Microbial Contamination of Hand Dug Wells and Pit Latrines in Fiapre in the Sunyani, Ghana.

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Abstract

Water is an essential resource and contributes immensely to the survival of man. This study investigated the microbial quality of hand dug wells sited near pit latrines in households of Fiapre community. Water samples were taken from five hand-dug wells sited near pit latrines over a period of 6 months. Analysis of the results based on selected physicochemical parameters showed that, SP4 was the most acidic (5.63) with SP5 being the most basic (6.15). The study also showed that, SP5 had the highest conductivity ($160.33\pm17.59 \mu$ s/cm) compared to the other 4 wells. With respect to microbial indicators of pollution, the study showed that, for total coliforms, SP4 was the most polluted with mean log_{10} of 1.9×10^2 colony forming units (cfu)/100mls. This was followed by SP3 ($log_{10} \ 1.44 \times 10^2 \ cfu/100$ mls) and then SP5 ($log_{10} \ of \ 1.09 \times 10^2 \ cfu/100$ mls). With respect to Escherichia coli, the results showed that, SP2 was the most polluted with cfu counts of 18.25cfu/100ml whiles drinking water from SP4 was the least polluted ($4 \ cfu/100$ mls). Analysis of the results based on Enterococci feacalis showed that, water from SP4 was the least polluted ($log_{10} \ 1.125 \ cfu/100$ mls). It was also observed there was a significant association between distances from dug-wells and the nearest pit latrine with respect to E. coli loads in the water samples. The study revealed that all the microbial indicators of pollution exceeded the EPA/WHO guidelines for drinking water making them unsafe for drinking.

Keywords

Pit latrine, hand dug wells, water quality, bacteriological parameters

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1. Introduction

Water is very essential and it plays a vital role in human daily life. Most of its uses include domestic, agricultural, industrial, recreation, power production and transportation (João, 2010). It is a fundamental commodity that determines the way of life and survival of humans. Water is therefore very vital as humans use almost every daily

activity. The human body is made up of about 70 percent water and this proportion of water has to be constantly replenish by more intake to sustain life as levels dips due to physical activities (Khandkar et al., 2002). But in this case, the water to be consumed should be potable and free from pathogens to avoid causing serious health implications (WHO, 2006). Even though water appears to be in plentiful supply on the earth's surface, it is still considered a rare and precious commodity. The World Health Organization (WHO) estimates that close to a billion people mostly living in the developing world do not have access to safe and adequate water (UNICEF/WHO, 2012). In fact only a small proportion of the earth's water reserves (approximately 0.03%) constitutes global water resource which is available for human use (Deniz, 2013). Currently, over 90% of water provided for some small towns in India for domestic use is extracted from groundwater (HimanshuKulkarni and Shankar, 2015). Groundwater supply include hand dug wells, driven and drilled wells, rock and earth springs as well as infiltration galleries. It is therefore not a surprise to uncover that groundwater is being currently being consumed in increasing quantities and is thus fast becoming affected by waste discharge (Klaus-Dieter Balke and Yan Zhu, 2008).

A major source of contamination to groundwater especially in West Africa is inappropriate human waste

exposure (UNICEF/WHO, 2012). This anomaly can be prevented through the provision and maintenance of appropriate environmentally friendly waste disposal systems and facilities (Shaheed et al., 2014). Good sanitation will not exist in the absence of safe and potable water. In terms of dealing with safe drinking water, the execution of bad sanitation systems may have a detrimental effect on human health since these two elements must correlate. Investigating the contamination of ground water pollution has been the centre of attention by numerous researchers for some time now (Howard et al., 2006; Priis-Ustun et al., 2004; Adejuwon 2011). All these researchers have concluded that, one major source of pollution to ground water is the leachate from pit latrine.

It has been established that pit latrine offers a safe and reliable method of excreta disposal. Pit latrines are especially suitable for rural areas where water is scarce (Adejuwon 2011). This is mainly because of its simplicity both in terms of operation and maintenance as it does not depend solely on water borne units. As a result, they are more valuable in areas where water may not be available (Howard et al., 2006). In spite of these advantages, pit latrines are one of the major contributing factors to groundwater pollution when situated near (< 30m) water sources such as shallow wells and boreholes. To correct this anomaly, city engineers have construction guidelines and regulations which require pit latrines to be built, 30 meters or more away from water sources (boreholes, streams etc) (Graham and Polizzotto, 2013).

