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Public Health Concern on Commercially Available Sachet Waters in Nigeria: A Mutagenicity, Genotoxicity and Health Risks Study

Okunola Adenrele Alabi

Department of Biology, Federal University of Technology, Akure, Ondo State, Nigeria.
https://orcid.org/0000-0002-0807-9521

Abstract

Sachet water is a common means of obtaining drinking water in many countries in Africa. However, there are concerns about the portability of this water type. This study investigated toxic metal concentrations in five commercially available sachet waters in Nigeria. The non-carcinogenic and carcinogenic risks were calculated. The Ames Salmonella fluctuation assay (Salmonella typhimurium: TA100 and TA98) and SOS chromotest (Escherichia coli PQ37) were used to assess the sachet waters' capacity to cause DNA damage. A higher level of Cr, Cd, As, and Fe in the sachet waters than the allowable limit was recorded. There were substantial carcinogenic and non-carcinogenic hazards for both adults and children, according to data on the total carcinogenic (THQ) and non-carcinogenic (TCR) risks of some of the hazardous metals examined in the sachet waters. The values of these metals surpassed the tolerable threshold. The mutagenicity of the sachet waters was demonstrated by the Ames Salmonella fluctuation assay data. Comparing the mutagenic index of TA 98 and TA100, the earlier was more sensitive to all the water samples. The data obtained in the SOS Chromotest was similar to the data of the Ames test. The five sachet water samples in E. coli PQ37 induced a significant SOS response which indicates that the samples are genotoxic. When the two microbial assays were compared, the Ames Salmonella fluctuation appeared to be a little more sensitive in detecting genotoxins and mutagens in this study. These results are indication of the mutagenic, genotoxic and health effects that might occur in exposed individuals.

Keywords: *Potable Water; Heavy Metals; Sachet Water; Non-Carcinogenic and Carcinogenic Risks; In Vitro Assay.*

INTRODUCTION

The well-being and existence of humans rely on water, with sustaining life necessitating clean, safe drinking water access. Many bodily functions, like digestion, circulation, and temperature control, rely significantly on water. Maintaining healthy skin, hair, and nails, as well as the normal functioning of organs such as the liver and kidneys, is crucial (WHO, 2017). Therefore, there is need for the provision of potable water for human consumption which is believed to be a fundamental human right. Potable water refers to water that is safe for humans to drink and free from harmful contaminants. Potable water must meet World Health Organization (WHO) standards, such as being free of radioactive hazards, chemicals, and pathogenic microbes (WHO, 2017). Drinkable water needs to undergo several procedures and examinations to ensure it is devoid of harmful substances and contaminants before it can be deemed safe for drinking. Examples of these treatments include filtration, disinfection, and chemical treatment (EPA, 2021).

In various regions of the developing world, water sachets, also called sachet water, are commonly used to distribute pre-filtered or sanitized water in plastic packets that are sealed with heat. They are especially popular in Africa (Lerner, 2020) due to the perceived safety when compared to underground water, which is a common drinking water source in many African countries. Sachet water is commonly used in homes, offices and at different social functions/ gatherings in Africa. In comparison to plastic bottles, water sachets are simpler to carry and cheaper to produce (Stoler et al., 2012). Water vendors across different countries have the option to label their sachet water as "pure water". It is sold in bulk, pieces, at shops and even by the roadsides. Despite being a basic human right, many individuals in Nigeria and Africa lack access to safe and clean drinking water. To ensure the availability of good quality drinking water for future generations, efforts must be made to conserve and preserve water resources because water pollution and contamination pose serious dangers to human health (IEA, 2020).

