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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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Protected water sources are designed to prevent contamination by human waste, unlike unprotected sources, which are vulnerable to seepage from agricultural and sanitation activities. Poor sanitation management increases the risk of diseases like cholera, typhoid, and schistosomiasis (Othoo et al., 2020). In developing countries, poor waste management contributes to intestinal worm infections affecting approximately 10% of the population (Saijuntha et al., 2021). In Kenya, sanitation-related illnesses cost the country about $27 million annually (Othoo et al., 2020).

Despite modest progress in improving sanitation facilities in Kenya, with coverage increasing from 25% in 1990 to 30% in 2015 (Gudda et al., 2019), significant disparities remain between rural and periurban areas. Peri-urban regions face serious water quality concerns, including excess nitrate, chloride, and microbial pathogens from pit latrines. Research indicates a steady decline in groundwater quality due to contamination from pit latrines, underscoring the need for robust policies to protect water sources and ensure safe drinking water in areas like Tigania West Sub County. Addressing these challenges requires evidencebased strategies to improve water availability, sanitation practices, and public health outcomes.

2.0 METHODOLOGY

2.1 Study Area

This research was carried out in Tigania West Sub-County, Meru County, in Kenya (Figure 1).

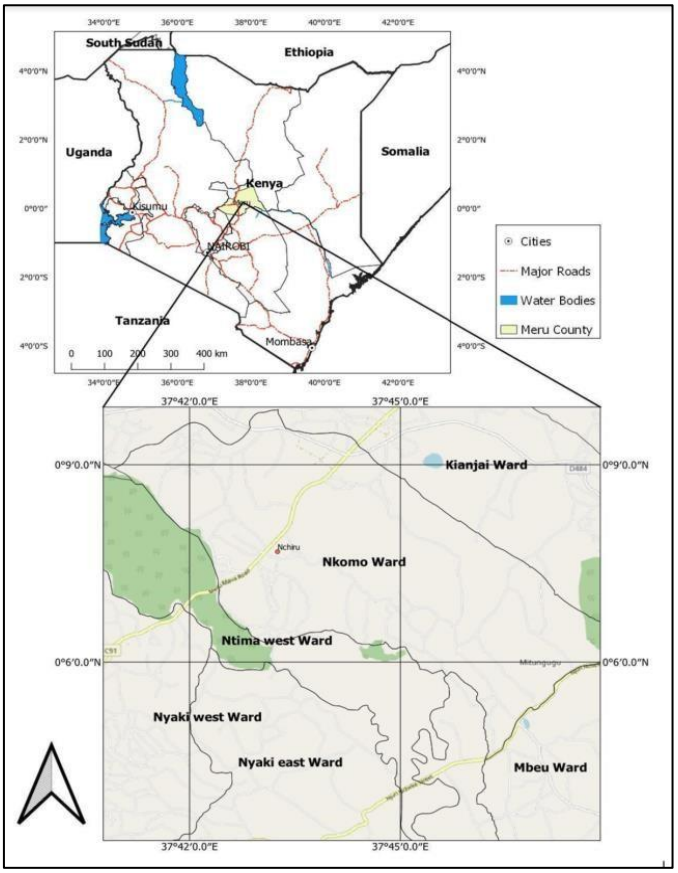

Figure 1. Map of Tigania West Sub- County, Meru County, Kenya

2.2 Chemical Reagents

Analytical grade reagents were used which included pH buffers, 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.

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2.3 Sampling

2.3.1 Sample Size

The sample size was 96 households, calculated using Fisher's formula for proportions:
Using this formula, the sample size for this research can be computed in the following manner:

$$n - \frac{Z^2 \times p \times (1 - p)}{d_2}$$

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 ≈ 96
To ensure proportional representation across the five wards Mbeu, Kianjai, Nkomo, Akithi, and Athwana the sample was distributed according to each ward's population size based on the most recent administrative household data as shown in Table 1. Proportional allocation was used to ensure fair representation.

Table 1: Sample size per Ward

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)

2.3.2 Inclusion Criteria

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

2.3.3 Exclusion Criteria

All households who did not consent to participate in the study were excluded (Shrestha & Dunn, 2019) People who had not resided in the study area for more than six months were excluded from the study.

2.3.4 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). Sample bottles were sterilized by autoclaving at 121°C for 15 minutes. A sterile 500 mL glass bottle was used, where the bottle was aseptically opened. The mouth of the bottles was flamed using alcohol cotton swab to ensure the mouth was sterile in order to avoid contamination. Water from wells was collected by sterilized bucket and aseptically poured into a collection bottle laid on the flat surface. Water from borehole 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.

2.4 Isolation and Identification of Enteric Pathogens

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

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2.4.1 Total Coliforms

Before the water was 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 incubated at 44 ºC for 24 hours. After one day the samples were observed for color change. Table 1 above shows the analysis set up for total coliforms using the MPN method (Bekuretsion et al., 2018).

