Microbial and Heavy Metal Evaluation of Sachet Drinking Water in the Hohoe Municipality, Ghana

ABSTRACT

Aim: To study microbial and heavy metal composition in sachet drinking water in the Hohoe municipality in the Volta Region of Ghana.

Place and Duration of Study: The study was conducted in the Hohoe Municipality in the Volta region of Ghana and laboratory analyses were carried out at the Microbiology laboratory of the Department of Biochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology between June 2018 and May 2019.

Study Design: Cross-sectional study

Methodology: The study was conducted on three samples each ofeight different brands of sachet water randomly selected across various towns from street vendors in the Hohoe Municipality for microbial and chemical analyses. 10ml of each sample was aliquoted under sterile conditions and cultured overnight in nutrient media. Enumeration and qualitative analysesof bacteria wereperformed using the pour plate method. For heavy metal analysis, 5 ml of concentrated HNO_3 was added to 40ml of each sample and heated at 100 °C for 20–30 minutes to reduce the volume to 20 ml. 30ml of distilled water was subsequently added and the mixture was analyzed using flame atomic absorption Spectroscopy.

Results: Out of total twenty-four samples analyzed, 50% of thesachet water was contaminated with total and fecal coliforms with the highest average coliform forming unit (cfu/ml) being 16.2 x 10^5cfu/ml and the least 0.01 x 10^5cfu/ml. There were*Klebsiella spp, andPseudomonas aeruginosa* contamination in 50% of the sachet drinking waters but*Salmonella spp and E. coli* were absent in all brands. Chromium was detected to be within the range of 0.40mg/L - 0.56mg/L in the sachet water while zinc was in the range of 0.03mg/L– 0.94mg/L. Cadmium and lead were below detectable limits (BDL).

Conclusion: This study indicated that some of the sachet drinking water sold in the Hohoe Municipality is impure for human consumption. Inappropriate water purification procedures and improper handling of sachet water play a significant role in determining the overall quality of sachet drinking water.

Keywords: Sachet water, heterotrophic bacteria, microbial quality, heavy metals

1. INTRODUCTION

Water is a requirement for every living organism and life forms. An adequate supply of non-polluted drinking water is of immense importance to human health and the need to access it is very imperative [1]. Unhygienic water treatment practices continue to be the cause of diarrhea in children and the global population as a whole [2]. According to [1], 80% of diseases and 30% of deaths are as a result of diseases acquired from drinking water. Sachet water or packaged water is any kind of water that has been produced under sterile conditions and is mainly targeted for human consumption after it has been sealed [3]. To ensure the safety of its consumers, the quality of the drinking packaged water must be of high quality [1]. Chemical and microbial composition analyses of water play a pivotal role in taking measures to protect the health of the public; however, analysis of good drinking water remains a challenge where there are few and limited resources [4]. Even though public health is of more concern, there is much consumption of unsafe drinking water due to unhygienic packaging practices.

Various studies have shown the availability of coliforms and other heterotrophic bacteria in bottled and sachet drinking water, which has by far exceeded national and international standards[5]. Heterotrophic bacteria are non-coliform bacteria species that utilize organic matter for their growth and their presence in water informs how unsafe drinking water may be. The mere presence of heterotrophic bacteria does not show the rate of contamination of the water but also the risk posed to individuals with compromised immune systems. Relatively, total coliform bacteria may pose a health threat to infants, children, and adults with immunocompromised systems [1]. Since the deterioration of water quality primarily involves microbial and chemical hazards, there is major evidence that water-related health problems are a result of microbial contamination of drinking water. Recently, the popularity of sachet and bottled drinking water has been rapidly increasing in several countries, with Ghana not an exception [6] [7].