The people of Fiapre in Sunyani in the Brong Ahafo Region of Ghana depend on hand dug wells for water for drinking and other domestic purposes. The sitting of hand dug wells in this community in many cases have violated the mandatory 30 meters distance from their pit latrines due to inadequate home land size. The siting of these two facilities in the Fiapre community (hand dug wells and pit latrines) pose a significant health risk due to possible seepage of feacal matter into the underground water system. In the face of the above mentioned, it is been speculated that ground water/hand dug wells from Fiapre could be microbially compromised. The urgent questions that require an immediate answer is that, are there microbial indicators of pollution in these hand dug wells? Could the close proximity of these latrine pits pose a significant public health hazard by virtue of pathogenic assault on the individuals who resort to water from these wells for drinking? These and many other research questions was the rationale for this study. The main objective therefore of this work was to investigate the microbial quality of hand dug-wells in relation to siting of the pit latrines in Fiapre in Sunvani in the Brong-Ahafo Region of Ghana as shown in Figure 1 below.

2. Materials and Methods

Fiapre is a town in the Sunyani West District in the Brong-Ahafo Region of Ghana. It is very close to the regional capital town of the Brong-Ahafo Region, Sunyani and lies between latitude 7^{o} 22 'N and 7^{o} 35 'N and longitude 2^{o} 21 'W and 2.3500°W with 932ft (284 m) above sea level. It shares boundary with Sunyani to the north, Nsoatre to the south, Odumase to the west and Bakoneaba to the east within the Suyani West. Fiapre, an urban community constitutes about 10.19% of Sunyani West district population.

2.1 Study Design

A cross section study relying on analytical laboratory approach was adopted for this study. Selected physiochemical and microbial indicators of pollution were employed for this research. The physicochemical parameters were limited to temperature, pH and conductivity. For microbial indicators of pollution, the study relied on total coliform, feacal coliform and Enterococci feacalis.

2.2 Study Area

Fiapre, a suburb of Sunyani within the Brong Ahafo Region of Ghana was chosen for the study based on the large proportion of inhabitants who depend on water for drinking from hand dug wells. Although this community has the supply of treated water from the Ghana Water Company, taps have failed to flow regularly due to low pressure experienced during most part of the year.



Figure 1. Study Area

2.3 Data Collection

With the help of surveyors' tape measure, distances between the hand dug- wells and the pit latrines were measured and recorded. The geographic positions of the selected hand dug-wells and their corresponding pit latrines were determined using a global position system (TotemTech, Shenzen, China, 2012) GPS device.

Table 1. GPS Designated Sampling Points of Hand Dug

 Wells

Designated Sampling Sites	Coordinates
Well 1 (SP 1)	N7°21'4.7412" W2°21'32.8248"
Well 2 (SP 2)	N7°21'10.4148" W2°21'31.3812"
Well 3 (SP 3)	$N7^{o}21'24.408" W2^{o}21'27.684"$
Well 4 (SP 4)	N7°21'31.4388" W2°21'5.2668"
Well 5 $(SP 5)$	N7°21'24.4872" W2°21'3.1572"

2.4 Sampling Procedure

Sampling were carried out monthly for a period of 6 months from September 2016 to March 2017. With the help of pre-washed sterilized 500ml sampling bottles, drinking water from 5 systematically selected hand dug wells were collected and appropriately labelled. Wells were labelled as SP1 to SP5 according to designated GPS locations of sampling points. With the help of PC 300 Waterproof Handheld pH/Conductivity/Temperature meter (CyberScan, Germany 2010) temperature, pH and conductivity were measured in-situ and recorded into field note book. Samples were then transported to the University of Energy and Natural Resources laboratory in an ice chest containing ice cubes within 1 hour of collection for analysis. Analysis were carried out within 2 hours after arrival from the field.

