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RESEARCH PAPER

Application of microorganisms to determine the impact of infiltration layer and season on pit latrine groundwater contamination

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Abstract. This study was necessitated as a result of the frequent cases of diarrhoea observed among the students of the researcher. The researcher employed faecal indicator bacteria to assess the impact of the infiltration layer and seasonal variations on groundwater contamination from pit latrines in the municipalities where the students reside. The main experimental materials consisted of water samples collected from 15 randomly selected wells in the Tano Districts of Ghana. Total coliforms, faecal coliforms, and enterococci were used as faecal indicators. The Most Probable Number (MPN) method was employed to determine the presence of faecal indicators in the water samples. The results showed that enterococci and faecal coliforms were reliable indicators of human faecal contamination than total coliforms. The study revealed that coliform level (indicating pit latrine groundwater contamination) increased with greater pit depth and lower static water levels. Based on these findings, it is recommended that the future studies on human faecal contamination prioritize enterococci and faecal coliforms over total coliforms as indicators. To mitigate groundwater contamination from pit latrines, the equation EC = 0.12(PLD) – 0.09(SWL) + 2.37 can be applied to predict a safer infiltration layer between the bottom of pit latrines and the water table.

Keywords: Faecal indicators; Groundwater; Infiltration layer; Pit latrine; Pit latrine depth; Static water level.

1. Introduction

Microorganisms are a group of living organisms that cannot be seen with the naked eye but can only be observed with the aid of a microscope. The ubiquitous nature allows them to survive in nearly every environment, including the human body (Sessitsch et al., 2023; Rappuoli et al., 2023). Microorganisms can be classified as either pathogenic or part of the normal flora of the gastrointestinal tract. While some microorganisms are adapted to cause diseases, others may become opportunistic pathogens when their host's immune systems are compromised or suppressed.

Faecal indicator bacteria are microorganisms that are normal inhabitants of the gastrointestinal tract of humans and other warm-blooded animals (Basu et al., 2020). These

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bacteria are utilized as faecal markers to show potential sewage pollution (Lenaker et al., 2024; Martin et al., 2024; Biguioh et al., 2020). This approach is reasonable because these microorganisms are typically present in human faeces. As such, they serve as a reliable criterion for assessing faecal contamination.

One effective strategy for mitigating human exposure to faecal contamination is improved sanitation (Mertens et al., 2023; Holcomb et al., 2020; Trimmer et al., 2020). Without proper sanitation, people are more likely to be exposed to faecal pollution and its associated harmful effects on human health. This includes illness and fatalities linked to intestinal infection caused by water contamination. The urgent need for effective sanitation strategies is particularly evident in underdeveloped and underprivileged regions, where human exposure to faeces contribute significantly to disease outbreaks and mortality (Wolf et al., 2022).

The choices of drinking water supplies continue to evolve, with a growing dependence on groundwater (WHO, 2023). This reliance can be attributed to groundwater being perceived as safe for human consumption. However, Awuah (2020) highlights a paradigm shift from the traditional belief that groundwater is completely uncontaminated, underscoring the need for further research into its quality. Contamination of groundwater by faecal matter can lead to severe gastrointestinal illnesses (Benediktsdóttir et al., 2020; Maramraj et al., 2020). Moreover, the presence of the faecal matter may also indicate the potential presence of other pathogenic microorganisms.

According to Graham & Polizzotto (2013), as nations strive to achieve the sanitation-related objectives of the Millennium Development Goals (MDGs), pit latrines - one of the most widely used methods for disposing of human waste in low-income countries, - are gaining increasing popularity. However, pit latrines, as a means of sanitation, can cause severe groundwater pollution if not properly sited (Gwenzi et al., 2023). To illustrate this, comparable research shows that leachate from pit latrines can seep into the ground and contaminate groundwater, posing significant risks, despite their affordability as one of the least expensive options for managing human waste and urine in low-income countries (Yahaya et al., 2023; Bakari et al., 2023).

There is growing concern that chemical and microbiological toxins from pit latrines may leak into groundwater, posing risks to human health. One significant factor contributing to groundwater contamination in pit latrines is the distance between the water table and the bottom of the pit (Ferrer et al., 2020).

This study defines the infiltration layer as the distance between the depth of the water table (static water level) and the base of the pit latrine. The infiltration layer refers to the depth of porous, unsaturated soil that lies between the bottom of a subsurface soil absorption system and a restricting or limiting feature, such as the water table, bedrock hardpan, undesirable fine-textured soils, or excessively permeable material (Awuah, 2020). The further the pathogen-containing water must travel to reach the water table, the more complex its journey becomes and the longer it remains in transit. The additional time allows a significant number of pathogens to naturally die off (Sugden, 2006). As a result, the likelihood of contamination decreases as the distance between the water table and base of the pit increases.

