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Full Length Research Article

DETERMINATION OF BACTERIA AND TRIHALOMETHANE COMPOUNDS IN SACHET WATER COMMONLY SOLD IN SAMARU, ZARIA

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ABSTRACT
Portable water is essential to humans and other forms of life. Poor treatment of drinking water results in the distribution of drinking water contaminated with bacteria and trihalomethane (THMs) compounds. Five brands of sachet drinking water were analyzed for coliform colony units and THM compounds concentration using Multiple Tube test and UV- spectrophotometer. The results showed the presence of *Enterobacter* which is a disease causing bacteria in all the water samples with W5 having the highest colony unit of 11. This was followed by W4 and W3 with five colony units and lastly W2 and W1 with less than two colonies. The concentrations of THMs were also determined with W5 having the highest concentration of 0.032 µg/cm^3, followed by W2 with a concentration of 0.02 µg/cm^3; W4 had a concentration of 0.011 µg/cm^3, W1 a concentration of 0.01µg/cm^3 and lastly, W3 having a concentration of 0.007 µg/cm^3. All samples had THM compounds with concentrations above the national permissible limits. The presence of *Enterobacter*, a disease causing organism and the high concentration of THMs in the water samples, are a cause for concern as they pose as a risk to human health; indicating that the waters evaluated are not in any way fit for consumption.

Keywords: Trihalomethanes, Coliforms, Pathogens, Sachet water, Bacteria

INTRODUCTION

Water, after air, is the most essential commodity to the survival of life. Human's life depends to a large extent, on water. It is used for an array of activities; chief among these being in drinking, food preparation, as well as for sanitation purposes (Miller and Juinor, 1997). Water covers 70.9% of the earth's surface, and it is vital for all known forms of life. On earth, it is found mostly in oceans and other large water bodies, with 1.6% of water below ground in aquifers and 0.001% in the air as vapour and precipitations. Oceans hold 97% of surface water, 2.4% for glaciers and polar ice caps, and, 0.6% for other land surface water such as rivers, lakes and ponds. A very small amount of the earth's water is contained within biological bodies and manufactured products (Lee *et al*., 2016).

Despite long time usage, portable water supply in urban centers is still a major problem (Holing r*et al*., 2014). In Nigeria for instance, several efforts from various governmental agencies have been made to improve on the safety of portable water supplied in its urban centers. Safe and portable water supplies in urban centers in Nigeria are still inadequate despite the long time usages. Therefore several efforts from various governmental agencies have been made to improve on the safety of portable water supplied in urban centers (Ajayi *et al*., 2008). The Urban Area, Zaria is blessed with abundant water resource both ground water and surface water and the distribution of this resource have very little variation in both time and space amongst the sub-settlements (Kassenga, 2007). There are two major river systems in the area; among them are the River Kubanni and River Saye, joined at a confluence to form River Galma. These Rivers together with their tributaries (Kamacha and Shika) drain the land area of Urban Zaria (Egwari *et al.,* 2005).

Despite the potentiality for good groundwater storage, there is incessant water shortage in the Urban Area of Zaria, because of the poor distribution and supply system of the treated water (Egwari *et al.,* 2005). This has made over 90% of the population rely on water provided from alternative sources other than the Zaria water scheme. Many people depend on water vendors for provision of water for domestic and daily needs and this has led to the advent of locally sourced low cost alternative packaged drinking water in 50-60cm^3 polyethylene sac called sachet water, commonly known as 'Pure water' (Egwari *et al.,* 2005). This packaged water is cheap and have increasingly become popular. The abuse in the production of these packaged water leads to a situation where sachet water is everything but pure. Poor treatment of packaged water results into distribution of contaminated water for human consumption. Possible water contaminants are bacteria, viruses and disease causing pathogens (Edema *et al*., 2011).

Bacteria are the smallest, simplest, single-celled microbes. They can exist as single cells, in pairs, chains or cluster. Relatively few bacteria are parasites or pathogens that cause disease in animals and plants (Katleen and Authur, 2001). *Escherichia coli*, also known as *E-coli,* are bacteria that live in, on, and around faecal matter. *E-coli* are rod- shaped, non-spore forming, gram-negative and lactose fermenting bacteria. They have the ability to grow at elevated temperatures. Most strains of *E-coli* are harmless and form part of the normal flora of the gut. Harmless *E-coli* strains benefit its host by producing vitamin k2. Some strains of *E-coli* can be harmful and can cause intestinal illnesses. They cycle between two principal habitats- intestines of warm blooded animals and water sediments. *E-coli* in drinking water indicate other pathogenic microbes (Durmishi *et al*., 2011). They also add excess organic material to water sources which decays to deplete the oxygen in the water source (Sonial *et al*., 2013).

Another water-borne pathogen of recent times is the bacteria-*Salmonella. Salmonella typhi* is a gram-negative enteric bacillus. It is a mobile, facultative anaerobe with high susceptibility. *Salmonella typhi* is strictly non-lactose fermenting. It causes the infection typhoid or enteric fever. *Salmonella typhi* can only infect human (Sadiq and Rodriquez, 2004). The main source of *Salmonella typhi* is from ingesting infected water. Encounter of humans with this bacterium can also be made via faecal-oral route from infected individual to a healthy one. Salmonella reproduces asexually with a cell division interval of 40 minutes (Sadiq and Rodriquez, 2004).