2.5 Laboratory Analysis

The pour plate count method was used for the Enterococci feacalis and Escherichia coli enumeration. Ten (10) ml of each sample was measured into a sterilised petri dish after serial dilutions of 10 to 10-5. Ten (10ml) of all serially diluted samples were then filter through 0.45 µm Millipore filter paper (Prat Dumas, France) after which the filter paper with trapped samples were placed on a sterilised petri dish with the help of sterilised forceps. Using the manufacturer's manual, about 10 ml of sterilised ready to use Oxoid MacConkey agar were added and placed in an incubator maintained at 37° C for 24 hrs. The Escherichia coli and Enterococci feacalis counts were estimated by counting the growth of colonies after 24hrs. Red colonies in the petri dishes signified E coli whiles pales pink colonies signified Enterococci feacalis growth.

For total coliform determination, the most probable number (MPN) method was used. Ten (10) ml of prepared ready to use Oxoid MacConkey broth was added to a test tube containing 10 ml of each serial dilution of water sample ranging from 10 to 10-5 dilution. Ordinary laboratory inverted Durham tubes were placed in each test tube after which the test tubes were swirled to mix and allowed to settle for about 10 minutes. All the inoculated samples were then incubated at $35^{\circ}C$ for 48 hours. Total coliform numbers were estimated using the MPN table based on the characteristics observed. These characteristics included colour changes, turbidity of mixture and collection of gas in the Durham tubes.

2.6 Data Analysis

Data acquired was first entered manually into Microsoft Excel. The means and standard deviations of the physiochemical parameters were calculated. Bar charts were generated to present results of the bacteriological parameters. The ordinary least squares (OLS) regression model was employed using Microsoft Excel 2016 package to assess the impact of distance of pit latrines from dug-wells and the levels of the bacteriological loads in the wells.

3. Results

Analysis of the results based on the physicochemical parameters of drinking water from wells under study with respect to pH showed that, SP4 was the most acidic (5.63 ± 0.29) followed by SP3 (5.65 ± 0.32) and SP2 (5.85 ± 0.27) as shown in Table 2 below. Drinking from these 3 hand dug wells exceeded the World Health Organization (WHO) and Ghana Environmental Protection Agency (GEPA) acceptable guidelines (Table 2). Hand dug well 5 (SP5) however recorded the highest pH (6.15 ± 0.13) and this fell outside the WHO/GEPA recommended standard which is 6.5-8.5 for portable water. The study sought to assess the temperature of drinking water from selected hand dug wells from Fiapre. Analysis of the results showed that, water collected from SP1 was the hottest $(30.38\pm 3.28)^{\circ}C$ compared to the rest of the wells as shown in Table 2 below. The study also showed that SP4 was the coldest as at the time of sampling $(28.73^{\circ}C)$ with a standard deviation of 3.83 (Table 2). This was followed by SP5 (29.38 $\pm 3.35^{\circ}C$) but these fell within the limit set by GEPA/WHO. When physicochemical parameters based on conductivity levels with respect to the five (5) wells were analysed, analysis of the results showed that, SP5 had the highest conductivity (160.33 ± 17.59) μ s/cm) compared to the other 4 wells. This figure however fell below the GEPA/WHO permissible guidelines for drinking water, as shown in Table 2 below. The study also showed that, SP 3 had the lowest conductivity value of 89 µs/cm with a standard deviation of ± 16.02 (Table 2).

Microbial quality of drinking water collected from selected hand dug wells from Fiapre were investigated as part of the study. Analysis of the results with respect to total coliforms showed that, SP4 was the most polluted $(1.9 \times 10^2 \text{ cfu})$ as shown in Figure 2 below. This was followed by SP 3 $(1.44 \times 10^2 \text{ cfu})$ and SP5 (1.09×10^2) (Figure 2). SP 2 was the least polluted with colony forming units of 0.923×10^2 cfu as shown in Figure 2. The study further showed total coliform numbers of drinking water from all these wells failed to meet the WHO/GEPA permissible guidelines for drinking water quality. To assess the extent to which hand dug wells in Fiapre were microbially contaminated, the presence and

