength of Broth
Untreated Water	1	50	Double
Treated Water	5	10	Double
Ground water	5	5	Single

Source: Researcher (2024)

Table 3.2 above shows the analysis set up for total coliforms using the MPN method (Bekuretsion *et al.*, 2018). The first column indicates the tested sample type (in this case, untreated water, treated water, and groundwater). The second column indicates the number of bottles used for each test. The third column indicates the volume of broth used for each test. The fourth column indicates the strength of the broth used for each test (double strength or single strength). The fifth column indicates the incubation time for each test.

The MPN method involves several steps to estimate the number of coliform bacteria in a water sample (Bekuretsion *et al.*, 2018). In the presumptive test, multiple lactose broth tubes are inoculated with water samples and incubated at 35°C for 48 hours. Tubes showing gas production are considered positive for coliform bacteria. In the confirmed test, positive tubes from the presumptive test are sub-cultured on selective media such as Eosin Methylene Blue (EMB) agar or Endo agar to confirm the presence of coliform bacteria. In the completed test, colonies from positive confirmed tests are sub-cultured on nutrient agar slants and subjected to biochemical tests such as Gram staining and indole production to confirm the presence of coliform bacteria. After 24 hours of

incubation, positive water samples were subcultured on Tryptone Bile X-glucuronide (TBX) agar to obtain pure colonies (Bekuretsion *et al.*, 2018).

3.9.2 *E. coli*

In accordance with the methodology outlined by Gebrewahd *et al.* (2020), the isolates was subjected to an Indole test, an effective method of confirming *E. coli* presence. The testing process began with careful inoculation of the organism into a bijou bottle container. This procedure requires precision to ensure an even distribution of bacteria in the medium and prevent any potential errors in the results.

Post-inoculation, the container was incubated at a consistent temperature of 37°C, mirroring optimal conditions for *E. coli* growth. After a period of up to 48 hours, Kovac's reagent was added. This chemical reacted with indole (if *E. coli* is present and has broken down tryptophan in the medium), yielding a red compound and thereby confirming the presence of *E. coli*.

3.9.3 Salmonella

The methodology included a systematic process for isolating and quantifying Salmonella from the water samples. The first step involved enriching the samples in selenite cystine broth, followed by incubation at 37°C for 24 hours (Brenner *et al.*, 2023). This specific enrichment and incubation helped to cultivate Salmonella while limiting other bacterial growth. The enriched samples were then streaked onto Xylose Lysine Deoxycholate (XLD) agar and incubated at the same temperature for a similar duration. This selective medium is ideal for the growth and identification of Salmonella colonies.

The quantification of Salmonella colonies was performed through the use of a colony counter or manually by counting the number of colonies that appear on the Xylose Lysine Deoxycholate (XLD) agar plates. This provided an estimate of the number of colony forming units (CFUs) per ml of the original water sample, which is a standard

unit of measurement for bacterial count. Suspected *Salmonella* colonies was subjected to further validation using specific biochemical tests such as Triple Sugar Iron (TSI) agar, and Lysine Iron Agar (LIA) for a more accurate determination. Each positive result from these tests represents one confirmed case of *Salmonella* in the tested water sample, thus providing a quantified measurement of *Salmonella* contamination (Rajkowski and Rice, 2022).

3.9.4 Shigella

In order to isolate *Shigella* from our water samples, standard microbiological techniques was utilised (Galanis, 2022; Gerhardt et al., 2023). Initially, the water samples were enriched in Gram-Negative (GN) broth, a medium that promotes the growth of Gram-negative bacteria like *Shigella* while inhibiting other types of bacteria. Following enrichment, the samples were incubated at a temperature of 37°C for a duration of 24 hours to foster bacterial proliferation.

During post-incubation, the enriched samples were streaked onto MacConkey agar, a selective and differential medium ideal for the isolation and differentiation of Gram-negative bacteria, including *Shigella*. These samples were incubated again at 37°C for 24 hours to allow for significant bacterial growth. Colonies suspected to be *Shigella*, based on their morphology and color, were subjected to further verification using biochemical tests such as Triple Sugar Iron (TSI) agar and Lysine Iron Agar (LIA), ensuring accurate identification of *Shigella*.

3.9.5 *Staphylococcus aureus*

For isolating and identifying *Staphylococcus aureus*, the water samples were initially be streaked onto Baird-Parker agar, a selective medium highly effective in promoting the growth of *S. aureus* while suppressing unwanted organisms. These samples were then

incubated at a steady temperature of 37°C for a period of 24-48 hours, facilitating the optimum growth of potential *S. aureus* colonies.

Following the incubation period, colonies that exhibit characteristics of *S. aureus* was selected and counted. The number of *S. aureus* colonies per plate was used to estimate the concentration of these bacteria in the original water sample, thereby quantifying the level of *S. aureus* contamination (Alexander *et al.*, 2022).

For definitive identification of *S. aureus*, colonies were subjected to further verification through biochemical tests such as coagulase and catalase tests. The coagulase test distinguishes *S. aureus* by its ability to clot plasma, and the catalase test helps differentiate it from other Gram-positive cocci by its ability to produce the enzyme catalase. This rigorous testing process ensures we accurately identify and quantify *S. aureus* in the samples.

3.9.6 *Streptococcus faecalis*

To isolate *Streptococcus faecalis* also referred to as *Enterococcus faecalis* from water samples, standard microbiological methods was applied. The procedure commences by streaking the water samples onto Bile Esculin Azide (BEA) agar, a medium specifically designed for the growth of Enterococcus species. Following this, the samples were incubated at a stable temperature of 37°C for 24-48 hours.

After the incubation period, colonies that visually resemble *E. faecalis* were selected for further testing. At this stage, the Pyrrolidonyl Aminopeptidase (PYR) test was employed for confirmation. This test identifies *E. faecalis* based on its ability to hydrolyze L-pyrrolidonyl- β -naphthylamide, a synthetic pyrrolidonyl- β -naphthylamide substrate, resulting in a color change that distinguishes it from other organisms.

The quantification of *E. faecalis* was performed by counting the number of validated colonies on each plate. The total count was then be used to calculate the concentration of *E. faecalis* in the original water sample, thus quantifying the extent of contamination.

This layered methodology facilitates both the accurate detection and quantification of *E. faecalis* in water samples. Utilising the BEA agar for primary isolation, followed by the PYR test for confirmation, ensures a high degree of specificity and reliability in the results (García-Garrote *et al.*, 2023).

3.9.7 Helminths

Standard parasitological techniques were employed to detect the presence of helminths in the water samples. Each water sample was passed through a 0.45 µm pore-sized membrane filter. This particular filter is designed to capture and concentrate microscopic entities such as helminth eggs and larvae, which are otherwise challenging to isolate from large volume water samples.

Post-filtration, the filter had to undergo microscopic examination to identify and verify the presence of helminth eggs and larvae. This technique allows for direct visualisation and thus provides a clear and accurate assessment of potential helminth contamination in the water samples.

Quantification of helminths in water samples involves counting the number of eggs and larvae visible under the microscope in the filter membrane. The total count is then adjusted based on the volume of water that was filtered. Results are often presented as eggs or larvae per litre of water, providing a measurable indication of the level of helminth contamination. It is essential to note that the quantification of helminths may vary based on the species and lifecycle stage (i.e., egg or larvae) of the helminths present in the samples (Robertson *et al.*, 2022).

3.10 Physicochemical Analysis of Ground Water

The assessment included key parameters such as pH, color, odor, turbidity and Biological Oxygen Demand (BOD), which are influential in determining water quality (APHA, 2020).

3.10.1 pH of ground water

The determination of the pH of water samples is crucial in understanding the acidic or alkaline nature of the water. This process was carried out using a pH meter that has been pre-calibrated using standard buffer solutions (APHA, 2020). Each water sample was placed in a separate clean beaker. The calibrated pH meter was then used to record the pH value. Each sample was measured in this manner, with the pH meter thoroughly rinsed with distilled water between measurements to prevent any form of cross-contamination (Morgan *et al.*, 2021).

3.10.2 Colour of groundwater

The color analysis of the water samples was performed using either a colorimeter or a spectrophotometer (APHA, 2020). Prior to testing, these instruments were calibrated using a standard solution with known color values. Each water sample was then placed in a cuvette and inserted into the colorimeter or spectrophotometer (Zhang *et al.*, 2018). The instrument measured the light absorbed by the sample, and this data was used to determine the color intensity of the water sample.

3.10.3 Oduor of groundwater

The odour assessment of the water samples was performed through a direct olfactory method. This involves warming each water sample slightly to allow for the release of any volatile compounds contributing to the odor. The researchers then directly smelled each sample, making detailed notes on the nature of the odour (Suffet and Rosenfeld, 2021).

3. 10.4 Turbidity of groundwater

To measure the turbidity of the water samples, a turbidimeter was used (APHA, 2020). Before measuring, the turbidimeter was calibrated using a formazin standard solution. Each water sample was then placed in a cuvette and inserted into the turbidimeter. The turbidimeter operates by shining light through the water sample and measuring the degree to which the light is scattered by any particles suspended in the water (Edzwald, 2021).

3. 10.5 Biological oxygen demand (BOD) of groundwater

BOD quantifies the amount of oxygen required by microorganisms to decompose organic matter in water. Elevated BOD values indicate a substantial level of organic pollution in water (Ngoc et al., 2020).

To measure the BOD of the water samples, the standard 5-day BOD test, also known as the BOD₅ test, was implemented. In this test, a given water sample is incubated in a BOD bottle along with a known volume of dilution water (also called seed water), typically supplemented with nutrients like phosphorus, nitrogen, and trace elements. These nutrients are added to stimulate microbial growth and are usually achieved by adding a reagent such as 1 mL of phosphate buffer, magnesium sulfate, ferric chloride, and calcium chloride solutions respectively.

The mixture was incubated for five days at 20°C. Before and after the incubation period, the dissolved oxygen concentration in the water was measured using a dissolved oxygen meter or titration (Metcalf and Eddy, 2014).

The difference in the initial and final dissolved oxygen concentrations represents the amount of oxygen consumed by microorganisms during the incubation period. This value is used to calculate the BOD of the water sample.