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Water toxicity in Nigeria is a serious health concern because of the pollution of water sources by various anthropogenic activities including industrial activities, agricultural practices, and domestic waste disposal. There are serious health dangers to people when hazardous chemicals and heavy metals contaminate water sources. Exposure to tainted water can cause gastrointestinal issues such as nausea, vomiting, diarrhea, and abdominal discomfort (Järup, 2003). Additionally, it may result in neurological issues like tremors, tingling, and numbness as well as kidney impairment. Infertility in both men and women has also been connected to exposure to harmful chemicals and heavy metals in water. Adebowale et al. (2019) found that men's sperm motility and count were reduced when exposed to lead and cadmium in drinking water. In a similar study, Onyema et al. (2020) found a link between heavy metals in drinking water and diminished ovarian function, along with a heightened risk of infertility in women. Additionally, Wigle et al. (2008) proposed that water contamination may cause reproductive issues like infertility, miscarriage, and birth defects resulting from exposure to heavy metals and endocrine-disrupting chemicals.

Since most of the sachet water producers in Nigeria use groundwater as their source of production, there is need for constant drinking water monitoring through chemical and biological analyses to ensure that such companies adhere to quality practices and the water sold for human consumption meet with international standard of drinking water. The analysis of water through chemical methods is an essential procedure that entails assessing the levels of different dissolved compounds found in the water (Singh et al., 2021). The assessment generally encompasses the evaluation of multiple parameters including hardness, total dissolved solids, dissolved oxygen, alkalinity, pH, along with the levels of different ions and minerals. Most studies use comparison of their chemical analysis of drinking water results with permissible limits, however, comparison with standards alone is insufficient to carry out a quantitative assessment of the health effect of drinking water contaminated with heavy metals. Therefore, in recent times, there is implementation of assessments models of human health risk to determine the possibility of increased adverse health outcomes as a result of exposure to heavy metals (USEPA, 2004, 2007; Sany et al., 2015). Also, it is insufficient to use only chemical analysis for the assessment the portability of drinking water, there is need for biological assays which can show the potential toxic effect of contaminated and polluted water in biological systems. The use of short term *in vitro* biological tests for toxicological study is generally acceptable and preferred with the rising call for alternative to animals in research. Among the short term in vitro assays commonly used in toxicology, Ames and SOS-Chromo tests are very common (Alabi and Esan, 2014).

Urban development, rising population, climate variations, and escalating water shortages pose significant challenges for potable water supply systems. By 2025, approximately 50% of the global population, especially in low- and middle-income nations, will reside in areas facing water stress (WHO, 2013). Hence, it is important to determine the concentrations of heavy metals in different drinking water sources to properly assess human health risks (EPA, 2012; WHO, 2013). In the present study, assessment of five sachet water commercially available in Nigeria was carried out by analyzing the presence and levels of some toxic metals in the water and their potential mutagenicity and genotoxicity using Ames and SOS-Chromo assays.

MATERIALS AND METHODS

Collection of Sachet Water

Five different commercially available sachet waters common in Nigeria were collected in Akure, Ondo State, anonymized and labeled samples 1, 2, 3, 4, and 5, respectively.

Chemical Analysis

The level of Cd, Fe, Cu, As, Zn, Mn, Cr, and selected physiochemical parameters (pH and Alkalinity) in the 5 different sachet water were analysed according to standard analytical methods (SON, 2017; WHO, 2019; USEPA, 2021). In summary, the digestion of 100 mL from each of the 5 distinct samples was performed by heating them with concentrated nitric acid (HNO_3) before concentrating the volume to 3-5 mL. This was subsequently raised to 10 mL using 0.1N HNO_3. The concentrations of the heavy metals were estimated using Buck Scientific 210VGP Atomic Absorption Spectrophotometer.

