STUDY OF PROGRESSIVE CHANGES IN BACTERIOLOGICAL CONSTITUENT OF HARVESTED RAINWATER FROM OYOKO COMMUNITY IN KUMASI, GHANA

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Abstract: The bacteriological characteristics of rainwater harvested from a typical rooftop were progressively studied for a period of nine (9) months. The study area, Oyoko, is a rural community considered as a typical farming area and located about 30 km from Kumasi, the capital city of Ashanti Region of Ghana. The collected rainwater samples were analyzed for *Escherichia coli* (*E. coli*) and *salmonella*. The entire *E. coli* counts varied from 0-60 CFU/100 m*l* and were absent on 39 observations out of total of 84 observations (replicate samples), representing 46.4%. Whiles the entire *salmonella* counts ranged from 0-78 CFU/100m*l* and only 10 observations out of the 84 were absent, which represents 11.9%. Both *E. coli* and *salmonella* showed higher concentration during early stages of continuous rainfall but, progressively reduced during later part of rainfall. The main cause of this phenomenon can be attributed to the deposition and accumulation of pollutant materials on the rooftop and catchment areas typically during the dry seasons as a result of wind-blown dirt particles and other environmental pollutants. The high bacteriological constituents in the early-stage harvested rainwater consequently have some proven significant health implications from their direct consumption. It is therefore imperative for the community to know the best time interval to harvest their rainwater as rainfall progresses, and also know any health implications associated with the harvested rainwater that goes into their storage tanks for consumption through progressive monitoring of the quality.

Keywords: Rainwater quality; Bacteriological characteristics; *Escherichia coli*; *Salmonella*; Environmental pollutants

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1. INTRODUCTION

Amid the increasing world population and its associated demand for water, rainwater has become an alternative source of water supply (WHO/UNICEF, 2006; Chukwuma *et al*., 2012). For those living in rural communities, there is high level of rainwater dependence purposely for drinking, as well as, for other domestic purposes. This is mostly attributed to the scarcity of public pipe-borne water supplies in these communities but the danger is that, in spite of this high level dependence, very little is done with regards to monitoring the quality of the harvested rainwater especially, in rural communities in Ghana (Yeboah *et al*., 2015).

Even though according to the 2010 Ghana MDG target 7C (Indicator 7.8), the proportion of population using an improved drinking water source for both rural and urban households in Ghana has improved remarkably since 1990, further classification by WHO/UNICEF Joint Monitoring Programme (JMP) into improved and unimproved water sources did not capture rainwater source (UNDP, Ghana and NDPC/GOG, 2012). According to Yeboah *et al*., 2015, the Oyoko community has no reliable public water supply network. And as a result, rainwater is traditionally used by the rural folks for drinking, food preparation, personal hygiene and other essential domestic purposes. Therefore, rainwater harvesting and storage is the main practice being followed to cover the growing water needs particularly, during the rainy seasons. However, following the results from Yeboah *et al*., 2015 which revealed that most physico-chemical constituents of rainwater samples collected from the Oyoko community were generally within the WHO threshold limit at all times during rain event, it is therefore imperative to consider the quality of the harvested rainwater either for drinking or for domestic purposes with regards to its bacteriological constituents. This will contribute to improved or basic drinking water source which according to WHO, should be available when needed and free of fecal and priority chemical contamination (WHO/UNICEF, 2014), which is a major priority with reference to the Goal 6 (Target 6.1) of the proposed Sustainable Development Goals (SDG), which aims to, by 2030, achieve universal and equitable access to safe and affordable drinking water for all (UNICEF/WHO, 2015).

Bacterial portability of water is basically determined by testing for indicator organisms which are bacteria whose presence or absence in drinking water indicates the availability or unavailability of pathogens (Hach, 2000; NH-DES, 2003). These are fecal coliform bacteria types basically used to detect and estimate the level of fecal contamination of water. These indicator organisms are easier to detect and test for, than the pathogens themselves therefore, analysis for their presence or absence, is the method of choice in testing for portable water. Though not dangerous to human health, the presence of these indicator bacteria in drinking water indicate possible presence of harmful disease-causing organisms (Muhammad, and Mooyoung, 2008; NH-DES, 2003), therefore can be used to indicate the presence or absence of health risk.