The BOD is calculated using the following formula:

$$\text{BOD5 (mg/L)} = (\text{D1} - \text{D2}) * (\text{Vt}/\text{Vs})$$

Where:

- D1 = Initial dissolved oxygen (DO) of the sample before incubation.
- D2 = Final DO of the sample after 5 days of incubation.
- Vt = Total volume of the sample and dilution water (mL)
- Vs = Volume of the undiluted sample (mL)

This formula gave the BOD in mg of O₂ consumed per liter of water over the 5-day period (Ngoc et al., 2020).

3.11 Relationship of Enteric Pathogens and Physicochemical Characteristics with Water-borne Diseases

To determine the correlation between isolated enteric pathogens, physicochemical characteristics of water, and the incidence of water-borne diseases in Tigania West Sub County, Meru County, Kenya (Objective iv), an integrated approach involving both laboratory data and structured questionnaires was employed.

The laboratory data consisted of information about enteric pathogens isolated from water samples and their respective physicochemical characteristics, including variables such as pH, temperature, Biological Oxygen Demand (BOD), and concentrations of specific contaminants. This provided a detailed snapshot of the quality of the sampled water.

Concurrently, data was collected from households within the community through structured questionnaires. These questionnaires gathered critical information about water treatment practices, sanitation facilities, and instances of waterborne diseases among household members. This was provided insights into the overall health impact and the extent of potential contamination of water resources in the community.

The collected data was then be analysed statistically. Cross-analysis methods were be employed to determine correlations between the presence and concentration of specific

pathogens or physicochemical factors in water and the reported instances of waterborne diseases. This helped establish a direct relationship between water quality parameters and the health of the community.

By examining these two sets of data together, a clearer understanding of how the presence of specific enteric pathogens and certain physicochemical conditions in water sources can contribute to waterborne diseases within the community can be established. This approach also assisted in developing evidence-based strategies to enhance sanitation and protect groundwater resources in the community.

3.12 Ethical Considerations and Research License

Permission to conduct the research was obtained from the county public health authorities, local authorities, and the Meru University of Science and Technology Institutional Research Ethics and Review Committee (MIRERC).

Research license was obtained from the National Commission for Science Technology and Innovation (NACOSTI).

Before any data is collected, informed consent was obtained from all participants. They were provided with information about the study's purpose, procedures, and their rights as participants, including their right to withdraw at any time without penalty. Consent forms were written in languages easily understood by the participants (English, Swahili, and Meru) and signed by both the participant and the researcher.

Confidentiality was ensured by assigning unique identification numbers to participants and storing all data in a secure location. Personal information that could identify participants, such as names or addresses, were not collected or recorded. Data was reported in aggregate form to protect individual privacy.

The study complied with all relevant ethical guidelines and regulations, including the Declaration of Helsinki and the guidelines of the Meru University of Science and Technology Institutional Research Ethics and Review Committee (MIRERC).

3.13 Quality Control

To ensure that the methods and data obtained provide valid and reliable results, several quality control measures were implemented throughout the study. Regarding the data collected from questionnaires, a crucial step is pretesting. The questionnaire was pretested in Ruiru Ward, which is different from the main study area. This pretest helped ensure that the questions are clear, comprehensible, and capable of eliciting the desired information. Any issues identified during the pretest were addressed and corrected before the full implementation of the questionnaire. It's important to note that the respondents involved in the pretest were not included in the main study to avoid any potential bias.

In terms of laboratory procedures, method validation was undertaken. This involved running standard controls alongside every batch of samples tested. Positive and negative control samples, which are samples with a known outcome, was included in each test run to verify the performance of the test method. Equipment and instruments used in the lab was calibrated regularly to ensure their precision and accuracy. This involved comparing the results produced by the equipment to a known reference standard.

Furthermore, to ensure data verification and integrity, multiple data entry personnel entered the data from questionnaires independently to reduce the risk of human error. The results were cross-checked to identify and rectify any discrepancies. Data integrity was maintained by implementing a secure data management system. All raw data was backed up regularly, and access to the data was restricted to authorised personnel.

Lastly, to ensure the reproducibility of the study, all procedures, calibrations, and data analyses was thoroughly documented. This allowed the study to be replicated in the

future, providing additional verification of the results. These measures contributed to the generation of reliable, valid, and reproducible results, therefore ensuring the credibility of the study's findings."

With this revision, you've addressed your supervisor's comment by including a detailed pretesting process for your questionnaires and explaining how it contributes to the overall quality control of your study.

3.14 Data Analysis

Information from the questionnaire and laboratory tests was entered into Microsoft Excel 2010 and analyzed using SPSS version 27. Descriptive statistics such as frequencies and percentages provided an overview of the data. Inferential statistics such as Analysis of Variance (ANOVA) and regression analysis were used to determine significant differences between groups and to identify relationships between variables.

ANOVA was used to compare the mean levels of enteric pathogens or physicochemical parameters in groundwater samples collected from different locations within Tigania West Sub-county. Regression analysis was used to examine the relationship between the presence of pit latrines and the levels of enteric pathogens or physicochemical parameters in groundwater samples.

The findings were presented as figures, tables, and graphs to provide a clear and concise summary of the results. The results of the data analysis were used to address the research objectives and to draw conclusions about the impact of pit latrines on enteric-pathogen contamination of groundwater in Tigania West Sub County, Meru County, Kenya.

CHAPTER FOUR: RESULTS

4.1 Introduction

This section provides a thorough presentation of the study's findings, encompassing the key results obtained through the pursuit of the research objectives outlined earlier. Through a systematic data analysis, the study delved into various aspects related to the impact of pit latrines on enteric-pathogen and physicochemical contamination of groundwater in Tigania West Sub-County, Meru County, Kenya.

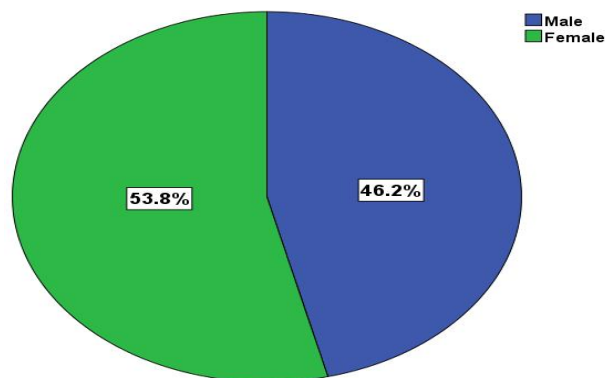
4.2 Surveying the Sites of Pit Latrines and of Groundwater Sources

4.2.1 Gender of respondents

Figure 4.1 provides a detailed breakdown of the gender distribution among the respondents in this study. This demographic information is crucial as it helps to understand the representation and diversity of the survey participants. By analyzing the gender of the respondents, we can ensure that the study encompasses a balanced perspective, which is important for the reliability and validity of the findings related to the impact of pit latrines on enteric-pathogen and physicochemical contamination of groundwater in Tigania West Sub-County, Meru County, Kenya.

Figure 4. 1

Gender of Respondents



Source: Researcher (2024)

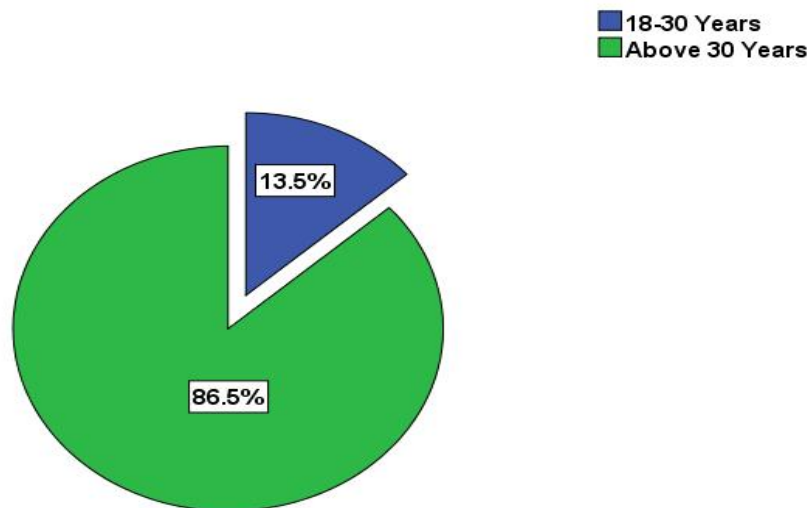
The results represent the distribution of participants by gender in the study. Out of the total $n = 96$ participants, $n = 44$ were male, representing (46.2%) of the sample, while $n = 52$ were female, constituting (53.8%) of the sample.

4.2.2 Age of respondents

Figure 4.2 illustrates the age distribution of the respondents in this study. Understanding the age demographics is essential for interpreting the data accurately, as different age groups may have varying levels of awareness, practices, and perceptions regarding pit latrine usage and groundwater contamination. By examining the age of the respondents, we can assess how age-related factors might influence the impact of pit latrines on enteric-pathogen and physicochemical contamination of groundwater in Tigania West Sub-County, Meru County, Kenya.