The movement of coliform organisms and chemical pollutants from contaminated trenches into groundwater (saturated circumstances) depends on a multitude of intricate and interrelated parameters (Awuah 2012; Banerjee, 2011). According to World Health Organization (WHO), the risk of groundwater contamination is minimal if there is more than 2 m of relatively fine soil between a pit and the groundwater table (Graham & Polizzotto, 2013). Lusk et al. (2017) observed that pathogens removal occurs during slowed passage through the soil due to bonding with particles and natural die-off caused by the unfavourable environment of aerobic soils and predatory soil organisms. Parameters such as soil moisture and soil type vary greatly across different area.

Therefore, studies must be locality-specific to provide evidence needed to determine the precise infiltration layer suitable for areas where pit latrines coexist with groundwater resources used as domestic water sources. In the Tano Districts, particularly in Zongo communities, most residents use pit latrines for sanitation and groundwater for household purposes (Awuah, 2020). Despite this, little research has been conducted on the impact of infiltration layer and seasonal variations on pit latrine-related groundwater contamination, leaving the inhabitants of these districts at risk. This lack of research may have contributed to the high rate of diarrhoea observed among students in the area under study. Faecal contamination of drinking water is a widespread issue and major contributor to diarrhoea cases (Maramraj et al., 2020; Loyola et al., 2020). This study, therefore, aims to employ microbial faecal indicators to assess the impact of the infiltration layer and seasonal changes on pit latrine groundwater contamination in the Tano Districts of Ghana.

2. Material and method

2.1. Study area

The study area encompasses the Tano Municipalities, collectively referred to as Tano North and Tano South Municipalities. Duayaw Nkwanta and Techire in Tano North, along with Techimantia in Tano South, were selected as the sample sites. The Tano North Municipal Assembly is located between latitudes 7°00′N and 7°25′N and longitudes 2°03′ W and 2°15′ W, covering a total land area of 876 square kilometres. This represents 10.3% of the Brong Ahafo Region's total land area of 8,505 square kilometres (Tano North Municipal Assembly, 2023). The municipality falls within the semi-equatorial climate zone, experiencing to peak rainfall period: from April to June and from September to November. The dry season, characterized by harsh weather conditions, occurs from December to March. On average, the municipality receives 1,308 mm of rainfall annually, with monthly temperatures typically ranging from 25°C (in August) to 33°C (in March). Relative humidity in the region is generally high, ranging from 75-80% during the rainy season to 70-72% during the dry season. The map of Tano North Municipality is shown in Figure 1.

Figure 1. Map of Tano North Municipality (Source: Awuah et al., 2020)

According to the Tano South Municipal Assembly (2021), the Municipality is located in the southern part of the Brong Ahafo Region, between latitudes 7°00" N and 7°25" N and longitudes 1°45" W and 2°15" W. It covers an area of approximately 635 square kilometres, representing of the 1.6% Brong Ahafo Region's total land area.

The municipality falls within a semi-equatorial climate zone characterized by double maximum rainfall periods. The first rainy season occurs from April to June, with the heaviest rainfall in June, while the second occurs from September and October. Annual rainfall ranges from 1,250 to 1,800 mm. The dry seasons begins in November and lasts through February. Typical monthly temperatures range from 26°C in August to 30°C in March. Relative humidity is generally high, varying from 50–70% during the dry season to 75–80% during the rainy season. A map of the Tano South Municipality is shown in Figure 2, and a contour map indicating the sample sites provided in Figure 3.