Table 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
Groundwater	5	5	Single

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 Most Probable Number (MPN) method was used to estimate the number of coliform bacteria in the water samples (Bekuretsion et al., 2018). During the presumptive test, multiple lactose broth tubes were inoculated with the samples and incubated at 35°C for 48 hours; tubes that produced gas were recorded as positive for coliforms. Positive samples were then subjected to a confirmed test, where they were sub-cultured onto selective media to verify the presence of coliform bacteria. In the completed test, colonies from confirmed positives were transferred to nutrient agar slants and analysed using biochemical tests, including Gram staining and indole production. After 24 hours of incubation, samples were sub-cultured on Tryptone Bile X-glucuronide (TBX) agar to isolate pure colonies.

2.4.2 E. coli

The water samples were subjected to an Indole test which is an effective method of confirming *E. coli* presence (Gebrewahd et al., 2020). The samples were inoculated into a bijou bottle container. Postinoculation was done by incubating the container at a consistent temperature of 37ºC, mirroring optimal conditions for *E. coli* growth.

2.4.3 Salmonella

The samples were enriched in selenite cystine broth, followed by incubation at 37°C for 24 hours (Brenner et al., 2000). 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 wass 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 appeared 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 Triple Sugar Iron (TSI) agar and Lysine Iron Agar (LIA) for a more accurate determination. Each positive result from these tests represented *Salmonella* contamination in the water sample (Neyaz et al., 2024).

2.4.4 Shigella

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 (Miles et al., 2024). 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.

2.4.5 Staphylococcus aureus

For isolating and identifying *Staphylococcus aureus*, the water samples were initially streaked onto BairdParker 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 2448 hours, facilitating the optimum growth of potential *S. aureus* colonies. Following the incubation period, colonies that exhibit characteristics of *S. aureus* were selected and counted. The number colonies per plate were used to estimate the concentration of these bacteria in the original water sample, thereby quantifying the level of *S.*

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aureus contamination (Alexander et al., 2009). For definitive identification of *S. aureus,* colonies were subjected to further verification through biochemical tests 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.

2.4.6 Streptococcus faecalis

The water samples were streaked onto Bile Esculin Azide (BEA) agar, a medium specifically designed for the growth of *Streptococcus faecalis* also known as *Enterococcus faecalis*. The samples were then incubated at a stable temperature of 37°C for 24-48 hours. After the incubation period, colonies that visually resembled *S. 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 *S. faecalis* was performed by counting the number of validated colonies on each plate. The total count was then used to calculate the concentration of *S. faecalis* in the original water sample, thus quantifying the extent of contamination. Utilising the BEA agar for primary isolation, followed by the PYR test for confirmation, ensured a high degree of specificity and reliability in the results (Garcia-Garrote et al., 2000).

2.4.7 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. The filter was examined under a microscopic to identify and verify the presence of helminth eggs and larvae. helminths in water samples were quantified by counting the number of eggs and larvae visible under the microscope in the filter membrane. The total count was then adjusted based on the volume of water that was filtered. Results were presented as eggs or larvae per litre of water, providing a measurable indication of the level of helminth contamination (Sandoval et al., 2015).

3.0 RESULTS AND DISCUSSION

Table 3 represents the microbiological quality of water samples across wards, prevalence of enteric pathogens in relation to WHO Guidelines in Tigania West Sub-County.

Table 3: Microbiological Quality of Water Samples Across Wards: Prevalence of Enteric Pathogens in Relation to WHO Guidelines

Ward	Sample Size	Enteric Pathogen	Positive Samples	Concentration (CFU/mL Count)	Enteric Pathogens Sample Size	WHO Permissible Limit
Mbeu	15	Total Coliforms	6	12 CFU/100 mL	0.80	<10 CFU/100 mL
		E. coli	7	1.8 CFU/100 mL	0.02	<1 CFU/100 mL
		Salmonella	6	11 Salmonella/250 mL	0.73	<10 Salmonella/250 mL
		Shigella	1	2 Shigella/100 mL	0.13	<1 Shigella/100 mL
		Staphylococcus aureus	7	90 CFU/100 mL	6	<100 CFU/100 mL
Kianjai	26	Total Coliforms	6	10 CFU/100 mL	0.38	<10 CFU/100 mL
		E. coli	5	2 CFU/100 mL	0.08	<1 CFU/100 mL
		Salmonella	5	10 Salmonella/250 mL	0.38	<10 Salmonella/250 mL
		Shigella	4	4 Shigella/100 mL	0.15	<1 Shigella/100 mL
		Staphylococcus aureus	5	98 CFU/100 mL	3.80	<100 CFU/100 mL
Nkomo	17	Total Coliforms	3	9 CFU/100 mL	0.53	<10 CFU/100 mL