Figure 4. 2

Age of Respondents



Source: Researcher (2024)

The results in Figure 4.2 illustrate the distribution of participants by age group in the study. Out of the total $n = 96$ participants, $n = 13$ fell within the age 18-30 years old, representing (13.5%) of the sample. Conversely, ($n = 83$) participants were above 30

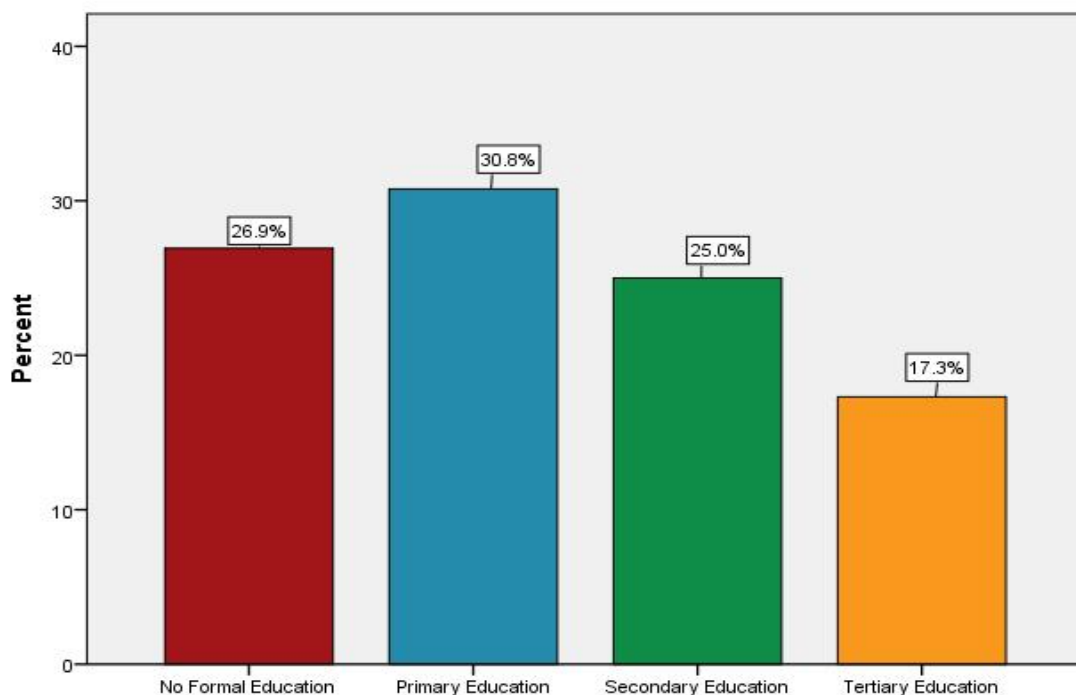
years, constituting 86.5% of the sample. The findings indicate that the majority of participants in the study were above 30 years of age.

4.2.3 Education level of respondents

Figure 4.3 presents the education level of the respondents in this study. Analyzing the educational background of participants is crucial as it provides insights into their knowledge, attitudes, and practices regarding water and sanitation. Education level can significantly influence awareness and understanding of the potential impacts of pit latrines on groundwater quality. By examining the education level, we can better understand how educational attainment affects the perception and behavior related to groundwater contamination in Tigania West Sub-County, Meru County, Kenya. This information is vital for designing effective educational and intervention programs to promote sustainable water management practices.

Figure 4. 3

Education Level of Respondents



Source: Researcher (2024)

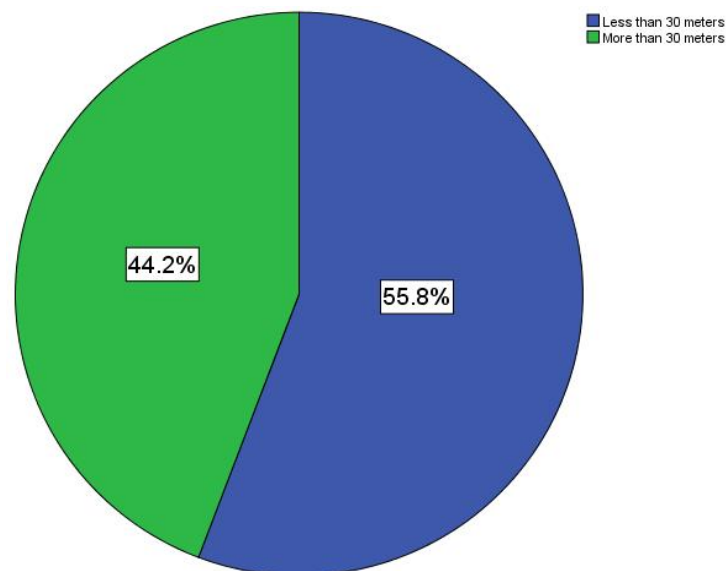
The results in Figure 4.3 depict the distribution of participants according to their education level in the study. Out of the total 96 participants, n = 26, (26.9%) individuals reported having no formal education. Additionally, n = 30, (30.8%) participants indicated that they had completed primary education. Moreover, n = 24, (25%) of participants had attained secondary education. Lastly, n = 16, (17.3%) of the participants reported having tertiary education.

4.2.4 Pit latrine distance to water sources

Figure 4.4 illustrates the distances between pit latrines and water sources in the study area. This information is pivotal for assessing the potential contamination risk of groundwater, as the proximity of pit latrines to water sources can significantly impact the infiltration of enteric pathogens and other pollutants.

Figure 4. 4

Pit latrine distance to water source



Source: Researcher (2024)

The results in Figure 4.4 represent the distribution of participants based on the distance of their pit latrines from water sources in the context of the study. Out of the total 96 participants, n = 54 individuals reported that their pit latrines were located less than 30

meters from a water source, representing (55.8%) of the sample. Conversely, n = 42 participants indicated that their pit latrines were situated more than 30 meters away from a water source, accounting for (44.2%) of the sample.

4.2.5 Locations of groundwater sources in Tigania-West Sub-County

Table 4.1 details the locations of groundwater sources in Tigania West Sub-County. Understanding the geographic distribution of these water sources is crucial for analyzing the potential impact of nearby pit latrines on groundwater quality. By mapping out these locations, we can better assess the risk of contamination and identify specific areas that may require targeted interventions to ensure safe and sustainable water supply in Tigania West Sub-County, Meru County, Kenya.

Table 4. 1

Locations of groundwater sources in Tigania-West Sub-County per ward

Ward	Distance to water source	Frequency	Percent
Mbeu (n = 15)	Less than 30m	13	86.67
	More than 30m	2	13.33
Kianjai (n = 26)	Less than 30m	14	53.85
	More than 30m	12	46.15
Nkomo (n = 17)	Less than 30m	13	76.47
	More than 30m	4	23.53
Akithi (n = 25)	Less than 30m	12	48.00
	More than 30m	13	52.00
Athwana (n = 13)	Less than 30m	9	69.23
	More than 30m	4	30.77

Source: Researcher (2024)

The result in Table 4.1 provides a breakdown of data by ward, specifically focusing on the distance of pit latrines from water sources in each ward within the study area. In Mbeu ward, out of the 15 respondents, the majority n = 13, (86.67%) reported that their pit latrines were located less than 30 meters from a water source, while only n = 2, (13.33%) reported a distance of more than 30 meters. Moving to the Kianjai ward, n = 14, (53.85%) of respondents reported that their pit latrines were less than 30 meters from

a water source, whereas n = 12, (46.15%) reported a distance of more than 30 meters. In the Nkomo ward, a significant majority n = 16, (80%) reported pit latrines located less than 30 meters from a water source, while n = 4, (20%) reported a distance of more than 30 meters. In the Akiathi ward, n = 12, (48%) of respondents reported pit latrines located less than 30 meters from a water source, while n = 13, (52%) reported a distance of more than 30 meters.

Lastly, in the Athwana ward, n = 9, (69.23% of respondents reported pit latrines located less than 30 meters from a water source, while n = 4, (30.77%) reported a distance of more than 30 meters.

4.3 Isolation and Identification of Enteric Pathogens

The isolation and identification of enteric pathogens were conducted using various microbiological techniques outlined in section 3.9. Analysis revealed the presence of multiple pathogens in the water samples, including total coliforms, *Escherichia coli* (*E. coli*), *Salmonella*, *Shigella*, and *Staphylococcus aureus*.

Table 4.2 provides an overview of the isolation and identification of enteric pathogens in water samples, along with the methods used for identification and the corresponding results.

Table 4. 2*Isolation and Identification of Enteric Pathogens*

Ward	Enteric Pathogen	Frequency	Results	WHO Permissible Limit
Mbeu (15)	<i>Total coliforms</i>	6	12 CFU/100 mL	<10 CFU/100 mL
	<i>E. coli</i>	7	1.8 CFU/100 mL	<1 CFU/100 mL
	<i>Salmonella</i>	6	11 Salmonella/250 mL	<10 Salmonella/250 mL
	<i>Shigella</i>	1	2 Shigella/100 mL	<1 Shigella/100 mL
	<i>Staphylococcus aureus</i>	7	90 CFU/100 mL	<100 CFU/100 mL
Kianjai (26)	<i>Total Coliforms</i>	6	10 CFU/100 mL	<10 CFU/100 mL
	<i>E. coli</i>	5	2 CFU/100 mL	<1 CFU/100 mL
	<i>Salmonella</i>	5	10 Salmonella/250 mL	<10 Salmonella/250 mL
	<i>Shigella</i>	4	4 Shigella/100 mL	<1 Shigella/100 mL
	<i>Staphylococcus aureus</i>	5	98 CFU/100 mL	<100 CFU/100 mL
Nkomo (17)	<i>Total coliforms</i>	3	9 CFU/100 mL	<10 CFU/100 mL
	<i>E. coli</i>	3	2 CFU/100 mL	<1 CFU/100 mL
	<i>Salmonella</i>	2	14 Salmonella/250 mL	<10 Salmonella/250 mL
	<i>Shigella</i>	2	3 Shigella/100 mL	<1 Shigella/100 mL
	<i>Staphylococcus aureus</i>	3	112 CFU/100 mL	<100 CFU/100 mL
Akithi (25)	<i>Total coliforms</i>	5	13 CFU/100 mL	<10 CFU/100 mL
	<i>E. coli</i>	4	3 CFU/100 mL	<1 CFU/100 mL
	<i>Salmonella</i>	3	15 Salmonella/250 mL	<10 Salmonella/250 mL
	<i>Shigella</i>	1	5 Shigella/100 mL	<1 Shigella/100 mL
	<i>Staphylococcus aureus</i>	2	120 CFU/100 mL	<100 CFU/100 mL
Ward	Enteric Pathogen	Frequency	Results	WHO Permissible Limit
Athwana (13)	<i>Total Coliforms</i>	7	14 CFU/100 mL	<10 CFU/100 mL
	<i>E. coli</i>	3	3 CFU/100 mL	<1 CFU/100 mL
	<i>Salmonella</i>	2	12	<10

		Salmonella/250 mL	Salmonella/250 mL
<i>Shigella</i>	3	5 Shigella/100 mL	<1 Shigella/100 mL
<i>Staphylococcus aureus</i>	2	94 CFU/100 mL	<100 CFU/100 mL

Source: Researcher (2024)

Table 4.2 shows the analysis of enteric pathogens in groundwater samples from various wards in Tigania West Sub-County revealed significant contamination, with several pathogens exceeding the permissible limits set for safe drinking water. In Mbeu Ward, the presence of Total Coliforms was recorded with a frequency of 6 and a result of 12 CFU/100 mL, which exceeded the permissible limit of <10 CFU/100 mL. Similarly, *E. coli* was found with a frequency of 7 at levels of 1.8 CFU/100 mL, surpassing the permissible limit of <1 CFU/100 mL. *Salmonella* was detected with a frequency of 6 at levels of 11 Salmonella/250 mL, above the permissible limit of <10 Salmonella/250 mL. *Shigella* was present at 2 Shigella/100 mL, exceeding the limit of <1 Shigella/100 mL. However, *Staphylococcus aureus* was within acceptable limits, recorded at 90 CFU/100 mL against the permissible limit of <100 CFU/100 mL.