	(Mean) WELL 1	(Mean) WELL 2	(Mean) WELL 3	(Mean) WELL 4	(Mean)	онм	GEPA GUIDELINES
PARAMETERS	(SP1)	(SP2)	(SP3)	(SP4)	WELL 5 (SP5)	STANDARD	
pH	6.07 ± 0.10	5.85 ± 0.27	5.65 ± 0.32	5.63 ± 0.29	6.15 ± 0.13	6.5 - 8.5	6.5-8.5
Temperature (^{o}C)	30.38 ± 3.20	$30.38 {\pm} 3.28$	29.88 ± 1.31	28.73 ± 3.83	29.38 ± 3.35	Ambient	Ambient
$Conductivity~(\mu s/cm)$	114.48 ± 9.86	$119.55 {\pm} 13.85$	89.73 ± 8.72	$98.43{\pm}16.02$	160.33 ± 17.59	400	400
Footnote: SP 1 to SP5	refers to Well 1 to	5., N=5, Figures	in each column re	fers to the means	whiles figure	s preceded by	+ refers to their standar
deviat	ions. WHO refers	to World Health (Drganization whil	es GEPA, Ghana	Environment	al Protection	Agency.

Table 2. Sampling wells with respective means and standard deviations of physiological Parameters and WHO standards

levels of Escherichia coli were investigated. Analysis of the results showed, SP2 had the highest number per 100 mls of $0.183 \ge 10^2$ cfu per 100mls of samples taken as shown in Figure 2. This was followed by drinking water sampled from SP1 with cfu of $0.158 \ge 10^2$ cfu. The study further showed that, SP5 followed the trail in terms of E. coli numbers related drinking water pollution (7.25 cfu/100mls of samples) with SP 4 being the least polluted 4 cfu/100 mls as shown in Figure 2 below. These values exceeded the WHO/GEPA guidelines of zero count for portable water. Another microbial indicator of drinking water pollution of importance assessed in this study was Enterococci faecalis. Results from Figure 3 below showed that, Enterococci faecalis counts in the hand dug wells ranged from 1.25 cfu/100ml to 3.50 cfu/100ml. The study also showed that, SP 2 was the most polluted (3.50)cfu/100ml). Our study has shown that, SP 4 was the least polluted of (1.25 cfu/100 ml) as shown in Figure 2 below. Enterococci faecalis in all the wells however exceeded the WHO/GEPA permissible limits of zero in drinking water.



Figure 2. A Graph of levels of microbial contamination levels in dug wells in Sunyani

The ordinary least square regression model based on the different microbial indicators of pollution for drinking water was assessed as part of the study. After analysis, the study showed that with respect to total coliform numbers isolated across all the wells, there was a negative correlation of the distance from the pit latrine and the level of pollution with a coefficient of (-2.650) and a standard error of (3.096) as shown in Table 3 below. The study further showed a standard error of 3.096 based on the distance between the hand dug wells and the pits latrines, a p value of 0.002 was recorded. This correlation was however not significant at a 95% confidence interval with a p-value of 0.403 (Table 3).

Table 3. Ordinary Least Squares Regression of the Effect of Distance on the Amount of Total Coliform in 5 Hand dug wells in Fiapre in Sunvani in the Brong Ahafo Region of Ghana

	Coefficients	Standard Error	t Stat	P-value Lo	wer 95%	Upper 95%
Intercept	159.495	43.901	3.633	0.002 67.264	-9.156	251.727
DISTANCE	-2.65	3.096	-0.856	0.403		3.855

The study sought to investigate the ordinary least squares regression of Escherichia coli pollution based on drinking water collected from hand dug wells in Fiapre. Analysis of the results showed that, a positive correlation existed between the Escherichia coli numbers isolated from the wells and the distance from the pit latrine with a coefficient of (0.623) and a standard error of (0.223) as shown in Table 4 below. This implied that, based on the intercept, a standard error of 3.159 was recorded with a p value of 0.427 at -4.070 lower 95% confidence intervals. However, with respect to distance, a coefficient of 0.623 at a standard error of 2.796 with an upper 95% confidence interval of 10.9 as shown in Table 3 below. This correlation was significant with a p-value of 0.012 at 95% confidence interval (Table 4).

Table 4. Ordinary Least Squares Regression of theEffect of Distance on the Amount of Escherichia coli in 5Hand dug wells in Fiapre in Sunyani in the Brong AhafoRegion of Ghana

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	2.567	3.159	0.813	0.427	-4.07	9.205
Distance	0.623	0.223	2.796	0.012	0.155	1.091

Enterococci feacalis in hand dug wells were investigated with respect to the locations of pit latrines in Fiapre using the analysis of ordinary least square regression model. Analysis of the results based on the intercept revealed that, a coefficient of 2.579 existed with a standard error of 10.2 as shown in Table 5. In addition to this, a significant positive correlation (p=0.021) was observed at 95% lower confidence interval of 0.435 as shown in Table 5 below. In spite of these, a negative correlation existed between the Enterococci faecalis loads in the wells and the distance from the pit latrines with a coefficient of (-0.015) with a standard error of 0.072. This correlation was however not significant at a confidence interval of 95% and a corresponding p-value of 0.835 as shown in Table 5 below.