According to Olobaniyi and Efe, 2007, rainwater acquires its salinity and bacteriological composition partly as it passes through the atmosphere by dissolving air-borne particulates and water soluble gases and also incorporating air borne microbes. However, the previous studies of Olobaniyi and Owoyemi, 2004 on the quality of water resources in tropical African environment have largely been restricted to surface and groundwater to the neglect of rainwater. This is due to the supposition that rainwater is usually pure and therefore needs very little investigation with regards to its quality. But, the likelihood of birds, insects and other climbing reptiles defecating on and/or transporting fecal matter upon roof catchment cannot be overlooked. Moreover, factors such as type of roof material, antecedent dry period (atmospheric deposition) and surrounding environmental conditions have been shown to influence concentrations of pollutants in roof runoff (Thomas and Greene, 1993). There is therefore the possibility that rainwater becomes polluted and hence the need to place its quality under examination.

Many research works have indicated probable health risks from ingestion of rainwater, which might results from microbiological or chemical contaminants in the water. But, the nature of microbiological risks is expected to be similar in municipal and rural settings however, there may be significant variances between urban and rural areas for chemical contaminants including (Sinclair *et al*., 2005).

According to Sinclair *et al*., 2005, some bacterial pathogens have been reported to have caused outbreak of infections such as gastroenteritis, which is directly related to rainwater consumption in Australia. There is therefore high vulnerability of rainwater to quality degradation from both anthropogenic and natural sources, and as a result, periodic assessment of harvested rainwater is essential. Meanwhile, water quality in general has been described by many researchers, including Johnson *et al*., 1997; Lucentini *et al*., 2013; and Egirani *et al*., 2014, to be dependent on physicochemical and bacteriological characterization of the available water resources. Therefore, following the progressive changes in physicochemical constituent of rainwater in the Oyoko rural community by Yeboah *et al*., 2015, subjecting harvested rainwater from the same community (within the same time frame) to progressive changes in bacteriological quality is a principal focus of this research work.

2. MATERIAL AND METHODS

2.1 Study Area

The study area, as described by Yeboah *et al*., 2015, is a rural community in the Sekyere-East District of Ashanti Region of Ghana. It is located at an elevation of 228 meters above sea level, approximately at coordinates 6°33'0" N and 1°34'0" W. The area is within a region which has an average annual rainfall of 1270 mm with two rainy seasons. The major rainy season starts in March, which intensifies in May. It slightly dip in July and a pick in August, tapering off in November. December to February is considered dry, hot, and dusty. The area has intensive agricultural land use where commercial fertilizers and manure are routinely applied to the field. The expected chemical pollutants in the environment are mainly that from fertilizers, pesticides, herbicides and other anthropogenic activities.

Yeboah *et al*., 2015 further described the Oyoko rural community to have no reliable public water supply network and as a result, and mostly in the rainy season, harvesting rainwater is a traditional practice by the rural folks purposely for drinking, as well as, for other domestic purposes. This is to compensate for the increasing water needs of the residents taking into consideration the growing population of the community.

2.2 Project Design and Sampling

Adopting the design and sampling procedures of Yeboah *et al*., 2015, this study employs an experimental design whereby rainwater samples were collected progressively from a single point source for a period of nine (9) months (December 2014 - August 2015) to represent both dry and wet seasons of Ghana. This experimental design is in accordance with the standard methods for the examination of water and wastewater (APHA, 1992, 1995), as well as, that of general water quality (ISO-29201, 2012).

2.3 Bacteriological Constituent

2.3.1 Test of *E. Coli* and *Salmonella* Constituents

The quality of the sampled harvested rainwater in the study was assessed by its bacteriological composition. *Escherichia coli* (*E. coli*) and *Salmonella* tests were used to assess bacteriological quality of the sampled rainwater. These tests are normally used to index hygienic quality because *E. coli* and *Salmonella* are usually associated with fecal pollution and thus, their numbers reflect the degree of pathogenic risk (Berg, 1978). The *E. coli* and *Salmonella* counts were carried out by means of standard plate count using Chromocult Coliform Agar (CCA) as a culture medium which specifically employs the membrane-filter culture medium method commonly known as M-FC medium. This process involves the use of growth or culture medium which represents a conducive environment where microorganisms can grow. There are two major types of growth-media. Thus, (1) the media used for cell culture, which use specific cell types that are derived from plants or animals, and (2), the media used for growing microorganisms, such as bacteria (Tauxe *et al*., 1997). For the purpose of this study, agar plates which is one of the most common growth media for microorganisms was used.