In Kianjai Ward, Total Coliforms were within permissible limits, recorded at 10 CFU/100 mL with a frequency of 6. However, *E. coli* exceeded safe levels with a frequency of 5 and a result of 2 CFU/100 mL. *Salmonella* was at the permissible limit with 10 Salmonella/250 mL, recorded with a frequency of 5. *Shigella* significantly exceeded safe levels at 4 Shigella/100 mL, with a frequency of 4. *Staphylococcus aureus* was within safe limits at 98 CFU/100 mL, with a frequency of 5.

In Nkomo Ward, Total Coliforms were within permissible limits, recorded at 9 CFU/100 mL with a frequency of 3. However, *E. coli* exceeded permissible limits at 2 CFU/100 mL, with a frequency of 3. *Salmonella* levels were particularly high at 14 CFU/100 mL, well above the permissible limit, with a frequency of 2. *Shigella* also exceeded

permissible levels at 3 Shigella/100 mL, with a frequency of 2. Staphylococcus aureus levels were above permissible limits at 112 CFU/100 mL, with a frequency of 3.

In Akithi Ward, Total Coliforms exceeded permissible limits at 13 CFU/100 mL, with a frequency of 5. *E. coli* levels were also above permissible limits at 3 CFU/100 mL, with a frequency of 4. Salmonella levels were high at 15 CFU/100 mL, exceeding permissible limits, with a frequency of 3. Shigella levels were significantly above permissible limits at 5 Shigella/100 mL, with a frequency of 1. Staphylococcus aureus levels were also above permissible limits at 120 CFU/100 mL, with a frequency of 2.

In Athwana Ward, Total Coliforms exceeded permissible limits at 14 CFU/100 mL, with a frequency of 7. *E. coli* levels were also high at 3 CFU/100 mL, with a frequency of 3. Salmonella levels were above permissible limits at 12 CFU/100 mL, with a frequency of 2. Shigella levels were significantly above permissible limits at 5 Shigella/100 mL, with a frequency of 3. However, Staphylococcus aureus levels were within acceptable limits at 94 CFU/100 mL, with a frequency of 2.

Table 4.3 presents the results of the isolation and identification of helminths from groundwater samples in various wards of Tigania West Sub-County. The data illustrates the prevalence and types of helminths found, providing insights into the extent of groundwater contamination by these parasites. The presence of helminths in groundwater is a significant public health concern, as it indicates potential exposure to parasitic infections for the local population.

Table 4. 3*Isolation and Identification of Helminths*

Ward	Enteric Pathogen	Identification Method	Results
Athwana (n = 13)	Flatworms (trematodes)	Microscopic Examination	Positive in 20 out of 26 ground water samples.
Akithi (n = 25)	Roundworms (nematodes)	Microscopic Examination	Positive in 12 out of 15 ground water samples.
Kianjai (n = 26)	Tapeworms (cestodes)	Microscopic Examination	Detected in 3 out of 17 untreated water ground water samples.
Nkomo (n = 17)	Roundworms (nematodes)	Microscopic Examination	Detected in 10 out of 25 ground water samples.
Mbeu (n = 15)	Tapeworms (cestodes)	Microscopic Examination	Detected in 9 out of 13 ground water samples.

Source: Researcher (2024)

The analysis of Helminths in various wards within Tigania West Sub-County revealed distinct patterns of contamination across untreated, treated, and groundwater samples.

The findings detailed in Table 4.3 provide a comprehensive overview of the isolation and identification of helminths across different wards, utilizing microscopic examination as the identification method.

In Athwana, out of 26 samples, flatworms (trematodes) were detected in 15 samples. Furthermore, 10 of these samples exhibited a high concentration of trematodes, indicating a significant presence of this enteric pathogen in the ward.

For Akithi, roundworms (nematodes) were identified in 12 out of 15 samples. The concentration of nematodes was moderate, suggesting a notable but not overwhelming prevalence in the samples analyzed.

Kianjai's analysis revealed the presence of tapeworms (cestodes) in 10 out of 17 samples. However, the concentration of cestodes was low, implying a relatively lower infestation level compared to other pathogens observed in different wards.

In Nkomo, roundworms (nematodes) were found in 20 out of 25 samples, with a high concentration noted in 12 of these samples. This indicates a significant infestation of nematodes in the Nkomo ward, highlighting it as a critical area of concern. Finally, in Mbeu, tapeworms (cestodes) were present in 8 out of 13 samples. The concentration of cestodes was moderate, indicating a considerable but not extreme prevalence of this pathogen. Refer to table 4.3.

4.4 Physicochemical Properties of the Groundwater

Table 4.4 provides a comprehensive overview of the physicochemical properties of groundwater across different wards within the study area.

Table 4. 4*Physicochemical Properties of the Groundwater*

Ward	Parameter	Results	Frequency	Percentage
Mbeu (n = 15)	pH	<6.5	1	6.67
		6.5-8.5	14	93.33
	Color	15 TCU	15	100
	Oduor	None	15	100
	Turbidity	>5 NTU	1	6.67
		5 NTU	12	80
<5 NTU		2	13.33	
Kianjai (n = 26)	pH	<6.5	2	7.69
		6.5-8.5	24	92.31
	Color	14 TCU	26	100
	Oduor	None	26	100
	Turbidity	>5 NTU	8	30.77
		5 NTU	6	23.08
<5 NTU		12	46.15	
Nkomo (n = 17)	pH	<6.5	0	0
		6.5-8.5	17	100
	Color	15 TCU	17	100
	Oduor	None	17	100
	Turbidity	>5 NTU	1	5.88
		5 NTU	15	88.24
<5 NTU		1	5.88	
Akithi (n = 25)	pH	<6.5	1	4
		6.5-8.5	24	96
	Color	14 TCU	25	100
	Oduor	None	25	100
	Turbidity	>5 NTU	5	20
		5 NTU	20	80
<5 NTU		0	0	
Athwana (n = 13)	pH	<6.5	0	0
		6.5-8.5	13	100
	Color	14 TCU	13	100
	Oduor	None	13	100
	Turbidity	>5 NTU	0	0
		5 NTU	0	0
<5 NTU		13	100	

Source: Researcher (2024)

The pH levels, in the Mbeu ward, the majority n = 14, (93.33%) of groundwater samples fell within the optimal pH range of 6.5-8.5, which is conducive to safe drinking water.

Only one sample n = 1, (6.67%) had a pH below 6.5, indicating slightly acidic conditions.

In the Kianjai ward, a similar trend was observed, with $n = 24$, (92.31% of groundwater samples falling within the optimal pH range of 6.5-8.5. Additionally, two samples ($n = 2$, (7.69%) had a pH below 6.5.

In the Nkomo ward, all groundwater samples had pH levels within the optimal range of 6.5-8.5, indicating neutral to slightly alkaline conditions. Similarly, in the Akithi ward, the vast majority $n = 24$, (96%) of groundwater samples fell within the optimal pH range of 6.5-8.5, with only one sample $n = 1$, (4%) having a pH below 6.5.

Lastly, in the Athwana ward, all groundwater samples had pH levels within the optimal range of 6.5-8.5, indicating favourable conditions for drinking water.

Regarding colour and odour, groundwater samples across all wards exhibited clear color and lacked any noticeable odor, indicating good aesthetic quality and absence of contaminants affecting color and odor.

However, when considering turbidity as an indicator of water clarity, variability was observed across wards. In the Mbeu ward, the majority $n = 12$, (80%) of groundwater samples had medium turbidity, while in the Kianjai ward, a higher proportion of samples $n=12$, (46.15%) had medium turbidity, with additional samples exhibiting high turbidity levels. In the Nkomo ward, most samples $n = 15$, (88.24%) had medium turbidity, while in the Akithi ward, a similar trend was observed, with $n = 20$, (80%) of samples exhibiting medium turbidity levels. Notably, in the Athwana ward, all groundwater samples had low turbidity levels, indicating clearer water quality.

4.5 Biological Oxygen Demand (BOD)

Table 4.5 provides the Biological Oxygen Demand (BOD) results for groundwater samples collected in Kinjai, Nkomo, Akithi, Athwana and Mbeu wards Tigania West Sub-County. BOD is a critical parameter for assessing the level of organic pollution in

water, as it indicates the amount of oxygen required by microorganisms to decompose organic matter.

Table 4. 5

Biological Oxygen Demand (BOD) Results

Ward	Frequency	BOD(mg/L)
Kianjai	2	2.0 - 2.9
	9	3.0 - 3.9
	4	4.0 - 4.9
	6	6.0 - 6.9
	5	7.0 - 7.9
Nkomo	1	2.0 - 2.9
	1	3.0 - 3.9
	3	4.0 - 4.9
	5	5.0 - 5.9
	6	6.0 - 6.9
	1	7.0 - 7.9
Akithi	5	3.0 - 3.9
	10	4.0 - 4.9
	6	5.0 - 5.9
	5	6.0 - 6.9
	1	7.0 - 7.9
Athwana	1	4.0 - 4.9
	2	5.0 - 5.9
	6	6.0 - 6.9
	4	7.0 - 7.9
Mbeu	2	3.0 - 3.9
	5	4.0 - 4.9
	5	5.0 - 5.9
	3	6.0 - 6.9

Source: Researcher (2024)

The results from Table 4.5 present the Biological Oxygen Demand (BOD) levels measured across different wards in Tigania West Sub-County, specifically Kianjai Ward, Nkomo Ward, Akithi Ward, Athwana Ward, and Mbeu Ward. In Kianjai Ward, BOD values predominantly ranged between 3.0 and 7.9 mg/L. Specifically, nine samples fell within the 3.0-3.9 mg/L range, four samples were between 4.0-4.9 mg/L, six samples were between 6.0-6.9 mg/L, and five samples exhibited the highest range of 7.0-7.9

mg/L. Only two samples had BOD values in the lowest range of 2.0-2.9 mg/L, indicating a relatively higher level of organic pollution.