Health-Risk Assessment

The sachet waters utilized for this research are typically consumed by local inhabitants in Nigeria, so the evaluation of health risks regarding both carcinogenic and non-carcinogenic risks from heavy metals was assessed based solely on the ingestion route. The formulas used were as follow (USEPA, 2004; Wang et al., 2021; Alabi et al., 2024):

$$CDI_i = C_i \times ED \times EF \times IR/AT \times BW \quad (1)$$

$$CR_i = CDI_i \times SF_i \quad (2)$$

$$TCR = \sum_{i=1}^{n} CR_i \quad (3)$$

$$HQ_i = CDI_i/RfD_i \quad (4)$$

$$THQ = \sum_{i=1}^{n} HQ_i \quad (5)$$

where BW = average body weight (kg); CDI_i = chronic daily intake (mg/kg/d); C_i = heavy metal "i" concentration (mg/L); CR_i = heavy metal "i" induced carcinogenic risk; IR = ingestion rate of water (L/d); THQ = total non-carcinogenic risk; AT = average time of exposure (d); SF_i = cancer slope factor of

heavy metal, i (kg/d/mg); EF = exposure frequency (d/a); TCR = total carcinogenic risk; RfD_i = reference dose of heavy metal, i (mg/kg/d); ED = exposure duration (a); and HQ_i = heavy metal "i" induced non-carcinogenic risk. Tables 1 and 2 show the values of EF, AT, SF_i, BW, ED, RfD_i, and IR for adults and children, respectively.

Table 1. Parameters of risk assessment of toxic metals for this study as adapted from Bai et al. (2022) and Alabi et al. (2024).

Description	Exposure parameters	Child	Adult
Average body weight (kg)	BW	15.00	80.00
Exposure duration (years)	ED	6.00	26.00
Exposure frequency (day/annum)	EF	350.00	350.00
Average time of exposure (days)	AT	2190.00	8760.00
Ingestion rate of water (Litre/day)	IR	0.78	2.50

Table 2. Adopted Reference dose ***(****RfDs)* and Cancer slope factor (SF) of heavy metals (mg kg^{-1} day^{-1}) in the present study (Bai et al., 2022; Alabi et al., 2024).

Metals	Cd	Fe	Cr	As
RfD_i	0.0005	0.3	0.003	0.0003
SF_i	6.1000	-	0.500	1.5000

"-" data not available in the literature.

For the non-carcinogenic risk, if THQ or $HQ_i > 1$, there is a possibility of deleterious effects in humans, whereas if THQ or $HQ_i < 1$, the possibility of any health effects in humans is negligible. For the carcinogenic risk, if TCR or $CR_i > 10^{-4}$, there is a possibility of increased human carcinogenic risk, if TCR or $CR_i < 10^{-6}$, there is a possibility of a negligible carcinogenic risk, and if TCR < 10^{-4} or 10^{-6} < CR_i, carcinogenic risk to humans is acceptable (USEPA, 2004; Alabi et al., 2024).

Ames Fluctuation Test

The sachet waters were sterilized by filtration using a cellulose nitrate filter (0.22-mm) before the mutagenicity test using Ames kit. Two S. typhimurium strains (TA 100 and 98) utilized for this test were sourced from Environmental Bio-Detection Products Inc. (Canada). The aseptic method developed by Maron and Ames (1983), modified by Alabi and Bakare (2017), was utilized for the test. Each sachet water was tested at 100, 75 and 50% (v/v, water/DMSO) concentrations. Dilution 1 was prepared by the mixture of each concentration of the sachet water (200 mL) and the reaction mixture (19.8 mL) which is made up of D-glucose, D-biotin, Davis-Mingioli salts made up of bromocresol purple, and L-histidine. The sterile culture tubes were filled with the reaction mixture followed by the sachet water before the bacteria were added. This mixture (200 mL) was then loaded into flat-bottomed 96-well microplates and incubated for 5 days sealed in plastic bags at 37 °C. After incubation, the microplates were examined for color change: all partially yellow, turbid, and yellow wells were recorded as positive and all purple wells as negative. χ^2 analysis was used to compare the total number of positive and negative wells per microplate in each sample and the controls (Gilbert 1980; Alabi et al., 2016a). If the count of positive wells in the sachet water plates is considerably greater than that in the control plates (Mutagenic Index, MI), the sachet water was classified as mutagenic. Additionally, the mutagenicity ratio was determined by dividing the count of positive wells in the sachet water plates by the count observed in the negative control plate (Alabi et al., 2016b). The experiment was conducted three times (±SD) using 2-nitrofluorene as the positive control and distilled water as the negative control.