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		E. coli	3	2 CFU/100 mL	0.12	<1 CFU/100 mL
		Salmonella	2	14 Salmonella/250 mL	0.82	<10 Salmonella/250 mL
		Shigella	2	3 Shigella/100 mL	0.18	<1 Shigella/100 mL
		Staphylococcus aureus	3	112 CFU/100 mL	6.58	<100 CFU/100 mL
Akithi	25	Total Coliforms	5	13 CFU/100 mL	0.52	<10 CFU/100 mL
		E. coli	4	3 CFU/100 mL	0.12	<1 CFU/100 mL
		Salmonella	3	15 Salmonella/250 mL	0.60	<10 Salmonella/250 mL
		Shigella	1	5 Shigella/100 mL	0.20	<1 Shigella/100 mL
		Staphylococcus aureus	2	120 CFU/100 mL	4.80	<100 CFU/100 mL
Athwana	13	Total Coliforms	7	14 CFU/100 mL	1.08	<10 CFU/100 mL
		E. coli	3	3 CFU/100 mL	0.23	<1 CFU/100 mL
		Salmonella	2	12 Salmonella/250 mL	0.92	<10 Salmonella/250 mL
		Shigella	3	5 Shigella/100 mL	0.38	<1 Shigella/100 mL
		Staphylococcus aureus	2	94 CFU/100 mL	7.23	<100 CFU/100 mL

Table 4 shows the Analysis of Enteric Pathogens in Water Samples by Ward (less than 30 meters) in Tigania West Sub-County.

Table 4 Analysis of Enteric Pathogens in Water Samples by Ward.

Ward	<30 mPit Latrines Distance (%)	Total Coliform/N	E-Coli/N	Salmonella/N	Shigella/N	Staphylococcus aureus/N
Mbeu	86.7	0.80	0.02	0.73	0.13	6.0
Kianjai	13.3	0.38	0.08	0.38	0.15	3.80
Nkomo	76.5	0.53	0.12	0.82	0.18	6.58
Akithi	48.0	0.52	0.12	0.60	0.20	4.80
Athwana	69.2	1.08	0.23	0.92	0.38	7.23

The results in Table 4 showed marked ward-level differences in microbial contamination of water sources for Pit Latrines less than 30 meters. Pit latrine proximity was highest in Mbeu (86.7%), followed by Nkomo (76.5%), Athwana (69.2%), Akithi (48.0%), and lowest in Kianjai (13.3%). *Total coliform* levels were highest in Athwana (1.08) and Mbeu (0.80), moderate in Nkomo (0.62) and Akithi (0.59), and lowest in Kianjai (0.38). *E. coli* occurrence was more in Athwana (0.23), was moderate in Nkomo and Akithi (0.12 each), and lowest in Mbeu (0.02). *Salmonella* contamination was highest in Athwana (0.92) and Nkomo (0.82), followed by Mbeu (0.60) and Akithi (0.42), with Kianjai (0.38) recording the lowest. *Shigella* was most prevalent in Athwana (0.38), moderate in Akithi (0.20), and lower in Kianjai (0.15) and Mbeu (0.13), with minimal detection in Nkomo (0.02). *Staphylococcus* aureus counts were highest in Athwana (7.23), Nkomo (6.58), and Mbeu (6.00), while Akithi (4.80) and Kianjai (3.80) recorded comparatively lower levels.

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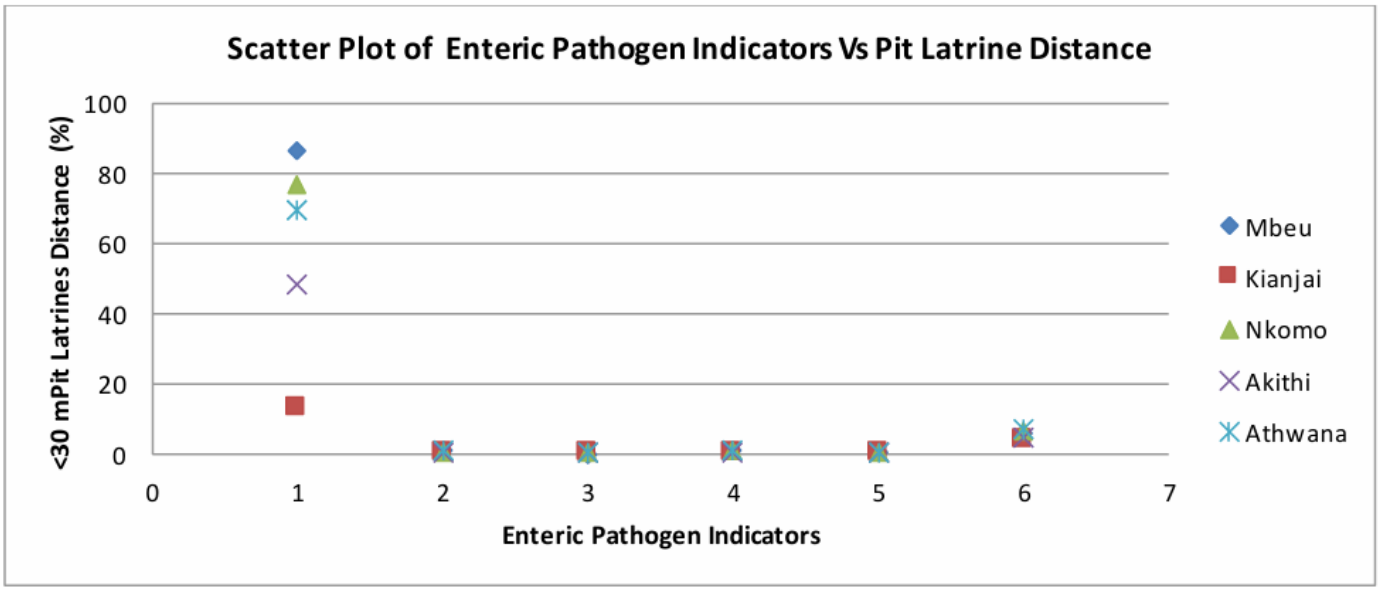