Nkomo Ward exhibited a broader distribution of BOD values. One sample fell within the 2.0-2.9 mg/L range, and one sample within the 3.0-3.9 mg/L range. Three samples were in the 4.0-4.9 mg/L range, five samples in the 5.0-5.9 mg/L range, six samples in the 6.0-6.9 mg/L range, and one sample had a BOD value in the 7.0-7.9 mg/L range. This distribution suggested significant variability in organic pollution levels across the ward.

In Akithi Ward, BOD values showed a concentration in the 4.0-4.9 mg/L range, with ten samples. Six samples were in the 3.0-3.9 mg/L range, five samples in the 5.0-5.9 mg/L range, five samples in the 6.0-6.9 mg/L range, and one sample in the 7.0-7.9 mg/L range. This indicated a moderate level of organic pollution with occasional higher values.

Athwana Ward demonstrated a pattern of higher BOD values, with most samples falling between 6.0 and 7.9 mg/L. Specifically; one sample was in the 4.0-4.9 mg/L range, two samples were between 5.0-5.9 mg/L, six samples between 6.0-6.9 mg/L, and four samples in the 7.0-7.9 mg/L range. This distribution suggested a higher level of organic pollution compared to other wards.

In Mbeu Ward, BOD values ranged between 3.0 and 6.9 mg/L. Two samples were in the 3.0-3.9 mg/L range, five samples in the 4.0-4.9 mg/L range, five samples in the 5.0-5.9 mg/L range, and three samples in the 6.0-6.9 mg/L range. This indicated moderate to high levels of organic pollution.

4.5 Associations between Isolated Enteric Pathogens to Disease Burden

Table 4.6 presents the associations between isolated high enteric pathogens and waterborne infections in Tigania West Sub-County. This table is vital for understanding the relationship between specific pathogens found in groundwater and the incidence of waterborne diseases in the community.

Table 4. 6*Associations between high enteric pathogens and waterborne infections*

Ward Name	Ward Coordinate	Enteric Pathogen %	Result	Waterborne Infection (%)	Observed Relationship
Mbeu	0.09790, 37.7606	Shigella (20%)	2 Shigella/100 mL	22	Comparable levels of pathogen and infections observed
Kianjai	0.173501, 37.75992	Shigella (40%)	4 Shigella/100 mL	40	Proportional increase in infection with pathogen presence
Nkomo	0.128471, 37.7194379	Staphylococcus aureus (12%)	112 CFU/100 mL	70	High infection burden despite relatively low pathogen prevalence
Akithi	0.186555, 37.7350	Shigella (50%)	5 Shigella/100 mL	59	High pathogen prevalence reflected in elevated infections
Athwana	0.1984182, 37.7677752	Shigella (50%)	5 Shigella/100 mL	55	High pathogen prevalence reflected in elevated infections
Correlation, r value	0.076				
p-value	0.903				

Source: Researcher (2024)

The results in Table 4.6 demonstrated the associations between high levels of enteric pathogens, the correlation coefficient of 0.076 indicates a very weak positive linear relationship between Enteric Pathogen (%) and Waterborne Infection (%). The p-value of 0.903 suggests that the observed correlation is not statistically significant. A p-value greater than 0.05 indicates that we fail to reject the null hypothesis, which means there is

no significant linear relationship between Enteric Pathogen (%) and Waterborne Infection (%).

4.6 Associations between Isolated Low Enteric Pathogens and Waterborne

Infections

Table 4.7 explores the associations between isolated low levels of enteric pathogens and the prevalence of waterborne infections. This table provides insight into how the presence of low levels of enteric pathogens may correlate with the occurrence of waterborne infections in different wards within Tigania West Sub-County.

Table 4. 7

Associations between Isolated Low Enteric Pathogens and Waterborne Infections

Ward Name	Ward Coordinate	Enteric Pathogen (%)	Waterborne Infection (%)
Mbeu	0.0405, 37.8141	Shigella (20%)	4
Kianjai	0.1520449, 37.7840433	Shigella(15%)	2
Nkomo	0.1271632, 37.7216082	Staphylococcus aureus (11%)	7
Akithi	0.1851234, 37.7667318	Shigella(5%)	10
Athwana	0.230457 ,37.746641	Shigella(5%)	7
Correlation, r value	-0.799		
p-value	0.105		

Source: Researcher (2024)

The results in Table 4.7 illustrated the associations between isolated low enteric pathogens and waterborne infections. The correlation coefficient ($r = -0.799$) indicates a strong negative correlation between Enteric Pathogen (%) and Waterborne Infection (%). This suggests that as the percentage of Enteric Pathogens increases, the percentage of Waterborne Infections tends to decrease.

However, this result is counter intuitive and may require further investigation to understand underlying factors. Additionally, the p-value is greater than 0.05, indicating that the correlation is not statistically significant at the 5% significance level. Therefore,

we cannot confidently conclude that there is a true correlation between Enteric Pathogen (%) and Waterborne Infection (%). This lack of statistical significance suggests that the observed relationship might be due to random variation in the data. Further analysis and validation with a larger sample size or additional variables may be necessary to clarify these findings.

4.7 Associations between Distance between Pit Latrines and Water Sources (<30m) verses Enteric Pathogens

Table 4.8 presents the associations between the distance from pit latrines to water sources (less than 30 meters) and the presence of enteric pathogens in Tigania West Sub-County. This table is important for understanding the impact of pit latrine proximity on groundwater contamination.

Table 4. 8

Association between distance between Pit Latrines and Water Sources (<30m) verses enteric pathogens

Ward	Water Sources <30m from Pit Latrines (%)	Enteric Pathogens (%)	Pathogen Category
Mbeu	86.67%	40	High
Kianjai	13.33%	47	High
Nkomo	76.47%	38	High
Akithi	48.00%	32	High
Athwana	69.23%	39	High

All wards with <30m distance show high prevalence (>25%)

Source: Researcher (2024)

The analysis examined the association between pit latrine proximity (<30m) and enteric pathogen contamination of groundwater across wards in Tigania West Sub-County. Results (Table 4.8) indicated that in all wards where water sources were located less than

30m from pit latrines, the prevalence of enteric pathogens was consistently high (>25%). For example, Mbeu and Nkomo wards recorded pathogen prevalence rates of 40% and 38%, respectively. This suggests that reduced separation distance between pit latrines and water points increases the risk of microbial contamination.

4.8 Associations between Distance between Pit Latrines and Water Sources (>30m) versus Enteric Pathogens

Table 4.9 presents the associations between the distance from pit latrines to water sources (greater than 30 meters) and the incidence of enteric pathogens in Tigania West Sub-County. This table is crucial for evaluating the effectiveness of recommended sanitation practices in mitigating health risks.

Table 4.9

Distance between Pit Latrines and Water Sources (>30m) versus Enteric Pathogens

Ward	Water Sources >30m from Pit Latrines (%)	Enteric Pathogens (%)	Pathogen Category
Mbeu	13.33%	14	Low
Kianjai	46.15%	20	Low
Nkomo	23.53%	15	Low
Akithi	52.00%	18	Low
Athwana	30.77%	22	Low

Source: Researcher (2024)

Table 4.9 presents the associations between the distance of pit latrines from water sources (>30m) and the prevalence of enteric pathogens in various wards of Tigania West Sub-County. Conversely, in wards where water sources were located at distances greater than 30m from pit latrines (Table 4.9), pathogen prevalence was consistently low ($\leq 25\%$). For instance, Akithi and Kianjai recorded prevalence rates of 18% and 20%, respectively. This pattern indicates that maintaining greater separation between pit latrines and groundwater sources substantially reduces the risk of contamination.

Table 4. 10

Chi-square Test of Association between Pit Latrine Distance and Enteric Pathogen

Prevalence

Distance	High	Low	High Pathogen	Low Pathogen
Category	Pathogen	Pathogen	(Expected)	(Expected)
	(Observed)	(Observed)		
<30m	5	0	2.5	2.5
>30m	0	5	2.5	2.5

Chi-square value (X^2): 6.40

Degrees of freedom (df): 1

p-value: 0.011

Source: Researcher (2024)

To statistically assess the association between pit latrine distance (<30m vs. >30m) and enteric pathogen prevalence (High vs. Low), a Chi-square test of independence was conducted (Table 4.10). Results revealed a significant association ($X^2 = 6.40$, $df = 1$, $p = 0.011$). Specifically, sources located within 30m of pit latrines were significantly more likely to have high prevalence of pathogens, while those located beyond 30m were significantly more likely to have low prevalence.

4.9 Associations between Biological Oxygen Demand (BOD), Enteric Pathogens and Waterborne Infections

Table 4.10 presents the associations between Biological Oxygen Demand (BOD) levels and the incidence of waterborne infections in Tigania West Sub-County. This table is essential for understanding the relationship between the organic pollution of groundwater, as indicated by BOD levels, and the prevalence of waterborne diseases.

Table 4. 11*Associations between Biological Oxygen Demand (BOD) and Waterborne infections*

Ward	Biological Oxygen Demand (BOD)	Enteric Pathogens	Waterborne Infections
Mbeu	5.08 mg/L	40	14
Kianjai	5.04 mg/L	47	20
Nkomo	5.39 mg/L	38	15
Akithi	4.91 mg/L	32	18
Athwana	6.45 mg/L	39	22
Correlation, <i>r</i> value	0.020		
Correlation, <i>r</i> value	0.568		
p-value	0.975		
p-value	0.318		

Source: Researcher (2024)

The analysis of the associations between Biological Oxygen Demand (BOD) and waterborne infections across different wards revealed significant findings. In Mbeu Ward, a BOD level of 5.08 mg/L was associated with 40% prevalence of enteric pathogens and 14% prevalence of waterborne infections.

In Kianjai Ward, a BOD level of 5.04 mg/L corresponded to 47% prevalence of enteric pathogens and 20% prevalence of waterborne infections. In Nkomo Ward, a BOD level of 5.39 mg/L was linked to 38% prevalence of enteric pathogens and 15% prevalence of waterborne infections. Akithi Ward showed a BOD level of 4.91 mg/L with 32% prevalence of enteric pathogens and 18% prevalence of waterborne infections. Athwana Ward had a notably high BOD level of 6.45 mg/L, associated with 39% prevalence of enteric pathogens and 22% prevalence of waterborne infections.