Heavy metals are constituents of the earth's crust and are persistent environmental contaminants because they cannot be degraded or destroyed by any form of bacteria [4]. Many of these compounds exist naturally, but their concentration has increased as a result of anthropogenic activities [8]. Whilst these elements occur naturally, they are often bound up in inert compounds. However, their concentrations have increased several-fold as a result of anthropogenic activities. Human exposure to harmful heavy metals can occur in many ways, ranging from the consumption of contaminated food, exposure to air-borne particles, and contact or consumption of contaminated water, and accumulating over some time [9]. Water-related diseases can often be attributed to exposure to elevated heavy metal concentrations of both organic and inorganic contaminants. Long-term exposure to these chemicals even at low concentrations can be harmful and this therefore gives the need for water to be treated for the health benefit of the public [10]. Therefore, this study aims to enumerate the microbial load, and types of heterotrophic bacteria and quantify the presence of selected heavy metals in the sachet drinking water.

2. MATERIALS AND METHODS

2.1 Study Area and Study Design

Hohoe is a town and the capital of Hohoe Municipality of the Volta Region of Ghana, its beautiful climate and tourist attraction make it the 35th most populous settlement in Ghana. It has an estimated population of over 150,000 people. It is about 78 km from the regional capital, Ho, and 220 km from the national capital, Accra [11]. The busy nature of the municipality makes it an area where there are about 10 companies producing sachet water.This study was a cross-sectional study.

2.2 Sample collection and Sample preparation

Sampling was performed in the Hohoe Municipality of Ghana. Three (3) samples from each of the 8 brands of sachet water, representing a total of twenty-four samples were randomly purchased from vendors, kept under sterile and temperate conditions, and transferred to the Microbiology laboratory of the Department of Biochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology for microbial and chemical analysis. The samples were sterilized with alcohol before being transferred into 50ml falcon tubes with each brand coded. 10ml of each sample was used for microbial cultures while 40ml of each sample was digested for heavy metal analysis.Each brand of sachet was coded as**CLD, CLV, CNT, HMY, AMZ, EMM, TPI, and EYB**.

2.3 Sample analyses

2.3.1Plating, coliform enumeration,and colony identification

The most probable number (MPN) method was used to determine the total and coliform load in the samples. Serial dilutions of 10^1 to 10^3 were prepared by measuring 1 mL of the sample into 9 mL sterilized distilled water. 100µl of each sample aliquot from each of the dilutions was plated on MacConkey, and mannitol salt media was incubated for 24 hours at 37 ºC to allow bacterial growth. The resulting colonies were counted. The samples were analyzed in triplicates and the average was recorded. Counts per 100 mL were calculated from the MPN from Petri dishes containing more than 30 colonies. Colonies were identified using bacterial colony morphology.

2.3.2Digestion and Analysis of Heavy Metals

40ml of each sample was transferred from falcon tubes to a 50ml glass beaker.5ml of concentrated HNO_3 was added to each sample and heated at 100 °C for 20–30 minutes to reduce volume to 20ml.Samples were allowed to cool and transferred to a 50 ml graduated tube. Distilled water was added to top up to the 50ml mark, capped, and mixed well. The final digestate was analyzed using Flame Atomic Absorption Spectra (FAAS).

3. RESULTS

3.1 Bacterial enumeration (Coliform count)

From **Fig. 1,** four of the sachet water brands were determined to be contaminated with fecal coliforms. The brand **CLD**was recorded to have the highest coliform count averaging 16.2 x 10^5 cfu/ml, followed by the brand **EYB**with an average coliform count of 0.55 x 10^5 cfu/ml. The brands **HMY** and **CLV** both produced an average coliform count of 0.01 x 10^5 cfu/ml while the brands **TPI, AMZ,** and **EMM** showed no observable colonies on media indicating the absence of coliform bacteria.