Save Our Soul (SOS) Chromotest

Identical concentrations to those used in the Ames test were utilized in four replicates for this test. The method of Quillardet and Hofnung (1985), modified by Alabi et al. (2014), was employed without metabolic activation. EBPI (Canada) provided the *E. coli PQ37* utilized for the examination. Sachet water (20 mL) was mixed with 600 mL of overnight culture dilution before incubation and agitation for 2 h at 37 °C. After incubation, there was centrifugation of the mixture for 20 min at 700 g, supernatant removed, before the re-suspension of the bacterial pellets in SOS Chromogen [200 mL; p-nitrophenyl phosphate for alkaline phosphatase (AP) and 5-bromo-4chloro-3-indolyl-b-D-galactopyranoside for β-galactosidase (β-gal)]. The plates for AP analysis were incubated again for 10 min and the plates for β-gal analysis for 60 min. β-gal and AP optical density (OD) readings were recorded at 620 and 405 nm, respectively. The method employed by Legault et al. (1996) and Alabi et al. (2016b) was utilized to determine the adjusted induction factors (CIF = IF/RF), β-gal induction factors (IFs) and AP reduction factors (RFs) thus:

CIF = IF⁄RF

RF = *X*OD405*tX*OD405*c*

IF = *X*OD620*tX*OD620*c*

where RF and IF are the values for background activities of the control, *t* and *c* refer to test dilutions and control, respectively, and *X* is the mean of four OD readings. Genotoxicity is said to be significant if the normalized IF is ≥ 1.1 (Legault et al., 1996). The positive control employed was 4-Nitroquinoline 1-oxide (4-NQO). To evaluate the β-gal activity adjusted for toxicity, the quotient of IF to RF units was utilized.

Statistical Analysis

All statistical analyses were carried out using SPSS 22.0. Dunnet test and ANOVA were used to calculate the differences between the controls and the different concentrations of the sachet water. Significance level at $p < 0.05$ was considered.

RESULTS

Heavy Metal and Physicochemical Analyses

The result presented in Table 3 displays the physicochemical properties and heavy metal attributes of the sachet waters analyzed in this study. The concentrations of As, Cd, and Cr in the five sachet waters exceeded the permissible maximum limits established by regulatory bodies (SON, 2007; WHO, 2017; UEPA, 2021). Nonetheless, the levels of Zn, Fe, Cu, Mn, pH, and total alkalinity in the water samples fell within the acceptable regulatory limits. The order of contamination of the sachet water based on the presence and concentrations of heavy metals is sample 3>2>4>1>5.

Table 3. Heavy metal and physicochemical characteristics of five common sachet waters in Nigeria

Samples	Cu	Cd	Cr	As	Fe	Mn	Zn	Alkalinity	pH
1	0.110	0.004	0.089	0.026	0.087	0.071	0.489	57.32	7. 60
2	0.118	0.005	0.174	0.002	0.094	0.002	0.721	60.53	7.53
3	0.119	0.006	0.120	0.020	0.081	0.092	0.579	56.21	7.67
4	0.110	0.007	0.079	0.013	0.110	0.079	0.543	59.40	7.74
5	0.118	0.004	0.116	0.014	0.078	0.097	0.494	54.00	7.77
Mean	0.115	0.005	0.116	0.015	0.090	0.068	0.565	57.49	7.66
[a] SON	2	0.003	0.05	0.01	-	0.4	5	-	6.5-8.5
[b] USEPA	1.3	0.005	0.1	0.01	0.3	0.05	5	-	6.5-8.5
[c] WHO	2	0.003	0.05	0.01	-	0.4	3	50-200	6.5-8.5

Except for pH which has no unit, all parameters are in mg/L. [a]SON [17], [b]USEPA [18], [c]WHO [19],. "-" data not available.