The calculations were based on Table 2.1 on WHO permissible limits. The correlation analysis between BOD and Enteric Pathogens yielded an r value of 0.020, indicating a very weak relationship between BOD levels and the prevalence of Enteric Pathogens. The correlation between BOD and Waterborne Infections is moderate (around 0.57), but the p-value is above the typical significance threshold (0.05), indicating that this correlation is also not statistically significant. All the p-values for enteric pathogens and waterborne infections were the above the typical significance threshold (0.05), indicating that the correlations were not statistically significant, suggesting that the associations was statistically significant.

CHAPTER FIVE: DISCUSSION

5.1 Introduction

The discussion aims to provide a comprehensive discussion of the findings presented in Chapter four in connection to existing literature, focusing on the impact of pit latrines on enteric-pathogen and physicochemical contamination of groundwater in Tigania West Sub-County, Meru County, Kenya. The section explores the implications of the findings in relation to the study objectives.

5.2 Relationship between Pit Latrine Locations to Groundwater Sources and the Prevalence of Waterborne Diseases in Tigania West Sub County in Meru County

The survey conducted on the sites of pit latrines in relation to groundwater sources within Tigania West Sub-County, Meru County, and revealed notable variability across different wards. The findings shed light on the spatial distribution of pit latrines relative to water sources, highlighting potential concerns regarding fecal contamination of groundwater.

Among the respondents, a significant proportion $n = 18$, (34.6%) expressed uncertainty regarding the proximity of their pit latrines to underground water points, indicating a lack of awareness or knowledge regarding the spatial relationship between sanitation facilities and water sources. Additionally, $n = 15$, (28.8%) of respondents reported that pit latrines were situated closer to water points which was less than thirty meters raising concerns about the potential for contamination through seepage or leaching of contaminants into groundwater sources. The setback distance according to other researchers is approximately 150 meters (Abanyie *et al.*, 2022; Back *et al.*, 2018)

Misuse and leakages from sanitation facilities, including pit latrines, pose significant risks of polluting groundwater resources with pathogens and other contaminants. These

contaminants can include fecal bacteria, viruses, and parasites, which can cause waterborne diseases such as diarrhea, cholera, and typhoid fever. Therefore, these findings are in line with the study by Fate Ali *et al.* (2019) which found that preventing and controlling contamination from pit latrines is crucial for safeguarding groundwater quality and protecting public health.

The observed variations in the distances between pit latrines and water sources underscore the need for targeted interventions to mitigate contamination risks in areas where latrines are located close to water sources. Strategies such as promoting the use of improved sanitation technologies, enforcing setback distances between pit latrines and water sources, and implementing regular monitoring and maintenance of sanitation facilities can help reduce the risk of groundwater contamination and protect the health and well-being of communities relying on groundwater for drinking and domestic purposes. Additionally, community education and awareness-raising initiatives are essential to promote proper sanitation practices and encourage behavior change to minimize contamination risks (Ndoziya, Hoko & Gumindoga, 2019; Lutterodt *et al.*, 2023).

5.3 Determination of the Groundwater Physicochemicals Properties in Tigania West Sub-County in Meru County, Kenya

The study investigated several physicochemical properties of groundwater within Tigania West Sub County, Meru County, aiming to assess their compliance with WHO drinking water quality guidelines. The results provide valuable insights into the overall quality of groundwater and its suitability for drinking and domestic use.

The analysis of pH levels in groundwater samples revealed that the majority fell within the optimal range of 6.5-8.5, which is in line with WHO guidelines for drinking water quality (World Health Organization, 2022). Adequate pH levels are crucial for ensuring

water safety and preventing corrosion of distribution pipes, as well as minimizing the risk of metal leaching from geological formations (World Health Organization, 2022). A lower pH level of groundwater indicates higher acidity, which may suggest contamination by acidic substances, such as organic acids or acidic by-products of microbial activity in pit latrines. This could be indicative of fecal contamination or the presence of organic matter leaching into the groundwater from pit latrines.

Conversely, a higher pH indicates alkalinity, which may suggest contamination by alkaline substances, such as ammonia or detergents, commonly found in pit latrine effluents. Additionally, alkaline conditions can promote the survival and growth of certain pathogens, further exacerbating contamination issues. Therefore, monitoring pH levels in groundwater can help assess the extent and nature of contamination from pit latrines, informing appropriate mitigation measures and safeguarding public health.

The clear colour and absence of noticeable Oduor in all groundwater samples indicate good aesthetic quality, which is an essential aspect of consumer acceptability and satisfaction with drinking water (Uddin *et al.*, 2021). However, the presence of high turbidity levels in some groundwater samples raises concerns about water clarity and potential contamination by suspended particles, which can harbor pathogens and compromise water quality (Gupta and Gupta, 2021). When comparing these findings with the World Health Organization's (WHO) permissible limits for turbidity in drinking water, which is typically less than 5 NTU (Nephelometric Turbidity Units), the observed elevated turbidity levels may indicate a deviation from the recommended standards, highlighting the need for further investigation and appropriate remedial actions.

The variability in turbidity levels across different wards suggests spatial differences in water quality and potential sources of contamination. High turbidity levels can result from various factors, including soil erosion, agricultural runoff, and inadequate treatment

of wastewater, highlighting the need for targeted interventions to address specific sources of pollution and improve water quality (Noori *et al.*, 2019). This underscores the importance of addressing the impact of pit latrines on groundwater quality through improved sanitation practices and proper waste management strategies.

When comparing these findings with the World Health Organization's (WHO) permissible limits for turbidity in drinking water, which is typically less than 5 NTU (Nephelometric Turbidity Units), the observed spatial variations indicate deviations from the recommended standards, necessitating targeted interventions to mitigate potential health risks associated with contaminated water sources.

Analysis of the BOD values revealed disparities in organic pollution levels among different areas within Mbeu Ward. BOD concentrations vary from 3.7 mg/L to 6.7 mg/L, suggesting differences in the extent of organic contamination in the water samples. Higher BOD values, such as (0.07521, 37.76069 = 6.7 mg/L), indicated elevated levels of organic matter, potentially stemming from sources like untreated sewage discharge or agricultural runoff. This aligns with the study by Zahmatkesh *et al.* (2023). This suggests a potential association between pit latrines and the observed organic contamination, highlighting the need for improved sanitation practices and waste management strategies to mitigate such pollution sources.

Higher BOD values in Akithi ward, such as in location (0.195324, 37.789161=7.2 mg/L), indicated elevated organic pollution levels, possibly stemming from sources like untreated sewage. Similarly, Locations with higher BOD levels in Athwana Ward, such as (0.215170, 37.750670 =7.3 mg/L), also signifies increased inputs of organic waste, potentially originating from sources like untreated sewage discharge. Given the presence of pit latrines in close proximity to water sources in these areas, it is plausible that leachate from these latrines contributes to the organic pollution, as organic matter from

human waste can infiltrate the groundwater. This underscores the importance of addressing sanitation issues and implementing proper waste management practices to mitigate the impact of pit latrines on water quality.

In conclusion, while most physicochemical properties of groundwater in Tigania West Sub County, Meru County, comply with WHO drinking water quality guidelines, the presence of high turbidity levels in some samples indicates potential risks to public health. Continued monitoring and management of groundwater quality are essential to ensure the provision of safe and clean drinking water to communities reliant on groundwater sources.

5.4 Examination of the Relationship among Enteric-Pathogens, Physicochemical, Properties and Location of Pit Latrines with Disease Burden within Tigania West Sub-County in Meru County, Kenya

The study successfully isolated and identified various enteric pathogens from potentially contaminated groundwater sources within Tigania West Sub-County, Meru County. The results provide valuable insights into the microbial quality of groundwater and the potential risks posed to public health due to fecal contamination.

The detection of total coliforms and *E. coli* in all untreated water samples is consistent with findings from previous studies highlighting the prevalence of these indicators of fecal contamination in groundwater sources (Stokdyk *et al.*, 2020; WHO, 2019). Total coliforms and *E. coli* are widely recognised as indicators of fecal pollution and are commonly used to assess the microbial quality of water and the potential presence of pathogens of fecal origin (Mendoza *et al.*, 2023). The results in Table 4.10 demonstrate a clear and statistically significant association between the proximity of pit latrines to water sources and the prevalence of enteric pathogens in Tigania West Sub-County. Water sources located within 30 meters of pit latrines consistently exhibited high prevalence of pathogens (>25%), whereas those located beyond 30 meters showed low

prevalence ($\leq 25\%$). The Chi-square test confirmed this association ($X^2 = 6.40$, $p = 0.011$), underscoring the public health risks posed by inadequate separation distances.

These results are consistent with previous studies conducted in sub-Saharan Africa, which have highlighted the contamination risks posed by pit latrines located too close to groundwater sources. The study by Gwenzi et al (2023) reported that shallow wells situated near pit latrines were frequently contaminated with fecal coliforms, particularly in densely populated rural settings. Similarly, a study in Kenya by Gudda et al. (2019) documented elevated pathogen levels in groundwater sources situated less than 30 meters from latrines.

This finding underscores the importance of proper sanitation infrastructure and highlights the potential risks associated with proximity between waste disposal sites and water sources. Moreover, the correlation coefficients, t -values, and p -values provide statistical evidence supporting the observed associations. These findings are consistent with previous studies (Ndoziya, Hoko & Gumindoga, 2019), which emphasize the critical role of sanitation in safeguarding water quality and public health.

The detection of *Salmonella*, *Shigella*, and *Staphylococcus aureus* in untreated water samples further underscores the potential health risks associated with groundwater contamination. These pathogens are known to cause a range of waterborne diseases, including salmonellosis, shigellosis, and staphylococcal infections, which can lead to gastrointestinal illnesses and other health complications (Olalemi *et al.*, 2021; Sekgobela *et al.*, 2023).

The absence of these pathogens in treated and ground water samples suggests that treatment processes and natural attenuation mechanisms may be effective in reducing microbial contamination and mitigating public health risks. However, the persistence of total coliforms and *E. coli* in treated water samples indicates that treatment may not

always fully eliminate fecal contamination, highlighting the importance of continuous monitoring and maintenance of water treatment facilities to ensure the provision of safe drinking water (Baharudin *et al.*, 2023).