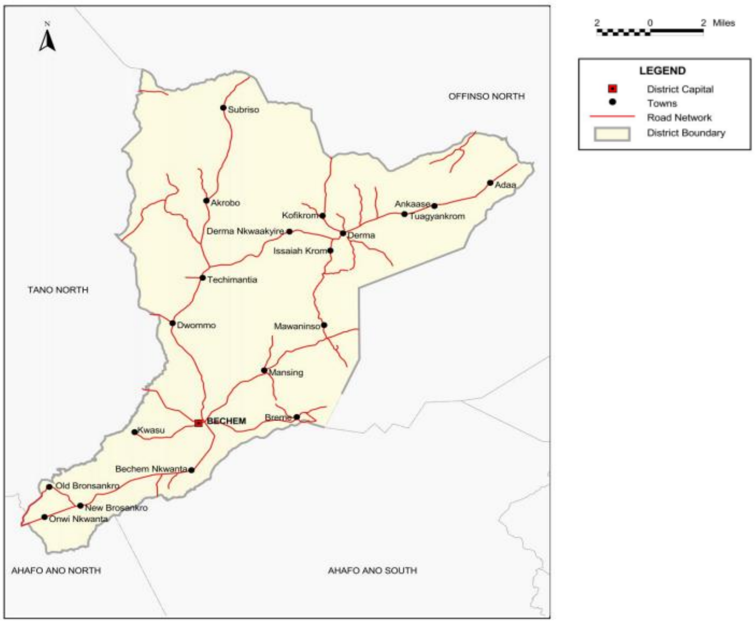

Figure 2. Map of Tano South Municipality (Source: Awuah et al., 2020)

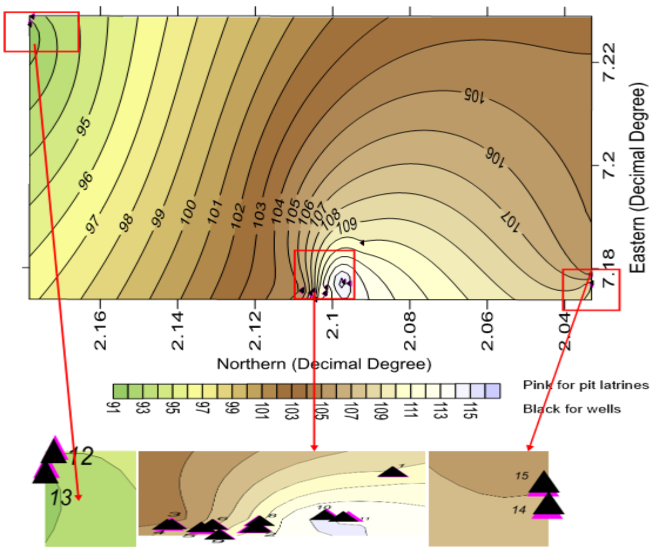

Figure 3: Area contour map with pits latrines and wells shown.

2.2. Data collection and sampling procedure

The water samples from the hand-dug wells and boreholes served as the primary experimental materials. For the investigation, 10 hand-dug wells and five boreholes were randomly selected. Water samples were collected using 2-litre screw-capped, rigid plastic bottles that had been properly sanitized. Four samples were periodically collected from every well. The sample were transported in an ice-filled cool box to the Science Laboratory of the Department of Theoretical and Applied Biology, the Faculty of Bioscience, Kwame Nkrumah University of Science and Technology, for analysis within two hours of collection. A simple random sampling method was used to select the 15 wells. This approach minimized biases as much as possible, ensuring each well had an equal and independent chance of being included in the sample.

2.3. Laboratory procedure

2.3.1. Faecal and total coliforms

To detect the presence of the total and faecal coliforms in the water samples, the Most Probable Number (MPN) approach was employed. This approach was chosen for its simplicity, allowing results to be easily understood through observation. Additionally, it is an effective technique for analysing highly turbid samples and enables the analysis of samples that cannot be e processed using membrane filtration (Aulya et al., 2020).

One millilitre of the sample material was taken and added to nine millilitres of sterile distilled water to create the 10^{-1} and 10^{-11} serial dilutions. A one millilitre aliquot from each dilution was used to inoculate five millilitres of MacConkey Broth (1:5) containing inverted Durham tubes. The samples were then incubated for eight to twelve hours at 35°C for total coliforms and 45°C for faecal coliforms. Both total and faecal coliforms were detected in the Durham tubes, indicated by a colour change from purple to yellow and the collection of gas inside the tubes after incubation. The counts per 100 millilitres were then determined using MPN tables.

2.3.2. Faecal enterococci

One millilitre of the material was taken and added to nine millilitres of sterile distilled water to prepare serial dilutions ranging from 10^{-1} to 10^{-11}. To aid bacterial resuscitation, one millilitre of aliquots from each dilution were inoculated on a Slanetz and Barltey Agar prepared in sterile Petri dishes and incubated at 37°C for four hours. Subsequently, the plates were incubated for a further forty-four hours at 44°C. Following incubation, all smooth, convex, red, maroon, and pink colonies were enumerated and identified as faecal enterococci.