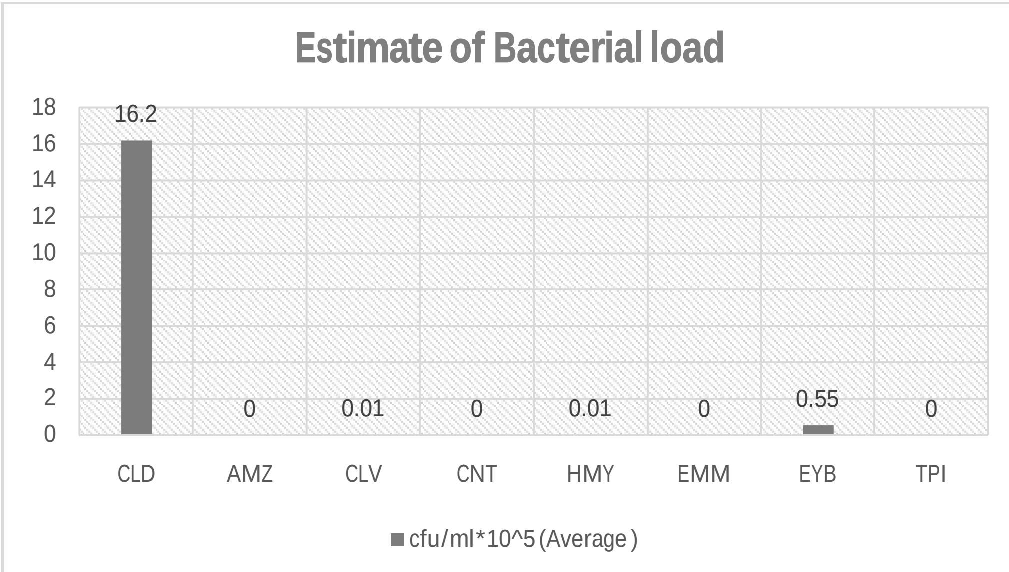

Fig. 1 Coliform count of the different brands of sachet drinking water

3.2 Qualitative analysis

3.2.1 Morphological characteristics of heterotrophic bacteria

The morphological characterization was performed to distinguish between lactose fermenting bacteria and non-lactose fermenting bacteria. Pink colonies indicated the presence of lactose fermenters (coliform bacteria) while the pale colonies indicated the availability of non-lactose fermenting bacteria. Growth of both lactose fermenters and non-lactose fermenting bacteria was observed in the sachet water brands **HMY, EYB, CLD,** and **CLV** but the brands **CNT, EMM, AMZ,**and **TPI**exhibited no color change in media, and hence the absence of either lactose or non-lactose fermenting bacteria.

3.2.2 Speciation of bacteria isolated

Bacterial identification was performed using the morphological characteristics of each bacteria class.*Salmonella spp*and *E. coli*were absent in all brands, however, *Staphylococcus aureus*and *Staphylococcus epidermidis* were present in 12.5% (**CLV** only) of the sachet water brands. *Pseudomonas aeruginosa and Klebsiella spp* were present in 50% (**CLV, EYB, HMY, CLD**) of sachet water brands.

Fig. 2 A plate showing the growth of bacteria on mannitol salt agar

Fig. 3.A plate showing bacterial colonies on MacConkey agar

3.3 Heavy Metal Analyses

Table 1 presents the concentrations of some selected heavy metals analyzed from the sachet water brands. Concentrations were determined using flame absorption spectroscopy. From the table below, the average concentration of zinc was highest (0.9403 mg/L) in CLD and the least concentration was 0.0315 mg/L in EMM compared to the blank. The highestaverage (0.5615 mg/L)chromium concentration detected was CNT while the lowest average concentration recorded was 0.4030 mg/L in the brand EMM compared to the blank.Cadmium and lead were both below detectable limits.