Apart from bacteria, chemicals equally pose as a source of water contaminant.

Trihalomethane is formed when water is disinfected with chlorine, is a potential water pollutant. The formation occurs when chlorine reacts with organic matter in water. These organic matters are commonly found in drinking water sourced from surface water. Trihalomethanes (THMs) represent only the very volatile fraction of Disinfection by-products (DBPs). Trihalomethane compounds have been tested to be associated with development of cancer (Periera, 2000; Souaya *et al.,* 2015). Some studies report an association between trihalomethane compounds and adverse birth outcomes. In view of the likely health hazards consumers of sachet water are exposed to, there arises the need for the evaluation of commonly consumed sachet water sold within Samaru area of Zaria, Kaduna state, Nigeria.

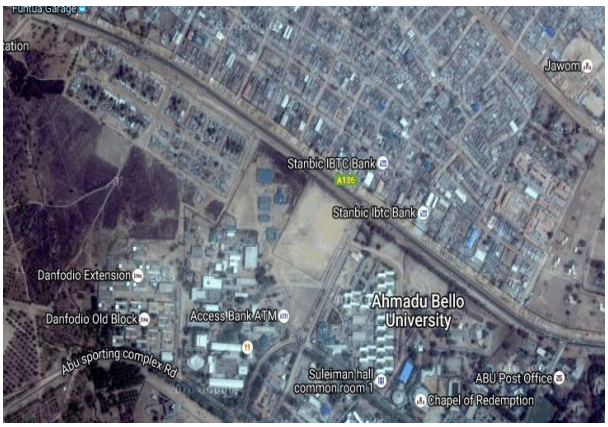
MATERIALS AND METHODS

Description of Study Area

Zaria is a major city in Kaduna state in Northern Nigeria, as well as being a local Government Area. Formerly known as Zazzau, it was one of the original seven Hausa city-states. The 2006 Census population was estimated as 406,990. Zaria's economy is primarily based on agriculture. Zaria is also the center of a textile industry that for over 200 years has made elaborately hand-embroidered robes that are worn by men throughout Nigeria and West Africa.

Sampling Site

This study was carried out in Samaru, Sabon-Gari Local Government Area of Kaduna state. Samaru is situated on latitude 112^{o} 12" N and longitude 07^{o} 37" E, at an altitude of 550–700 meters. It is about 13 km from Zaria city on the Sokoto road, 8 km to Shika and 7 km from Basawa.



Map 3.1: Sampling site

Sample Collection

Five brands of sachet water were randomly collected and labeled W1 –W5. A total of 15 samples, 3 from each of the five brands of sachet water were bought. The water samples were collected in triplicates. These samples were then taken to the laboratory within twenty four hours of collection for analysis. The bacteriological analysis was carried out within twenty four hours of collecting the samples at the Department of Microbiology, Ahmadu Bello University, Zaria. Prior to analysis, each water sample was thoroughly mixed and a portion of the sachet passed through a flame to effect sterilization of the sachet itself.

Apparatus

An Autoclave (Astell, ENGLAND), an Economy incubator (Gallenkamp, ENGLAND), a Agilent UV/VIS spectrophotometer, Metler weighing balance (Toledo WB, CHINA), and a Microscope (CET 1, BELGIUM) were used for the analysis carried out in this work.

Reagents

Reagents used were of analytical grade except where otherwise stated. Distilled water was used in the preparation of all aqueous solutions. Solutions prepared were stored in amber coloured bottles. All concentrations were prepared according to the manufacturers' specification. Agars were weighed using the Metler Toledo weighing balance. After preparation, each broth in their respective volumetric flasks was stuffed with lids to avoid any form of interference and was then inserted into the autoclave for 24 hours.

Preparation of Reagents

- Mac Conkey's Broth (single strength); prepared by dissolving 10.5 g of the agar in 300 cm^3 of distilled water.
- Mac Conkey's Broth (double strength); prepared by dissolving 21 g of the agar in 300 cm^3 of distilled water.
- Brilliant Green broth; prepared by dissolving 2 g of the agar in 50 cm^3 of distilled water.
- Salmonella-Shigella Broth; prepared by dissolving 6.3 g of the agar in 100 cm^3 of distilled water.
- EOS in methylene blue Broth; prepared by dissolving 3.8 g of the agar in 100 cm^3 of distilled water.

Agars were weighed using the Metler Toledo weighing balance. After preparation, each broth in their respective volumetric flasks was stuffed with lids to avoid any form of interference and was then inserted into the autoclave for 24 hours.

Preparation of 50 % w/v Sodium Hydroxide Solution

12.5 g of sodium hydroxide pellets were dissolved in 25 cm^3 volume of distilled water to give a concentration of 50 % weight to volume of sodium hydroxide solution.