2.4. Data analysis

Analysis of variance (ANOVA) was used to determine whether the microbial counts varied significantly with the infiltration layer. The data were statistically assessed using *p*-values to determined their significance, with significant *p*-values defined as those less than 0.05 at a 95% confidence level. Lorowitz et al. (2005) noted when comparing more than two samples, ANOVA is a better option and less prone to errors than repeated *t*-tests. ANOVA considers two forms of variance: error variance (within-groups variance) and treatment variance (between-groups variance) these advantages of ANOVA necessitated its use in this study. Additionally, the effect of groundwater contamination caused by the vertical distance between the pit's bottom and the groundwater table (static water level) was analysed using multiple regression.

3. Result and discussion

3.1. Infiltration layer and groundwater contamination

The study found no significant relationship ($p > 0.05$) between the total coliform counts in the water and the infiltration layer, defined as the layer between the water table and the bottom of the pit latrines (Table 1). Notwithstanding, a noteworthy impact of the infiltration layer was observed on the counts of enterococci and faecal coliform ($p < 0.05$). The microbial in relation to the infiltration layer in the dry season is illustrated in Table 1.

There may be other sources of the total coliform apart from the pit latrines in this study area. Miezah et al. (2015) and WHO (2008), in their studies on water and sanitation, observed that total coliforms are easily isolated in tropical areas from locations remote from human activities and may also be isolated from the soil and plant materials.

Therefore, the present study suggests that total coliform may not be a reliable indicator of faecal pollution and health risks related to pit latrine groundwater contamination. This claim aligns with previous research demonstrating that total coliform bacteria are not accurate indicators of faecal pollution and human health risks because they can be readily isolated in tropical regions far from human-populated areas and are also be found in soil and plant matters (Awuah, 2020). This observation might explain why total coliform counts did not show significant variation with the infiltration layer between the bottom of the pit latrines and the static water level, which represents the water table in this study.

It could be deduced from this investigation that the presence of total coliform may not always indicate faecal contamination. This assertion is supported by the work of Solaiman et al. (2020), who suggested that bacteria most commonly found in faeces might be more suitable indicators than total coliforms. Total coliforms were discovered unreliable indicators of faecal contamination, which is the most likely source of pathogens in water.

However, according to Murei et al. (2024), when selecting a diagnostic tool for managing faecal pollution of water sources in rural areas and monitoring microbiological water quality, a statistically significant positive correlation was found between faecal contamination and total coliform bacteria counts. The disparity between this study and earlier ones may be attributed to differences in the environments where the investigations were conducted and the inclusion criteria applied.

While the present study was conducted in the Tano Municipalities of Ghana, focusing solely on water samples from groundwater resources, Solaiman et al. (2020) conducted their research in the United States using alternative irrigation water sources. In contrast, Murei et al. (2024) conducted their study in the South Africa's Limpopo Province, specifically in Vhembe District Municipality, using water samples from rivers and boreholes. In general, sites with longer infiltration layers found to have lower microbial counts in water samples compared to those with shorter layers (Tables 1, 2, and 3).

The multiple regression analysis (Table 2), which shows that EC = 0.12(PLD) -0.09(SWL) + 2.37, summarizes the impact of the vertical separation between the bottom of the pit and the groundwater table (static water level) on groundwater pollution, as indicated by this study. Here,

Table 1. Means of microbial counts in relation to infiltration layer (Dry season)

IL/m	**Frequency**	**Log_{10} Geo mean counts/100ml**		
		TC	**FC**	**EC**
0.1 - 10.1	9	3.01(0.64)	1.96(0.52)	2.08(0.30)
10.2 - 20.2	5	2.40(1.06)	1.67(0.41)	1.57(0.41)
Above 20.2	1	2.62(0.00)	0.00(0.00)	0.00(0.00)
Pr	Not applicable	0.419	0.008	0.001

TC = total coliform, FC = faecal coliform, EC = enterococci.

Table 2: Multiple regression of static water level and pit latrine depth (infiltration layer) on microbial counts

Parameter	**Coefficients**	**Standard error**	**Significance**
Intercept	2.37	0.21	<0.001
SWL	-0.09	0.01	<0.001
PLD	0.12	0.04	0.0016

R = 0.91, P<0.001, EC = 0.12(PLD) – 0.09(SWL) + 2.37, EC = enterococci, PLD = pit latrine depth, SWL = static water level

SWL stands for static water level, PLD for pit latrine depth, and EC for enterococci count. According to this formula, enterococci count (EC) is inversely proportional to static water level (SWL) and directly proportional to pit latrine depth (PLD). This indicates that as pit latrine depth increases, enterococci count rises, and as static water level increases, enterococci count decreases. In other words, a higher pit latrine depth brings the pit bottom closer to the water table, thus shortening the infiltration layer. As a result, the amount of faeces contaminating the groundwater in pit latrines increases, which is reflected by the EC (faecal indicator bacteria) entering the groundwater more quickly.