Table 1. Concentration of Heavy Metals

	Concentration (mg/L)			
Sample ID	**Chromium**	**Zinc**	**Cadmium**	**Lead**
Blank	0.3146	0.0129	BDL	BDL
AMZ	0.4291	0.7752	BDL	BDL
TPI	0.4631	0.0357	BDL	BDL
CLD	0.4514	0.9403	BDL	BDL
CLV	0.4858	0.0524	BDL	BDL
HMY	0.4786	0.0419	BDL	BDL
EMM	0.4030	0.0315	BDL	BDL
EYB	0.4961	0.1209	BDL	BDL
CNT	0.5615	0.4276	BDL	BDL

BDL = Below Detection Limit

4. DISCUSSION

A significant and pressing issue in many developing nations is ensuring the availability of sufficient and safe drinking water for the population. However, the safe supply of drinking water remains a challenge in many developing countries as water treatment methods have been relegated to the responsibility of the manufacturers[12]. As a result, daily exposure and consumption of unsafe drinking water is on the rise. The World Health Organization (WHO) stipulated that water-borne diseases account for an estimated 4.1% of the total daily global burden and it is responsible for the deaths of 1.8 million people every year [13]. It was estimated that 88% of that burden is attributable to unsafe water supply, sanitation, and hygiene[14]. This study was therefore conducted to determine the microbial and chemical quality of sachet drinking water sold in the Hohoe Municipality in Ghana. Even though all brands of sachet water have the set standard of colorless and odorless, the amount of bacterial and heavy metal contents in the different brands of sachet water provides a clear inference that half of the presumed "clean" water was contaminated by harmful bacteria and heavy metals, which are indicators for water-borne and other lethal diseases.The current study recorded coliforms in half of the sachet water brands with CLD having the highest coliform count, followed by the brand EYB HMY and CLV brands in concentrations of 16.2 x 10^5 cfu/ml, 0.55 x 10^5 cfu/ml and 0.01 x 10^5 cfu/ml respectively while TPI, AMZ, EMM had no coliform bacteria. These concentrations indicate that the sachet water brands have been contaminated with fecal coliform contrary to what the WHO stipulated, that drinking water should be devoid of fecal coliforms. The finding in the current study is consistent with the results obtained [7] [15] who conducted a study in Kumasi to determine the microbial quality of sachet drinking water sold on the streets of Kumasi, Ghana. Similarly, [16] conducted a microbiological quality of packaged water in Accra, Ghana, and found that more than half of the sachet water samples were contaminated with heterotrophic bacteria exceeding the W.H.O standards. Comparatively,[17] obtained results were consistent with the current findings.Fecal coliform is considered as an indicator of total and fecal contamination hence its presence is indicative of contamination with animal or human intestinal faeces [18]. Even though the coliform counts for these sachet waters are low, they exceeded the limit of the World Health Organization which states that the colony-forming units in any portable drinking water should be zerocfu/100 ml [19].

The morphological characterization of each sample indicated the presence of lactose and non-lactose fermenting bacteria. *Salmonella spp* and *E. coli* were absent in all brands, however, *Staphylococcus aureusand Staphylococcus epidermidis* were present in 12.5% of the sachet water brands. *Pseudomonas aeruginosa and Klebsiella spp* were present in 50% of the sachet water brands. Comparatively, [20] through their assessment of the microbial quality of sachet water in Nigeria obtained results similar to the current findings where they found that *Staphylococcus epidermidis* contaminated 12.5% of their study samples. These results were also consistent with the results obtained from the studies conducted by [21] [22] [23]. The bacteriological characteristics of sachet water deteriorate considerably and significantly as products move farther from the chain of distribution [24]