Human Health-Risk Assessment

Carcinogenic Risk

Table 4 illustrates the cancer risk associated with the consumption of Cd, Cr, and As via sachet water for both adults and children. The data indicated a significant risk of developing cancer from the consumption of Cr, Cd, and As for both children and adults, with children showing a greater carcinogenic risk [Cd (mean = 0.0018), Cr (mean = 0.003), and As (mean = 0.0012)] compared to adults [Cd (mean = 0.0012), Cr (mean = 0.0019), and As (mean = 0.0008)], since the CR values surpassed the threshold of 1×10^{-4} for both groups. The sequence of carcinogenic risk for the metals is: Cr > Cd > As for adults and children alike.

Table 4. Chronic Daily Dose, carcinogenic and non-carcinogenic risks of heavy metals from the tested sachet water by adults and children via ingestion

		Children					Adults				
Element		CDI	CR	TCR	HQ	THQ	CDI	CR	TCR	HQ	THQ
Cd	Min	0.0002	0.0012		0.400		0.0001	0.0006		0.2000	
	Max	0.0004	0.0024		0.800		0.0002	0.0012		0.4000	
	Mean	0.0003	0.0018		0.600		0.0002	0.0012		0.4000	
Fe	Min	0.004			0.013		0.0025			0.0083	
	Max	0.006			0.020		0.0036			0.0120	
	Mean	0.005			0.017		0.0029			0.0097	
As	Min	0.0001	0.0002		0.333		0.00007	0.0001		0.2333	
	Max	0.0013	0.0020		4.333		0.0009	0.0014		3.0000	
	Mean	0.0008	0.0012		2.667		0.0005	0.0008		1.6667	
Cr	Min	0.004	0.0020		1.333		0.0026	0.0013		0.8667	
	Max	0.009	0.0045		3.000		0.0057	0.0029		1.9000	
	Mean	0.006	0.0030	0.0060	2.000	5.284	0.0038	0.0019	0.0039	1.2667	3.3431

Non-Carcinogenic Risk

The HQ values for the non-carcinogenic risks associated with Cd, As, Cr, and Fe in sachet waters indicated that Cd and Fe had non-carcinogenic risks lower than 1, whereas the values for Cr and As exceeded 1 for both children and adults. The sachet waters contained heavy metals with average HQs arranged as follows: As > Cr > Cd > Fe (Table 4). Relative to the adults, children displayed a greater non-carcinogenic risk for each metal, with the highest average non-carcinogenic risk observed for As in children.

Total Health Risk

Total Non-Carcinogenic Risk

Data for the total Hazard Quotient (THQ) attributed to non-carcinogenic heavy metals in sachet waters for both children and adults were > 1 (Table 4). The THQ values for children and adults were 5.28 and 3.34, respectively. Arsenic posed the greatest overall non-carcinogenic risk among heavy metals that are non-carcinogenic, representing 50.47% and 49.87% of the THQ for children and adults, respectively. This was followed by Cr, which represented 37.85% and 37.89% of the THQ for children and adults, respectively.

Total Carcinogenic Risk (TCR)

In the current study, the total carcinogenic risk values for As, Cr, and Cd for both children and adults exceeded 1 x 10^{-4} in the sachet waters (Table 4). The value determined for the children is roughly double the value determined for the adults. Chromium posed a significantly greater carcinogenic risk compared to As and Cd, representing 50% and 48.72% of the TCR for children and adults, respectively.