Table 5. Ordinary Least Squares Regression of theEffect of Distance on the Amount of Enterococci Faecalisin 5 Hand dug wells in Fiapre in Sunyani in the BrongAhafo Region of Ghana

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	2.579	1.02	2.527	$\begin{array}{c} 0.021 \\ 0.835 \end{array}$	0.435	4.723
Distance	-0.015	0.072	-0.211		-0.166	0.136

4. Discussion

The effects of acids and alkalis depend on the strength of the acid or alkali and the concentration. Strong concentrated acids or alkalis are corrosive, whereas dilute and weak acids and alkalis are not corrosive. Ordinarily, pH alone is not the primary determinant of adverse effects. In

fact the pH of stomach fluid, which contains hydrochloric acid, is between 1.0 and 3.5, with a mean of approximately 2.0. There is a range of commonly encountered foods that are also of low pH. These include lemon juice, with a pH of 2.4, and vinegar, with a pH of 2.8. Because these are weak acids, they pose no threat to health from their consumption. A direct relationship between human health and the pH of drinking water is impossible to ascertain, because pH is so closely associated with other aspects of water quality. However, because pH can affect the degree of corrosion of metals as well as disinfection efficiency, any effect on health is likely to be indirect due to increased ingestion of metals from plumbing and pipes or inadequate disinfection. Although pH usually has no direct impact on water consumers, it is one of the most important operational water quality parameters. Careful attention to pH control is necessary at all stages of water treatment to ensure satisfactory water clarification and disinfection. It has been reported that, high levels of pH can cause a bitter taste in drinking water, results in pipes and appliances to become encrusted and this reduces the effectiveness of chlorine disinfection (Joyce and Dhillon, 1994). Temperature is the measure of how much heat is present in the water. It is desirable that the temperature of drinking water from wells should not exceed $15^{\circ}C$ because the palatability of water is enhanced by its coolness. Low water temperatures offer a number of benefits (Benson et al., 2015). A temperature below $15^{\circ}C$ tend to reduce the growth of nuisance organisms and hence minimise associated taste, colour, odour and corrosion problems (Nasir et al., 2016). Temperature of water sources depends upon several factors amongst which are time, season and water depth. Variations in temperatures in this study followed the ambient temperature pattern (Nasir et al., 2016) and were high because sampling was conducted in the dry season as at the time the rains had not set in. This was in consistent with the results of this study. Conductivity values recorded for the sampled wells were low. It usually expected that for siting of hand dug wells in close proximity to pit latrines, leaching of nitrates, nitrites and ammonia from these latrines into the wells could increase the dissolved ions in the water. Conductivity is a measure of water's capability to pass electrical flow. This ability is directly related to the concentration of ions in the water (Hui et al., 2015). These conductive ions came from dissolved salts and inorganic materials such as alkalis, chlorides, sulphides and carbonate compounds. Compounds that dissolve into ions are also known as electrolytes. The more the ions present, the higher is the conductivity of water. Likewise, the fewer the ions in water, the less conductive it is. Research has shown that, high conductivity of water is attributed to the presence of high concentrations of dissolved ions in the water which could give the water an abnormal saline taste (Parra et al., 2015).

The presence of total coliforms recorded in all the wells was expected. Personal observation during sampling revealed that all the wells were closer than the recommended distance of 50 metres to the nearby pit latrines as mandated by the Community Water and Sanitation Agency of Ghana. As a result, there was the likelihood of leachates leaching through the soil cover into the underground water systems. In addition to this could be the activities of livestock and other stray domestic animals that induced contamination since the receptacles used to draw water from these wells were mostly left uncovered on top of the wells. Total coliform group of bacteria has been the most frequently used indicator of biological water quality. Coliform groups consist of all aerobic and facultative anaerobic, gram-negative, non-spore forming and rod-shaped bacteria that ferment lactose in a broth medium with gas formation within 48 hours at 35 °C (Ahmad et al., 2015). Most coliforms also produce enzyme B-D galactosidase which can be detected with a colour forming reagent. The group generally comprise of genera klebsiella, enterobacter and citrobacter. The presence of these bacteria in drinking water is indicative the level of microbial pollution in these hand dug wells. It has been reported through research that total coliforms in the wells is an indication of the presence of pathogenic bacteria in the which can cause disease (Eassa and Mahmood, 2012). This assertion was in line with this research.