Heavy metals exert toxic effects on the health of humans and animals. Due to their persistent nature, they bioaccumulate in animals and human beings [25]. Heavy metals influence the residency of microorganisms, however, accumulation to a certain amount may not be ideal to support the growth of microorganisms. From the current study, zinc and chromium were detected in all brands of the sachet drinking waters while cadmium and lead were below detectable limits. The concentration of chromium and zinc weredetectedwithin the range 0.40mg/l - 0.56mg/l and zinc in the range 0.03mg/l– 0.94mg/l respectively compared to the WHO standard for chromium (0.05mg/l)and zinc (3.00 mg/l) stipulated by the World Health Organization for potable drinking water.The findings from the heavy metal analysis for the current study are consistent with the results obtained by [26] for the concentrations of zinc and chromium in sachet drinking water. Similarly, the findings from the current study arecomparable with the results of [28]. However, [27] obtained contrasting results for the concentration of chromium in sachet drinking water sold in Nigeria. [28] obtained results similar to this present study for the concentration of chromium in drinking water. Even though levels of zinc were below the standard limits, continuous accumulation of heavy metals over a period poses a threat to the health of its consumers.

5. CONCLUSION

The results of this study reveal the extent of contamination of sachet drinking with heterotrophic bacteria and heavy metals such as zinc and chromium. These sachet water should be recommended for appropriate quality control by the relevant regulatory government authorities to lessen their microbial and heavy metal contamination. All water samples intended for human consumption should be treated adequately to reduce the levels of microorganisms and heavy metals.

REFERENCES

1. Kalpana, D.V., Balaji, M. and Victor, K. (2014). Microbial Analysis of Packaged Drinking Water Sold in Chennai. *International Journal of Medical Science and Public Health*, 3(4): 472-475.

2. Usman, M., Gerber, N. and von Braun, J. (2016). The impact of drinking water quality and sanitation behavior on child health: Evidence from rural Ethiopia. *ZEF-Discussion Papers on Development Policy*, 221.

3. Fisher, M.B., Williams, A.R., Jalloh, M.F., Saquee, G., Bain, R.E. and Bartram, J.K. (2015). Microbiological and chemical quality of packaged sachet water and household stored drinking water in Freetown, Sierra Leone. *PloS one*, 10(7), p.e0131772.

4. Bain R., Bartan J., Elliot M, Mathews R., Menahan L., Tung R., Chuag P and Gundry S (2012). A Summary Catalogue of Microbial Drinking Water Tests for Low and Medium resource Settings. *International Journal of Environmental Research and Public Health*, 9: 1604.

5. Allevi, R. P, Krometis L, Hagedorn C, Benham B, Lawrence A, Linmg J.E and Zoeglaer, P.E (2013). Quantitative Analysis of Microbial Contamination of Private Drinking Water Supply System. *Journal of Water and Health*, 11(2): 244-245.

6. Moosa, M.E, Khan, M.A, Alalami, U. and Hssain, A. (2005). Microbial Quality of Drinking Water from Water Dispenser Machine. *International Journal of Environmental Science Development*, 6 (9): 710-713.

7. Obiri-Danso K, Okore-Hanson, A and Jones, K. (2003). The microbial quality of drinking water sold on the streets in Kumasi, Ghana. *Letters in Applied Microbiology*, 37 (4):275-280.

8. Sunderland, E.M (2012). Mercury biogeochemistry cycling in the ocean and policy implications. *Environmental Research Journal*,19:101–117.

9. Huang, Z, Pan, X.D, Wu, P.G, Han, J.L and Chen, Q (2014). Heavy metals in vegetables and the health-risk to population in Zhejiang, China. *Food Control*, 36: 248–252.

10. Borah, P., Kumar, M. and Devi, P. (2020). Types of inorganic pollutants: metals/metalloids, acids, and organic forms. *In Inorganic pollutants in water*:17-31. DOI: 10.1016/B978-0-12-818965-8.00002-0.

11. Ghana Statistical Service (2013). 2010 population & housing census: National analytical report. Ghana Statistics Service.

12. Sobsey, M.D., Stauber, C.E., Casanova, L.M., Brown, J.M. and Elliott, M.A. (2008). Point of use household drinking water filtration: a practical, effective solution for providing sustained access to safe drinking water in the developing world. *Environmental science & technology*, 42(12): 4261-4267.