Ames Salmonella Fluctuation Test

Data shown in Table 5 represents the mutagenicity result of five sachet waters in *S. typhimurium* (TA 100 and TA 98). Generally, both strains showed mutagenicity that was concentration-dependent in the five sachet waters with 100% (highest) concentration of each water causing the highest mutagenicity. However, sample 3 induced the highest mutagenicity, followed by samples 1, 2, 5 and the least was sample 4. The mean of the mutagenicity of the sachet waters ranged from 0.21±0.03 in TA 100 of sample 4 to 4.57±0.10 in TA 98 of sample 3. The results showed that both tester strains were responsive and sensitive to the mutagens in the sachet water samples, however, TA 98 showed a higher sensitivity than TA 100 in all the samples. In both microbial strains, MI of >1.5 was recorded for the water samples with the 100% concentration producing the highest induction in each of the sachet waters (Figures 1-5).

Table 5. Mutagenicity of different concentrations of five sachet waters in Ames Fluctuation Salmonella assay.

Conc. of water (%)	**Mean±SE[a]**	
	TA98	**TA100**
Negative (DMSO)	0.48±0.04	0.76±0.02
	Sample 1	
50	0.36±0.01	0.31±0.04
75	1.85±0.27*	1.80±0.01*
100	3.28±0.32*	3.01±0.03*
	Sample 2	
50	1.76±0.02*	1.56±0.34*
75	2.51±0.01*	2.37±0.07*
100	2.93±0.41*	2.89±0.71*
	Sample 3	
50	2.29±0.06*	2.01±0.02*
75	3.01±0.40*	2.95±0.05*
100	4.57±0.10*	4.28±0.03*
	Sample 4	
50	0.25±0.01	0.21±0.03
75	1.01±0.06*	1.00±0.09*
100	1.89±0.09*	1.76±0.02*
	Sample 5	
50	1.54±0.05*	1.42±0.01*
75	1.98±0.02*	1.86±0.09*
100	2.41±0.05*	2.31±0.02*
Positives	**2-NF**	**NaN_3**
	5.67±0.08*	6.96±0.07*

[a]Number of histone$^+$ per plate = mean values of at least three experiments± standard deviation. 2-NF = 2-nitrofluorene; NaN_3 = sodium azide. * data significant at $p<0.01$ compared to the negative control.

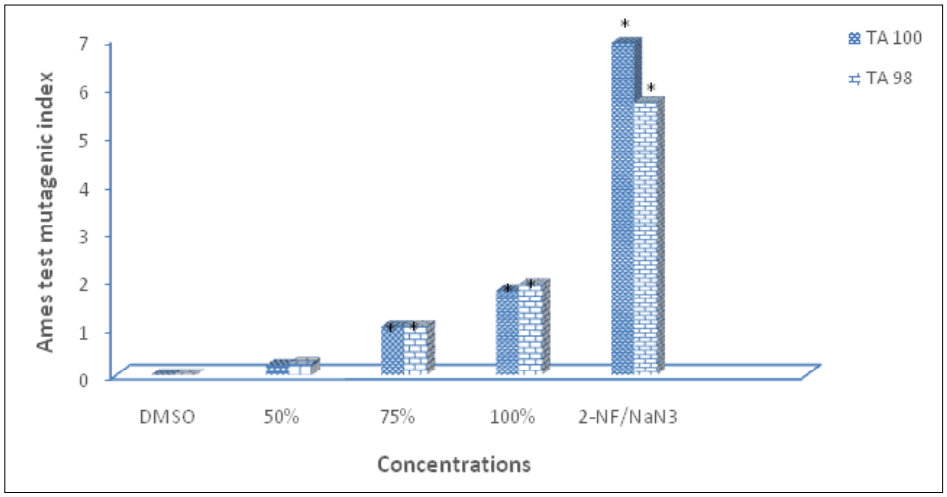

Figure 1. The mutagenic index observed in *Salmonella typhimurium* after treatment with various concentrations of sample 1 sachet water

Mutagenic index = number of sachet water's histone$^+$ revertants/number of negative control's histone$^+$ revertants. * data significant when compared with the negative control at 0.01.