Escherichia coli and Enterococci feacalis were detected in drinking water collected from all the hand dug-wells investigated during our research. Escherichia coli is a gram negative rod shaped bacterium that is normally found in the lower intestines of warm blooded organisms. E coli and bacteria constitute about 0.1% of gut flora (Garrison et al., 2016). Faecal oral transmission is the major route through which pathogenic strains of the bacterium causes diseases. These feacal indicators of pollution are able to survive outside the body of warm blooded humans and animals for a limited amount of time. The presence of these indicator organisms make drinking water abstracted from these wells unsafe for drinking as inhabitants could be exposed to serious public health treat. This is because E. coli is present in very high numbers in human and animal faeces and is rarely found in the absence of faecal pollution (Figueras and Borrego, 2010). Faecal streptococci are a bacterial group that has been used as an index of faecal pollution in drinking water. This group include species of different sanitary significance and survival characteristics. Streptococci species prevalence differs between animal and human faeces. Furthermore, the taxonomy of this group has been subjected to extensive revision. The group contains species of two genera- Enterococcus and Streptococcus. Although several species of both genera are included under the term enterococci, the species most predominant in the polluted aquatic environments are Enterococcus feacalis, Enterococcus feacium and Enterococcus durans (Petersen and Dalsgaard, 2003). The International Organization for Standardization has defined the intestinal enterococci as the appropriate subgroup of the faecal streptococci to monitor (i.e., bacteria capable of aerobic growth at $44^{\circ}C$ and of hydrolysing 4-methylumbelliferyl-b-D-glucoside in the presence of thallium acetate, nalidixic acid and 2,3,5triphenyltetrazolium chloride, in specified liquid medium) (Figueras and Borrego, 2010). But it must also be noted that, the microbial indicator levels observed at these sampling sites give an indication of contamination of these wells by faecal matter of both humans and other warm blooded origin. This observation offers a direct link to the pit latrines and makes the water unsuitable for drinking, posing a significant health risks to humans and other livestock that might depend on it on daily basis. Even though E. coli and Enterococci faecalis counts were relatively low, their presence in the water sample also gives an indication of the presence of other potentially harmful bacteria in the water. It can also be inferred that, the presence of E. coli in drinking water indicates possible presence of pathogenic bacteria, virus and protozoans.

5. Conclusion.

The study has revealed that, all the physiochemical parameters with the exception of pH (Temperature, Conductivity) of water samples analysed from the hand dug wells fell within the WHO/GEPA standard for drinking water. All the five (5) hand dug-wells tested positive to total coliforms. The presence of Enterococci faecalis and Escherichia coli implies that there is faecal contamination of the dug-wells, thus making the dug- wells unsuitable and therefore water abstracted from them not recommended as potable water for drinking. It was also observed that no significant association existed between distances from dug-wells to the nearest pit latrine and the bacteriological loads in the water samples.

The OLS regression model revealed that, there was a significantly positive correlation between E coli and distances between pit latrines and hand dug wells. It was also concluded that, there could be an alternative sources of contamination other than the pit latrines' proximity to the dug wells. These alternative sources of contamination may be associated to nearby dumpsites and dead animals/humans that may have been buried some years ago before the dug wells were constructed. It is therefore being recommended that, the Community Water and Sanitation Agency, Ghana in the district should ensure that regulations regarding the hydraulic characteristics of the soil where the pit latrine and the dug well would be sited is adhered to. The presence of high levels of feacal streptococci in in drinking water from wells is a matter that requires urgent attention. The study however did not investigate the health status of inhabitants of the study

area to ascertain whether there is a linkage between continues consumption of microbial contamination and the prevalence of diarrhoea diseases within the community.

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7. Competing interest

The authors will like to state that no competing interest exist.

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