13. World Health Organization. Guidelines for Drinking Water Quality; World Health Organization: Geneva, Switzerland, 2008.

14. Grabow, W.O.K., 2009. Water and Health. *Water and Development*-Volume I, 219.

15. Obiri-Danso, K., Adjei, B.K.N.S., Stanley, K.N. and Jones, K., 2009. Microbiological quality and metal levels in wells and boreholes water in some peri-urban communities in Kumasi, Ghana. *African journal of environmental science and technology,* 3(3): 059-066.

16. Abena Safoa Osei, Mercy J. Newman, J.A.A. Mingle, Patrick F. Ayeh-Kumi and Mubarak Osei Kwasi (2013). Microbiological quality of packaged water sold in Accra, Ghana, *Food Control,* 31(1):172-175.

17. Addo, B.E., Amankwaa, G. and Gyasi, R.M., 2019. Physicochemical and bacteriological quality of sachet water used by Ghanaian university students: implications for public health. *Journal of water, sanitation and hygiene for development*, 9(1): 56-63.

18. Odonkor, S.T. and Ampofo, J.K., 2013. Escherichia coli as an indicator of bacteriological quality of water: an overview. *Microbiology research*, 4(1): p.e2.

19. Mmuoegbulam, O.A., Bassey, I.U., Unimke, A.A. and Igwe, E.E., 2017. Bacteriological and physicochemical quality of Malabor hostel tap water-University of Calabar, Nigeria. *Annual Research & Review in Biology*, 13(5): 1-12.

20. Agi, V. N., Aleru, C. P. and Azike, C. A. and Ollor, O. A. and Alonyenu, D. U. (2020) Antimicrobial Activity of Honey on Some Bacterial Isolates from Selected Brands of Sachet Water Sold within Port Harcourt, Nigeria. *European Journal of Nutrition & Food Safety*, 12 (2): 40-46.

21. Abayasekara, C. L., WHMAT, H., Adikaram, N. K. B., Chandrajith, R., Illapperuma, S. C., & Sirisena, A. S. (2007). Microbiological quality of bottled water in Sri Lanka: A preliminary survey. *Proc Perad Univ Res Sess Sri Lanka*, 12: 49-50.

22. Kuitcha, K., Kamgang, K. B. V., Sigha, N. I., Lienou, G., & Ekodeck, G. E. (2008). Water supply, sanitation and health risks in Yaoundé, Cameroon. *African Journal of Environmental Science and Technology*, *2*(11): 379-386.

23. Khaniki, G. J., Zarei, A., Kamkar, A., Fazlzadehdavil, M., Ghaderpoori, M., & Zarei, A. (2010). Bacteriological evaluation of bottled water from domestic brands in Tehran markets, Iran. *World Applied Life Sciences Journal*, 8:274-278.

24. Ohanu M.E, Iniekong, P. and Eleazer C.I (2012). Microbial Analysis of sachet and tap water in Enugu STATE, Nigeria. *Advances in microbiology*, 2: 547-551

25. Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B.B. and Beeregowda, K.N. (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary toxicology*, 7(2): 60-72.

26. Denkok, Y., Linus, V.G., Abara, U.S. and Oyebade, K.F. (2022). Assessment of Heavy Metal Contamination and Physicochemical Parameters of Sachet Water Consumed in Bukuru Metropolis of Jos South Local Government Area of Plateau State, Nigeria. *Asian Journal of Biotechnology and Genetic Engineering*, 5(1): 52-60.

27. Halilu, M., Modibbo, U.U. and Haziel, H. (2011). Determination of heavy metal concentration in sachet water sold in Gombe metropolis.*African Journals Online*, 8 (1): 15-19

28. Alhassan, A.J., Imam, A.A. and Yakasai, H.M. (2008). Quality assessment of sachet water packaged around Kano Metropolis, Nigeria. *Bayero Journal of Pure and Applied Scienc*es; 83-87.