Considering a step-by-step procedure, a culture dish was first prepared and left overnight (about 12 hours) with a clear intention to detect any contamination prior to the experiment. A filtration unit made up of filtration equipment was then set-up and sterilized using autoclave at a temperature of 121 ˚C. Afterwards, the whole set-up was allowed to cool to room temperature (about 23˚C). The entire working environment was however sterilized by cleansing with alcohol. Sterilized and preserved membrane filter papers (pore size 0.45 nm, diameter 47 mm) were subsequently transferred onto the filtration funnel with the help of sterilized forceps. 100 ml of each sample was filtered through the membrane filter paper placed in the filtration funnel. This was to retain the microorganisms on the filter paper. The filter papers and the filtrate were then placed on the prepared culture dishes. These dishes were finally closed, overturned, and incubated at a temperature of 35- 37 ^{0}C for 18-24 hours

2.3.2 Observations

Figure 1 shows colonies of various colours produced by the bacteria on the M-FC medium. The violet colony is typically indicated by *E. coli* whiles colonies produced by *salmonella* are various shades of green-blue, whereas the coliforms are indicated by the deep-brown.

The various colonies were then enumerated by normal counting of those formed by each species presented in a unit of counting as colony form unit per 100 litre (CFU/100L) of water.

3. RESULTS AND DISCUSSIONS

3.1 E.coli and *Salmonella* counts

Table 1 shows the mean monthly count of the *salmonella* and *E. coli* constituents recorded from the sampled harvested rainwater throughout the study period, from December 2014 through to August 2015.

The entire *E. coli* counts varied from 0-60 CFU/100 m*l*, with the minimum of 0 CFU/100 m*l* recorded throughout in the months of December and July. Whiles the maximum value (60 CFU/100 m*l*) was recorded in the month of January during the first 6 minutes of rainfall. The *E. coli* counts were however generally absent on 39 out of the total of 84 observations, which

Table 1. Mean monthly count of Bacteriological Constituents of Rainwater

Parameter (CFU/100 ml)	**Month**								
	December	*January*	*February*	*March*	*April*	*May*	*June*	*July*	*August*
E. coli	0	31	24	8	19	1	1	0	1
Range (Min-Max)	0-0	14-60	11-40	2-15	1-28	0-2	0-4	0-0	0-6
Salmonella	9	36	28	19	10	7	8	6	1
Range (Min-Max)	5-20	20-78	12-66	2-42	0-38	1-23	2-30	0-17	0-5

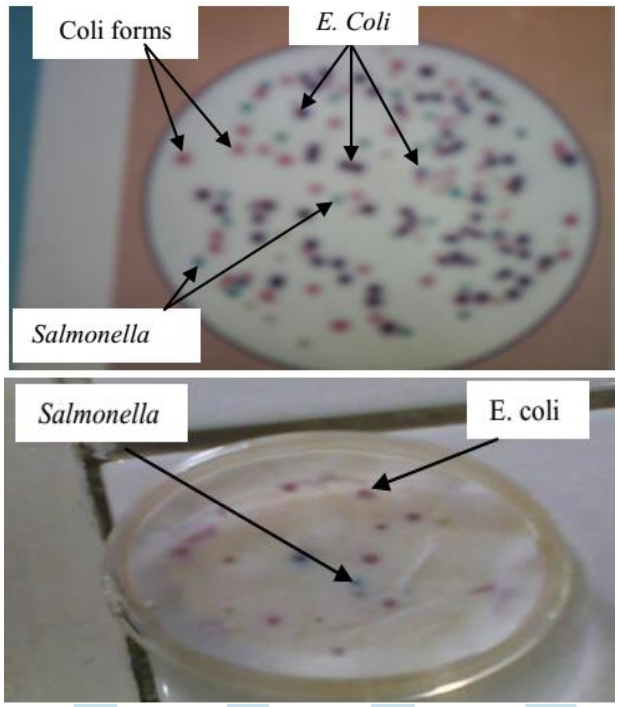

Fig. 1 Bacterial growth on membrane filter paper (mix-culture media)

represents 46.4%. On the other hand, even though the entire *salmonella* counts ranged from 0-78 CFU/100m*l*, no single month recorded entirely 0 CFU/100m*l* with exception of April, July and August recorded some few zero counts during the latter part of rainfall. However, the maximum *salmonella* count of 78 CFU/100m*l* was also recorded in the month of January during the first 6 minutes of rainfall. Only 10 observations out of the 84 were absent for *salmonella* counts, which represents 11.9%.