Preparation of Standard Stock Solution

1 cm^3 volume each of Chloroform ($CHCl_3$) and bromoform ($CHBr_3$) were transferred into a 100 cm^3 volumetric flask and diluted to mark with 100 cm^3 of methanol (CH_3OH) to obtain a concentration of 43700 µg/cm^3. 1 cm^3 of this resulting solution was further diluted to 100 cm^3 of distilled water to give the stock solution concentration of 437 µg/cm^3.

RESULTS AND DISCUSSION

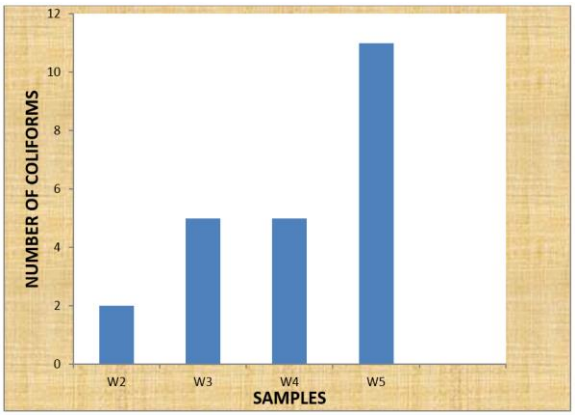


Figure 1: The Coliform Count in each Sachet Water Sample

The results in Figure 1 showed that the water sample with code W 5 had the highest number of coliform colonies (11 colonies).This was followed by W 3 and W 4 with 5 coliform forming colonies, and lastly, W 1 and W 2 with less than 2 coliform colonies). This implies that W 5 has the highest bacterial contamination. High coliform populations in sample W5 is are an indication of poor sanitary conditions in the sample. Inadequate and unhygienic handling of solid-wastes in the environment could have generated high concentration of microbial organisms.

Table 1: The Biochemical Confirmatory test result

SAMPLE NO	CITRATE UTILISATION TEST	MR/VP TEST	INDOLE TEST	INFERENCE
WS1,2 and 3	+	_	_	Enterobacter spp
WS 4 and 5	+	_	_	Enterobacter spp

The result for the Biochemical test carried out to confirm the presence of *E-coli* in the water samples are presented in Table 1 above. The test result confirms the presence of *E-coli* only if there is a positive change in the indole and methyl red test and a negative change to the citrate test. The results from the Table 1 confirms the presence of bacterial contamination within the samples. The result equally showed that this contamination was due to the presence of *Enterobacter* (which is a disease causing bacteria) and not *E-coli*. Coliform populations are indicators for pathogenic organisms which should not be found in drinking water but are usually present in surface water, soil and faeces of humans and animals. High coliform counts appear to be characteristic of the ground water quality in Nigeria, consistent with the works of other investigators who worked on bacteriological and chemical characteristics of sachet water supplies in other parts of the country (Ajayi *et al*., 2008).

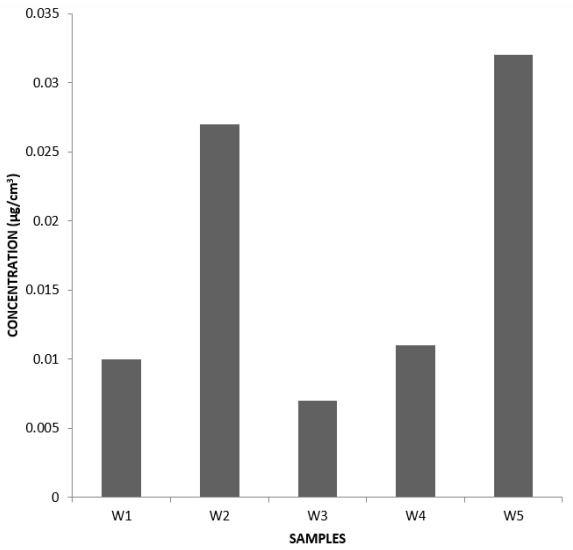


Figure 2: Concentrations of the Trihalomethanes in the Samples

The concentrations of the Trihalomethanes (THMs) detected in sachet water samples from the five different products sold in Zaria are recorded in Figure 2. The national standard permissible limit for THMs is 0.01 µg/cm^3. The International stipulated regulations to trihalomethane by EU and WHO, limit the permissible concentration to a range of 0.01-0.034 µg/cm^3. The results show that all the five samples have the concentration of trihalomethane lying within the international permissible limits but above the national permissible concentration. Also, the results of the analysis showed that the highest concentration value was found in sample W5 with the mean value of 0.032 µg/cm^{3}followed by W2 with a mean value of 0.027 µg/cm^3 and the lowest concentration of THMs in sachet water samples was detected for samples W3 with the mean value of 0.007 µg/cm^3. Although the concentrations of these THMs are relatively low, their consumption in chlorinated drinking water does result in daily and chronic exposure to a mixture of halogenated organics.

Conclusion

The findings from the study showed that all the sachet water samples were all contaminated by the presence of faecal coliform bacteria known as *Enterobacter,* though the samples did not contain *E-coli and Salmonella typhi*. The sample with code W5 had the highest microbial pollutants with a most probable number of 11coliform colonies. The results also showed that the water samples contained high concentrations of Trihalomethane compounds, higher than the national stipulated limits.

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