Figure 1

3.1 DISCUSSION

The study revealed significant bacteriological contamination of groundwater across all five wards in Tigania West Sub-County, Kenya, linked to pit latrine seepage, with frequent and often severe exceedance of WHO drinking water standards for key pathogens. While *Staphylococcus aureus* levels were within permissible limits in Mbeu, Kianjai, and Akithi wards, they were exceeded in Athwana and Nkomo wards. More critically, contamination by enteric pathogens indicative of fecal pollution was widespread. *Total Coliforms* exceeded limits in Mbeu and Athwana. *E. coli*, a direct indicator of recent fecal contamination, surpassed safe levels in Athwana ward, with Mbeu being the only ward marginally lowest. *Salmonella* levels were high in four wards (Mbeu, Nkomo, Akithi, Athwana), with notably lower concentrations in Kianjai. *Shigella* contamination was high in Athwana, moderate in Akithi, and lower in Kianjai and Mbeu, with minimal detection in Nkomo.

These findings aligned strongly with global and regional studies on groundwater contamination from pit latrines and on-site sanitation, while highlighting specific local severity. The high prevalence of *E. coli* and Total Coliforms mirrors studies in similar settings globally (Graham & Polizzotto, 2013) and across Africa (Mkwate et al., 2017), confirming pit latrines as a major pathway for pathogens into shallow groundwater. The consistent and significant prevalence of *Shigella* and *Salmonella* limits across *all* wards are particularly alarming. While studies often find fecal indicators (like *E. coli*), detecting specific pathogens like *Shigella* is less commonly reported at this scale within a single study area. This suggests a very high disease risk potential in Tigania West Sub County. Similar high enteric pathogen detection linked to pit latrines has been observed in densely populated peri-urban areas(Burgess et al., 2010). The variation in contamination levels between wards (e.g., Mbeu and Nkomo showing low levels of *E. coli* ; Athwana showing high levels for multiple pathogens) reflects findings that contamination risk is highly dependent on local hydrogeology (soil type, depth to water table), latrine density/design, and population density. Studies in Kenya (Babb, 2016;Babb et al., 2018) and in Cameroon (Mabvouna et al., 2023) emphasize these factors. The widespread contamination, even where standard setback distances between latrines and wells might be assumed, supports previous research (e.g., in South Africa: Dzwairo et al., 2006) showing that recommended minimum distances are often insufficient, especially in areas with high water tables, permeable soils, or intense rainfall. A recent study in a Kenyan informal settlement (Anampiu et al., 2023) also found significant contamination despite latrines being >30m from wells in some cases. The mixed results for *S. aureus* in some wards and compliance in others align with studies suggesting it originates from both human sources (skin, nasal passages) and potentially environmental sources, making it a less specific indicator of fecal contamination compared to *E. coli* or enteric pathogens.

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4.0 CONCLUSION

This study concludes that pit latrines significantly impact groundwater contamination in Tigania West SubCounty, Meru County, Kenya, through both enteric-pathogen. The proximity of many pit latrines to water sources raises concerns about fecal contamination, and the presence of pathogens such as *E. coli* and *Salmonella* indicating health risks. While some groundwater parameters met WHO standards, others showed potential contamination, underscoring the need for improved sanitation practices, regular water quality monitoring, and regulatory enforcement. To safeguard public health, coordinated interventions by government agencies, local communities, and stakeholders are essential to mitigate groundwater pollution and ensure access to safe drinking water.

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