The results obtained from this research are true reflection of insanitary conditions at the roof catchment area and its surroundings, since both *E. coli* and *Salmonella* are known to originate from the exposure to human and warm-blooded animals' fecal matter, as well as animal sourced bacteria and accidental contamination (Thomas and Martinson, 2007; Muhammad and Mooyoung, 2008). The presence of these fecal coliforms is therefore a clear indication of fecal contamination of the sampled harvested rainwater from the study area. From **Fig. 2**, it was observed that both *E. coli* and *Salmonella* had highest counts from the months of January to April but declined drastically from the months of May to August. However, in Ghana, the weather and climatic conditions are such that, prolonged dry-season is usually observed from January-March hence, more pollutant load is likely to be deposited on roof-catchment areas within such periods. It is therefore not surprising to observe both parameters (*E .coli* and *salmonella*) recording astronomical counts during the month interval of January to April. The mean-monthly bacteriological results (according to **Fig. 2**) moreover indicates that, rainwater harvested between the months of May-August needs little treatment in order to be used for drinking purposes whereas, for domestic purposes other than direct drinking, the water harvested between the months of December-April can be advised.

3.2 Bacteriological variations within rain events

The progressive monitoring of *E.coli* and *Salmonella* counts from the sampled harvested rainwater at specific time interval were recorded and the mean values presented as shown in **Table 2**. From the exponential correlation of *E.coli* and *Salmonella* constituents as a function of time (**Fig. 3**), statistically significant R^2 values of 0.9604 and 0.7948 were respectively determined for both *E.coli* and *Salmonella*.

Table 2. Progressive mean count of *E.coli* and *Salmonella* in rainwater

Parameter (CFU/100 ml)	**Time** (minutes)						
	6	*12*	*18*	*24*	*30*	*36*	*42*
E. coli	16	13	9	8	8	8	3
Salmonella	35	18	11	10	7	5	4

Using the Pearson product-moment correlation, negative correlation coefficients (-*r*) were recorded for both parameters (*E.coli* and *Salmonella*) with *P-value* below 0.050 for both. This is an indication that, as time progresses both variables (*E.coli* and *Salmonella*) decreases significantly (**Table 3**).

However, interacting *E.coli* with Salmonella, the PPMCC produced positive correlation coefficients (+*r*) with *P-value* also below 0.050. This tend to indicate that, both parameters or variables (*E.coli* and *Salmonella*) increase or decrease together (depending on the time of sampling) and can be considered to have significant relationship.