Moreover, when static water level (SWL) increases, the water table is forced further from the pit bottom, thickening the infiltration layer. As a result, it takes a longer for the coliforms to reach the water table, lowering the rate of contamination. This explains why, as seen in Tables 1, 2, and 3, coliform levels are higher when the infiltration layer is thinner and lower when the infiltration layer is thicker. To put it succinctly, the coliform level increases with rising pit latrine depth (PLD) and decreases with increasing static water level (SWL) under constant circumstances.

According to Sugden (2006), the longer the pathogen-containing water must travel to reach the water table, the more convoluted its route becomes, the more time spends there, which results in a higher natural die-off pathogen. Pit latrines have a higher chance of contaminating groundwater if their bottoms are located close to the water table (Baloyi & Diamond 2019; McGill et al 2019; Díaz-Alcaide & Martínez-Santos, 2019).

The WHO recommendation for an infiltration layer of more than two metres between the water table and the bottom of the pit latrine's is also called into question by the findings of this current study. This is understandable, given that Table 3 of the research shows evidence of pit latrine groundwater contamination even with an infiltration layer of 10–20 metres. Because an approach has proven more effective in one context than another, it is consequently extremely risky to apply it to a different context. With regard to groundwater contamination, environmental conditions vary with the safe vertical distance between the water table and the bottom of the pit latrine. Only an environment with conditions identical to those of the recent study might benefit from the implementation of the aforementioned innovation, EC = 0.12(PLD) – 0.09(SWL) + 2.37.

3.2. Season and pit latrines groundwater contamination

The averages of the microbial counts relative to the infiltration layer measured throughout the dry season are displayed in Table 1, while Table 3 shows that during the wet season, greater microbial counts were reported than during the dry season.

Because the water table rises during the rainy season, drawing the groundwater extremely close to the pit latrine's bottom and reducing the infiltration layer, it is possible to explain why greater microbial counts are observed during that season. It now takes less time and effort for microorganisms in pit latrines that use water as a medium of transportation to reach groundwater. In other words, water acts as a conduit for the movement of microbial contaminants; the farther the pit's base is from the water table, the lower the risk of contamination (Ferrer et al., 2020; Capone et al., 2020; Gauld et al., 2020; Gokçekuş et al., 2020; Ercumen et al., 2020). According to Bartram et al. (2003), when the environment becomes drier, groundwater levels are prone to decrease, which gives pit latrines more resilience by strengthening the possi-

Table 3. Mean microbial counts in relation to infiltration layer (Wet season)

IL/m	Frequency	Log_{10} Geo mean counts/100ml		
		TC	FC	EC
-1 - 9	9	4.09 (0.92)	2.55 (1.05)	2.52 (0.62)
10 - 20	5	3.65 (1.63)	2.14 (0.74)	2.05 (0.82)
Above 20	1	3.62 (0.00)	0.00 (0.00)	0.00 (0.00)
Pr	Not applicable	0.780	0.074	0.015

bility of pathogen attenuation or death. Microorganisms require water, therefore even small amounts can encourage their spread (Sepehrnia et al., 2017; Humphries et al., 2020; Martínez-Santos et al., 2017). Table 1 shows the microbial counts in relation to the infiltration layer during the dry season. The microbial counts in the wet season are displayed in Table 3 in relation to the infiltration layer.

4. Conclusion and recommendation

Overall, the study shows that when the infiltration layer and static water level decrease, the amount of coliform, or contaminated pit latrine groundwater, increases. It was shown that enterococci and faecal coliforms were superior to total coliforms as human faecal contamination. This unique equation of this study, EC = 0.12(PLD) – 0.09(SWL) + 2.37, describes how the infiltration layer affects groundwater faecal contamination. This holds true for environmental with conditions similar to the study area of the current investigation.

It is recommended that, in studies to determine human faeces as a contaminant, preference should be given to the enterococci and faecal coliforms as indicators of human faecal contamination, rather than the other coliforms employed in this study.

To reduce pit latrine groundwater contamination, it is necessary to apply the multiple regression analysis, EC = 0.12(PLD) – 0.09(SWL) + 2.37 derived from this study, to predict a safer infiltration layer between the bottom of the pit and the water table. Therefore, when wells coexist with pit latrines, it is recommended to apply the multiple regression analysis, EC = 0.12(PLD) – 0.09(SWL) + 2.37 to increase the vertical separation between the bottom of the pit and water table, thereby reducing groundwater contamination.

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