The findings of the study emphasize the importance of regular monitoring of groundwater quality and the implementation of measures to prevent and control fecal contamination of water sources. Strategies such as improved sanitation infrastructure, protection of groundwater recharge areas, and source water protection programs can help minimize the risk of groundwater contamination and protect public health (Stańczyk-Mazanek & Stępnik, 2021).

In conclusion, the isolation and identification of enteric pathogens from potentially contaminated groundwater in Tigania West Sub County, Meru County, provide valuable information for assessing microbial risks and informing interventions to safeguard public health. Continued research and monitoring are essential to ensure the provision of safe and clean drinking water to communities reliant on groundwater sources.

The study aimed to explore impact of pit latrines on enteric-pathogen and physicochemical contamination of groundwater within Tigania West Sub County, Meru County.

Numerous studies have highlighted the critical role of inadequate sanitation, including the use of pit latrines, in contributing to the spread of enteric pathogens and the contamination of groundwater sources. For example, research by Graham & Polizzotto (2013) emphasizes the link between poor sanitation facilities, such as pit latrines, and the transmission of diarrheal diseases, which are often caused by enteric pathogens like *Salmonella*, *Escherichia coli*, and *Giardia*.

Studies like that of Saxena & Den (2022) have demonstrated how the improper disposal of human waste, particularly in areas with high population density and inadequate

sanitation infrastructure, can lead to fecal contamination of water sources. This contamination introduces enteric pathogens into the environment, posing significant health risks to communities reliant on contaminated water for drinking, cooking, and sanitation purposes.

Regarding physicochemical contamination, research by Sila (2019) has shown that pit latrines and other forms of sanitation can contribute to elevated levels of biological oxygen demand (BOD), turbidity, and other indicators of organic pollution in groundwater. These contaminants not only compromise the aesthetic quality of water but also create favorable conditions for the survival and proliferation of waterborne pathogens (Kirby *et al.*, 2016). These findings are consistent with the study by Kirby *et al.* (2016) and show the complexity of the interplay.

Similarly, there were no significant connections discovered between the distance of pit latrines from water sources, water treatment procedures, and the presence of enteric pathogens. These findings highlight the multifactorial nature of waterborne disease transmission, which is characterized by the fact that contamination risks are impacted by a mix of environmental, infrastructural, and behavioral factors (Bain *et al.*, 2014).

Nevertheless, the presence of discernible alterations in water, such as alterations in colour or odour, was found to be significantly associated with a greater burden of diseases that are transmitted through water. The importance of monitoring water quality and identifying changes in water characteristics at an early stage as indicators of potential contamination and health risks is brought into focus by this discovery. Previous studies have reported similar associations between changes in water quality and the incidence of waterborne diseases. These findings highlight the importance of proactive surveillance and response strategies in order to protect public health (Daly and Harris,

2022). The findings of previous research have demonstrated that there is a substantial connection between enteric pathogens and the burden of disease (Chard *et al.*, 2020).

Individual and infrastructure factors may not directly influence the presence of enteric pathogens, the findings suggest that changes in water quality can serve as important indicators of contamination and potential health risks. This is the conclusion that can be drawn from the findings. At the same time, it is essential to emphasize the significance of water quality monitoring and early intervention in order to protect public health, effective strategies for the prevention of waterborne diseases should center on holistic approaches that address environmental, socio-economic, and behavioral determinants of disease transmission.

CHAPTER SIX: CONCLUSION, RECOMMENDATIONS, AND PUBLICATION

6.1 Introduction

This chapter contains conclusions and offers recommendations based on the results obtained and presents the dissemination of research outcomes through publication.

6.2 Conclusion

Based on the three objectives outlined in this study, several key conclusions can be drawn regarding the impact of pit latrines on enteric-pathogen and physicochemical contamination of groundwater in Tigania West Sub-County, Meru County, Kenya.

The survey of pit latrine sites in relation to groundwater sources revealed significant spatial variability within Tigania West Sub-County. A considerable proportion of pit latrines were found to be in close proximity to water sources, within 30 m, raising concerns about potential fecal contamination of groundwater. This highlights the need for improved sanitation and regulatory measures to prevent the contamination of groundwater sources by pit latrines.

The assessment of physicochemical properties of groundwater revealed variations in parameters such as pH and turbidity, with some samples not fully complying with WHO drinking water quality guidelines. While most samples fell within acceptable ranges, the presence of high turbidity levels in some groundwater samples suggests potential contamination by suspended particles, highlighting the need for further investigation and remediation measures.

The isolation and identification of enteric pathogens from potentially contaminated groundwater samples underscored the presence of microbial contaminants in the water sources within the study area. The detection of pathogens such as total coliforms, *E. coli*, *Salmonella*, and *Shigella* indicates potential health risks associated with groundwater

consumption, emphasizing the importance of regular water quality monitoring and treatment to ensure safe drinking water provision.

The analysis of associations between location of pit latrines, isolated enteric pathogens, physicochemical characteristics of groundwater, and disease burden within provided insights into the complex interactions between pit latrines and public health outcomes.

While were significant correlations found between individual variables, the presence of noticeable changes in water quality was associated with a higher burden of waterborne diseases, indicating the importance of water quality monitoring and early detection of contamination.

This study highlights the significant impact of pit latrines on enteric-pathogen and physicochemical contamination of groundwater in Tigania West Sub-County, Meru County, Kenya. Addressing the challenges posed by inadequate sanitation infrastructure and groundwater pollution requires coordinated efforts from government agencies, local communities, and relevant stakeholders to ensure access to safe and clean drinking water for all residents. Further research and interventions are needed to mitigate the risks associated with fecal contamination of groundwater and safeguard public health in the study area and similar settings.

6.2 Recommendations

Based on the findings of the study, several key recommendations can be made to address the identified challenges and improve water quality and public health outcomes in the study area, these include:

Improve Sanitation Infrastructure: Enhance the construction and maintenance of pit latrines to ensure they are situated at safe distances from groundwater sources.

Implement regulations and guidelines for the siting and construction of pit latrines to minimise the risk of fecal contamination of groundwater.

Promote Safe Water Practices: Increase awareness and education among local communities on the importance of safe water practices, including proper sanitation and hygiene measures. Encourage the use of improved sanitation facilities and the adoption of water treatment methods to reduce the risk of waterborne diseases.

Strengthen Water Quality Monitoring: Establish a comprehensive water quality monitoring program to regularly assess the microbial and physicochemical parameters of groundwater sources. Conduct routine testing and analysis to identify potential contamination sources and trends in water quality over time.

Implement Water Treatment Measures: Invest in water treatment infrastructure and technologies to ensure the provision of safe and clean drinking water to local communities. Implement appropriate treatment processes, such as filtration, chlorination, and UV disinfection, to remove microbial contaminants and improve water quality.

Enforce Regulatory Measures: Enforce existing regulations and policies related to water quality and sanitation to prevent groundwater contamination. Strengthen regulatory oversight and enforcement mechanisms to ensure compliance with standards and guidelines for drinking water quality.

Foster Community Engagement: Engage local communities in water resource management and decision-making processes to promote ownership and sustainability of water and sanitation initiatives. Foster partnerships between government agencies, Non-Governmental Organisations (NGOs), community-based organisations, and other stakeholders to collaboratively address water quality challenges.

Conduct Further Research: Conduct additional research to investigate the sources and pathways of groundwater contamination in the study area. Explore the interactions between land use practices, hydrogeological conditions, and water quality parameters to develop targeted interventions and management strategies.

Address Socio-Economic Factors: Address underlying socio-economic factors contributing to inadequate sanitation and water quality issues, such as poverty, inadequate infrastructure, and limited access to resources. Implement poverty alleviation programs and livelihood improvement initiatives to empower communities and enhance their resilience to water-related challenges.

Enhance Capacity Building: Build the capacity of local institutions, water utilities, and community organisations to effectively manage and maintain water and sanitation infrastructure. Provide training and technical support to local stakeholders on water quality monitoring, sanitation practices, and sustainable water management.

Monitor and Evaluate Interventions: Establish mechanisms for monitoring and evaluating the effectiveness of interventions aimed at improving water quality and sanitation. Monitor key performance indicators and outcomes to assess progress towards achieving water quality targets and inform adaptive management strategies.

Collaboration and concerted efforts from all sectors of society are essential to ensure access to safe and clean drinking water for all residents.

Overall, this research study aimed to contribute to the body of knowledge on water sanitation and public health in Tigania West Sub-County and similar settings, providing evidence-based insights to inform policy-making and intervention strategies aimed at improving water quality and reducing disease burden in the community.

6.3 Publication

Stephen, M. E., Kagendo, D. & Mungai, J. N. (2025). Impact of Pit Latrines on Enteric-Pathogen Contamination of Groundwater in Tigania West Sub-County, Kenya. *Internation Journal of Current Science Research and Review*, 8(9), 4423-4431. <https://doi.org/10.47191/ijcsrr/V8-i9-03>

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APPENDICES

Appendix A. Research Permit

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Appendix B. Publication

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Impact of Pit Latrines on Enteric-Pathogen Contamination of Groundwater in Tigania West Sub-County, Kenya

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ABSTRACT: Access to safe water and sanitation remains a challenge in many developing regions, where pit latrines are the dominant sanitation option. Their proximity to groundwater raises concerns about fecal contamination and related health risks. This study examined the effect of pit latrines on enteric-pathogen and physicochemical contamination of groundwater in Tigania West Sub-County, Meru County, Kenya. A cross-sectional design was applied, with water samples collected from 96 households across five wards. Groundwater samples were obtained from boreholes and wells located within 30 meters and beyond the recommended setback distances from pit latrines. Microbiological analyses targeted total coliforms, *Escherichia coli*, *Salmonella*, *Shigella*, and *Staphylococcus aureus*, while physicochemical parameters including pH, turbidity, colour, odour, and biochemical oxygen demand (BOD) were measured. The results revealed high levels of microbial contamination across wards. Total coliforms were most prevalent in Athwana (1.08/100 ml) and Mbeu (0.80/100 ml), while *E. coli* contamination was highest in Athwana (0.23/100 ml). *Salmonella* was widespread, with Nkomo (0.82/100 ml) and Athwana (0.92/100 ml) showing the greatest occurrence. *Staphylococcus aureus* ranged from 3.8/100 ml in Kianjai to 7.23/100 ml in Athwana, exceeding WHO permissible limits. Overall, wards with pit latrines situated within 30 meters of water sources such as Mbeu (86.7%) and Nkomo (76.5%) recorded significantly higher contamination. These findings underscore the role of pit latrines in groundwater pollution in Tigania West and highlight the urgent need for improved siting, design, and management of sanitation facilities to safeguard public health.