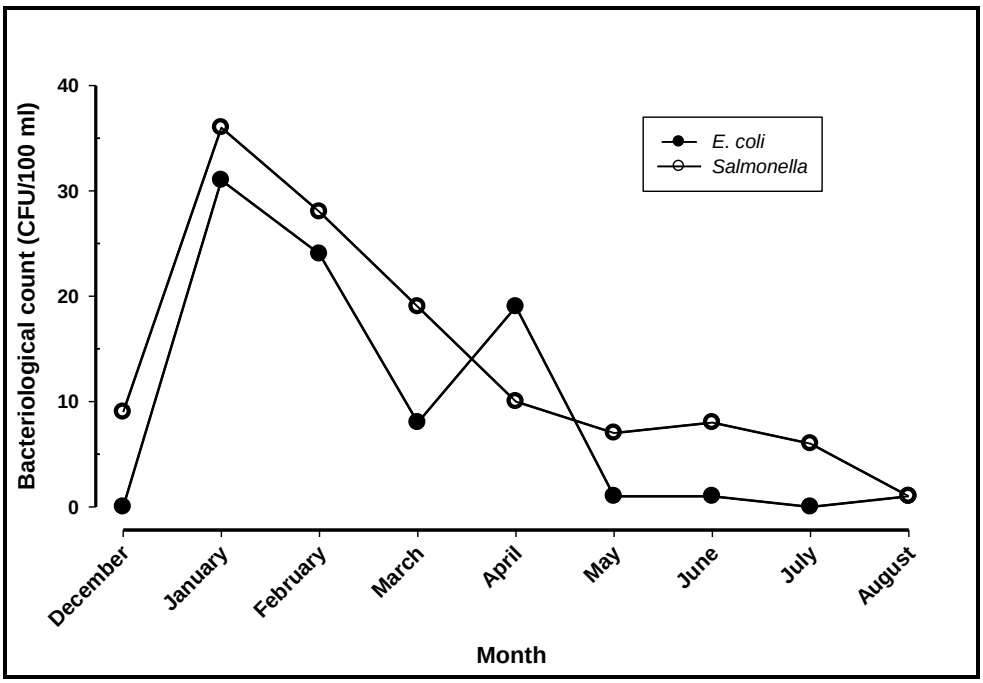

Fig. 2 Monthly bacteriological constituents

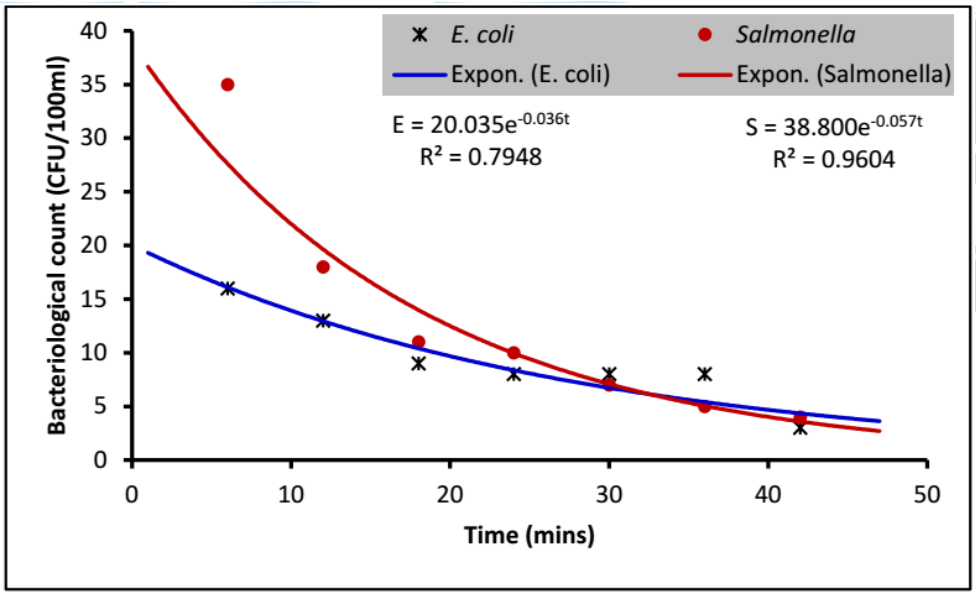

Fig. 3 Graph of bacteriological count against time

Table 3. *Correlation (Pearson) coefficient for E.coli and Salmonella* (p<0.005)

	Time	***E. coli***	***Salmonella***
Time	1		
E. coli	-0.929 *0.00247	1	
Salmonella	-0.877 *0.00957	0.910 *0.00448	1

*p-value

This study consequently revealed that, concentrations of both *E.coli* and *Salmonella* were high in the early stages of rainfall but decreased rapidly and significantly as the rains progressed. This outcome is in accordance with Yaziz *et al*., 1989, who evaluated two different types of roof catchment for fecal coliforms in collected rainwater in Malaysia, who stated that, concentration of various pollutants were high in the first litre of rain but decreased in subsequent samples with few exceptions.

The initial high concentration may be linked to the deposition of bird droppings, as well as, feces of reptiles and insects which may be on the roof catchment just before the start of rain. However, as rain progresses,

surfaces are washed, resulting in low values obtained in the subsequent collected samples. But, it should not be notion that, the raining water can completely clean the roof catchment off all bacteria (especially, the coliform bacteria) pollution. This is due to the fact that, though the progressive changes in bacteriological constituent decreased consistently, it never recorded zero values (as shown in **Fig. 3**).

3.3 Seasonal effects on Bacteriological composition

Samples collected during the dry season showed higher count for both *E. coli* (**Fig. 4**) and *Salmonella* (**Fig. 5**) compared to those in the rainy or wet season.

The significant increase in the percentage of microbial indicators in the dry season can easily be interpreted by the antecedent dry season, where a large amount of micro-organisms, animal droppings and other pollutants would have accumulated on the rooftop and its catchment areas due to the long absence of rainfall.