KEYWORDS: Enteric-Pathogen Physicochemical Analysis, Groundwater Contamination, Pit Latrines.

1.0 INTRODUCTION

Globally, about 2.6 billion people lack access to adequate sanitation facilities, which are crucial for separating humans from their fecal waste and preventing the spread of diseases. Common sanitation methods, particularly in low-income areas, include water-based toilets and pit latrines, with the latter being the most widespread (Garn et al., 2017). Improved sanitation has been linked to reduced incidences of diarrhea, lower morbidity and mortality rates, and decreased soil-transmitted helminths (Kelly et al., 2020). Despite these benefits, open defecation persists among nearly one billion people worldwide, highlighting the need for targeted policies under the United Nation Sustainable Development Goals (SDGs) aimed at achieving universal access to safe sanitation by 2030. These policies emphasize prioritizing marginalized populations and underserved settlements.

Proper sanitation services also enhance dignity, economic productivity, and environmental protection. When communities have reliable access to safe water, they reduce the time and effort spent on water collection, allowing for greater productivity and better health outcomes (Kelly et al., 2020). Improved sanitation decreases disease burdens, boosts children's school attendance, and leads to economic savings by reducing healthcare expenses (*Water, Sanitation and Hygiene (WASH)*, n.d.). However, Fecal Sludge Management (FSM) poses challenges, as poor handling of pit latrine waste can result in contamination of waterways and open drains, contributing to environmental and public health hazards (Mariwah et al., 2022).

Pit latrine construction faces technical challenges, particularly in flood-prone or high-water-table areas, where raised latrine designs may be necessary (Garcia-Becerra et al., 2021). Selecting appropriate construction sites is critical, with recommended horizontal distances of at least 30 meters from drinking water sources to limit microbial contamination (Garcia-Becerra et al., 2021). Factors such as soil type, aquifer properties, and environmental conditions also influence pathogen removal effectiveness, making it difficult to establish universally safe distances (Paul et al., 2020). WHO guidelines suggest a minimum vertical separation of two meters between the pit bottom and groundwater, although some experts recommend up to 50 meters depending on local conditions (Gokçekuş et al., 2020).

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Appendix C. GPS Table for all sampled sites in every ward

Ward	Location (Coordinates)	
Kianjai	0.160194 ,37.748254	
	0.170296 ,37.740978	
	0.173501 ,37.759922	
	0.1690222 37.751539	
	0.162050 ,37.741209	
	0.152610 ,37.778445	
	0.160452 ,37.769147	
	0.163786 ,37.781058	
	0.16812 ,37.768927	
	0.178773 ,37.759221	
	0.1542263, 37.737669	
	0.1569971, 37.412082	
	0.1579705, 37.7498541	
	0.1710907, 37.7584782	
	0.1608285, 37.6837212	
	0.1664691, 37.7531649	
	0.1828947, 37.7644553	
	0.1520449, 37.7840433	
	0.1512132, 37.7906239	
	0.1520449, 37.7840433	
	0.1506547, 37.7847072	
	0.1664691, 37.753149	
	0.1569971, 37.7412082	
	0.1617441, 37.7463468	
	0.1630219, 37.7405439	
	0.1710907, 37.7584782	
	Nkomo	Location (Coordinates)
		0.1158, 37.71
		0.1222, 37.71511
		0.154684, 37.740039
		0.156818, 37.725768
		0.139588, 37.7475
		0.128520, 37.725597
0.124112, 37.731347		
0.118051, 37.719436		
0.137105, 37.714750		
0.129663 , 37.720200		
0.1267042, 37.7192824		
0.1232732, 37.7189338		
0.1271632, 37.7216082		
0.128471, 37.7194379		
0.1324853, 37.7229372		
0.1141744, 37.7402117		
0.1455579, 37.7320164		
Location (Coordinates)		

Akithi	0.174848, 37.762754 0.192121, 37.785011 0.187535, 37.7240 0.195324, 37.789161 0.191279, 37.761832 0.185081, 37.753283 0.182745, 37.74158 0.186555, 37.7350 0.16665004, 37.7392152 0.1850924, 37.7667376 0.1859072, 37.7667571 0.1828947, 37.7644553 0.1984182, 37.7677757 0.1750432, 37.7528328 0.1851234, 37.7667318 O.1750432, 37.7528328 0.2013803, 37.7870308 0.2043649, 37.797652 0.2071758, 37.7873627 0.2043649, 37.797652 0.2018421, 37.7876946 0.1664691, 37.7531649 0.2071856, 37.7872627 0.1524914, 37.7920097 0.1985182, 37.767767
Athwana	Location (Coordinates) 0.215170, 37.750670 0.219071, 37.830953 0.243970, 37.832467 0.246112, 37.826768 0.234164, 37.724730 0.220277, 37.724138 0.053543 ,37.648399 0.230457 ,37.746641 0.1984182, 37.7677752 0.1984182, 37.7677757 0.2112119, 37.7544932 0.2160574, 37.7507234 0.2202481, 37.74443614
Mbeu Ward	Location (Coordinates) 0.02983, 37.78625 0.07521, 37.76069 0.09790, 37.7606 0.0842, 37.7778 0.0463, 37.6559 0.0480, 37.6865 0.0483, 37.7571 0,0453, 37.7146 0.0405, 37.8141 0.0775, 37.7291

0.5147, 37.6456
0.1370, 38.2457
0.1199, 37.8380
0.1066, 37.7635
0.0359, 37.8486

Appendix D. Structured Questionnaire

Study Title: Effect of Pit Latrines on Enteric-Pathogen and Physicochemical Contamination of Groundwater in Tigania West Sub-County, Meru County, Kenya

Participant's Name

Participant's Contact

Interview Date

Questionnaire Code

Socio-demographic profile of the participant

(Tick and answer the applicable)

1. What gender is the respondent? *(Tick the applicable)*

- Male
- Female

3. What is the highest level of education attained? *(Tick the applicable)*

- No formal education
- Primary education
- Secondary education
- Tertiary education

4. What is the age (years) of the respondent *(Tick the applicable)*

- Youths (18-30)
- Adults (> 30)

KNOWLEDGE, ATTITUDE AND PRACTICES (KAP) TOOL

Knowledge on the impact of pit latrines on groundwater hygiene *(Tick the applicable)*

- Do you understand the meaning of underground water hygiene?

Yes No Uncertain

- Do you have a pit latrine?
 - Yes No Uncertain
- Did you monitor the construction of your pit latrines?
 - Yes No Uncertain
- Do water sources pass near your pit latrine (s)?
 - Yes No Uncertain
- If yes, how do you ensure that the water source (s) near pit latrine(s) is/are safe?

Attitudes toward safety of groundwater

- We should ensure underground water is safe.
 - Yes No Uncertain
- Pit latrine(s) should be near water source(s).
 - Yes No Uncertain
- There is need for more water safety training programmes.
 - Yes No Uncertain
- Policy implementation of pit latrine construction should be implemented
 - Yes No Uncertain

Practices on use and construction of pit latrines and water safety

- Is your pit latrine close to any underground water point (s)?
 - Yes No Uncertain

If yes, approximately how many meters is the pit latrine away from the water source?

- Less than 30 meters
- More than 30 meters
- Do you treat your water before use?
 - Yes No Uncertain

- Do you have a pit latrine license?
 Yes No Uncertain
- Do you have a sewage treatment point?
 Yes > No

Thank you for participating in the survey.

Appendix E. Informed Consent Form

Study Title: Effect of Pit Latrines on Enteric-Pathogen and Physicochemical Contamination of Groundwater in Tigania West Sub-County, Meru County, Kenya

Researcher: Evans Monyancha Stephen

Introduction

My name is Evans Monyancha Stephen. I am currently a postgraduate student at Meru University of Science and Technology. As part of my studies, I am conducting research **on the impact of pit latrines on enteric-pathogen contamination of groundwater in Nkomo Ward, Meru County, Kenya.** The information obtained from this study will be used to address this problem.

If you agree to participate in this study, you will be asked questions regarding your knowledge, attitudes, and practices related to pit latrine hygiene. Participation in this research is voluntary.

Purpose of the Study

The purpose of this study is to assess the impact of pit latrines on enteric-pathogen contamination of groundwater in Nkomo Ward, Meru County, Kenya, and to understand how this relates to underground water hygiene and safety.

Study Procedure

If you agree to participate in this study, the researcher will provide you with a questionnaire to complete. You will be asked to respond to the questions on the questionnaire. The questionnaire should take approximately 15 minutes to complete.

Confidentiality and Privacy

Any information you provide about your identity will be treated with confidentiality. The information provided will only be used for the purposes disclosed in this study.

Benefits

As a participant in this study, you will receive hygiene advice to help reduce the extent of water contamination.

Compensation

No compensation will be offered for participation in this study. If you have any questions regarding your participation in the study, please feel free to contact the researcher or supervisors at any time.

Researcher: Evans Monyancha Stephen

Tel: 0706673863

Supervisors:

Dr. Dorothy Kagendo Kithinji

Tel: 0721431374

Dr. George N. Mungai

Tel: 0771551040

Consent

I have read and understood the purpose and procedures of this study. I hereby agree to participate in this study as explained to me.

Participant's Signature: _____ Date: _____

Appendix F. Photos of Water Analysis Enteric pathogens done at the Laboratory



Appendix G. Photos of Water Analysis on Physicochemical Properties of the Groundwater done at the Laboratory



Appendix H. Photos of Water Analysis on Biological Oxygen Demand (BOD) done at the Laboratory



**Appendix I. Photos of Water Analysis Isolation and Identification of Helminths
done at the Laboratory**



Appendix J. Photos of Water Analysis on Isolation and Identification of Enteric Pathogens done at the Laboratory



Appendix K. Plagiarism Report