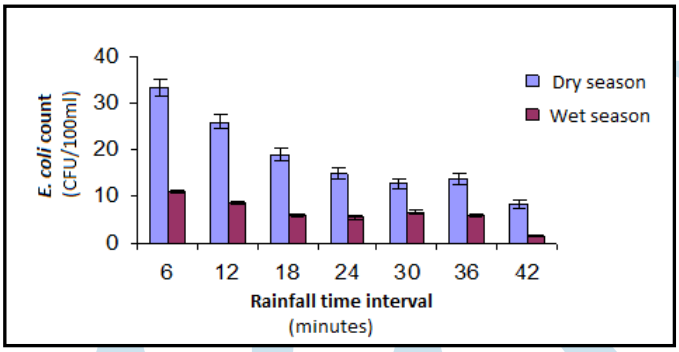

Fig. 4 Seasonal variations of progressive E.coli mean count

Conversely, during the rainy season, rainwater often comes in contact with the catchment surfaces, and washes many types of bacteria, algae, dust, leaves, bird droppings and other contaminants into the reservoir containers. Most observations obtained for *E. coli* and *salmonella* contradicts the recommended threshold limit value of 0 CFU/100 ml by WHO for no risk (WHO, 2008).

4. CONCLUSION

While it is desirable that *E. coli* and *Salmonella* be totally absent from drinking water, this was not practically feasible, considering the samples analyzed from the Oyoko rural community.

Out of a total 84 observations, *E. coli* counts were detected in 45 (53.6%) of them and ranged between 1-60 CFU/100*l*, whereas, the entire *salmonella* counts ranged from 0-78 CFU/100m*l* and were found to be present in 74 (88.1%) out of the total of 84 observations though all in low concentrations. Hence, 46.4 % (39 observations) and 11.9% (10 observations) of *E. coli*

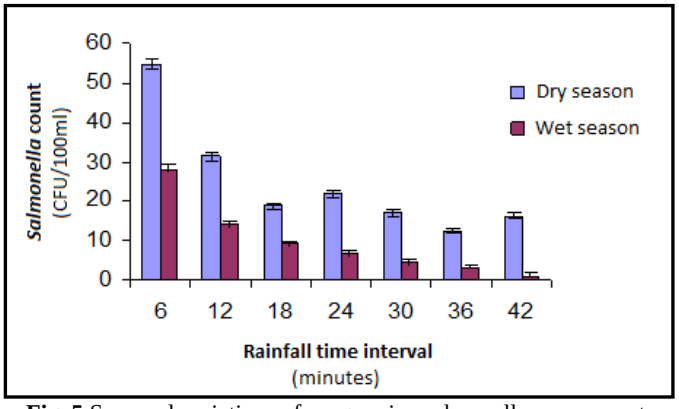

Fig. 5 Seasonal variations of progressive salmonella mean count

and *salmonella* respectively, were absent. The presence of both fecal coliforms (*E. coli* and *Salmonella*) in the sampled rainwater can partly be explained generally by its mode of collection onto the roof catchment area.

Although the rainwater samples were not tested for specific pathogens, the high microbial population present in the sampled rainwater may pose significant health risk to consumers. It is therefore not surprising to notice that, gastroenteritis is one of the major OPD disease cases reported by both adults and infants within the Oyoko Community in the Ashanti region, upon verbal interaction with residents and further visits to major health facilities in the area. The study also revealed that, *E. coli* and *salmonella* concentrations were higher during the dry seasons (December to March) than in the rainy seasons (April to September) and that, the concentrations of deposit materials (pollutants) on roof catchment were high at the early stages of rainfall (below 6 minutes) but reduced rapidly as the rains progresses (beyond 30 minutes). This is a clear indication of unsanitary environmental conditions at sampled the locations.

In conclusion, the bacteriological composition from the fecal coliform counts, exceeded the WHO standard for portable water which is 0 counts in 100 m*l* of water (0 CFU/ 100 m*l*) at all stages of rainfall. As such, the sampled rainwater recorded unsatisfactory and unacceptable concentrations in bacteriological constituents during the rain events. This unacceptable results could be attributed to direct contamination of fecal droppings from birds, animals, insects and wind-blown dirt on the roof catchment areas. Therefore, harvested rainwater from the study area should go through proper treatment before using for portable purposes. It is also imperative for further studies to be conducted to assess the health risk that may be associated with the consumption of such untreated rainwater by the rural folks, as well as, to assess the specific pathogenic species that may be present in the sampled rainwater from the study area in relation to *salmonella* and *E. coli* in particular.

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