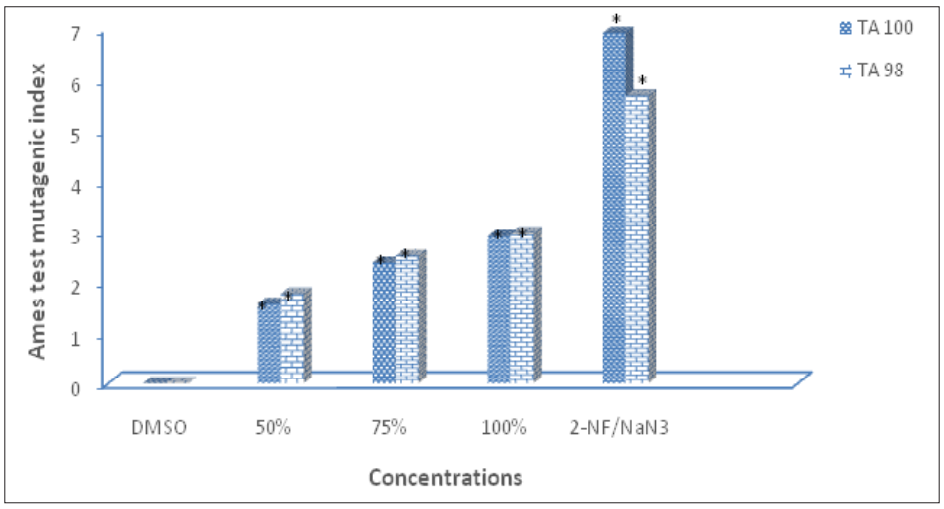

Figure 2. The mutagenic index observed in *Salmonella typhimurium* after treatment with various concentrations of sample 2 sachet water

Mutagenic index = number of sachet water's histone$^+$ revertants/number of negative control's histone$^+$ revertants. * data significant when compared with the negative control at 0.01.

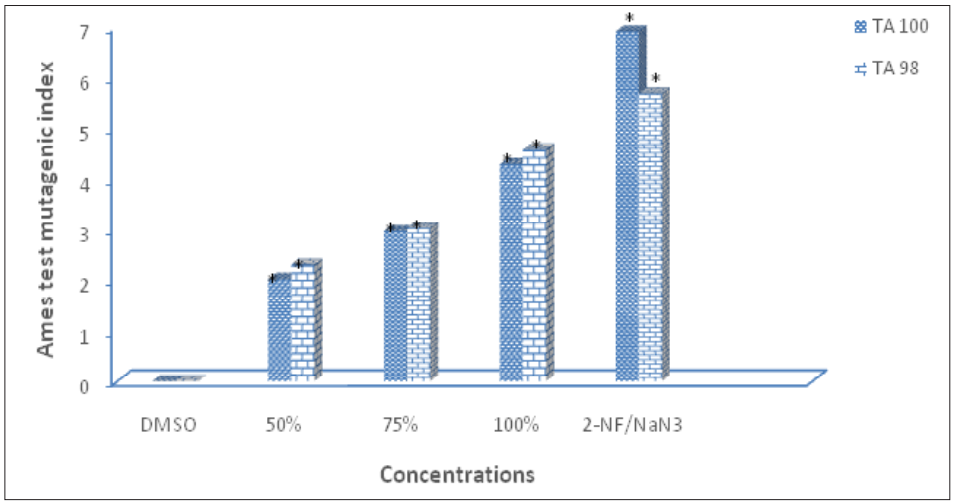

Figure 3. The mutagenic index observed in *Salmonella typhimurium* after treatment with various concentrations of sample 3 sachet water

Mutagenic index = number of sachet water's histone$^+$ revertants/number of negative control's histone$^+$ revertants. * data significant when compared with the negative control at 0.01.

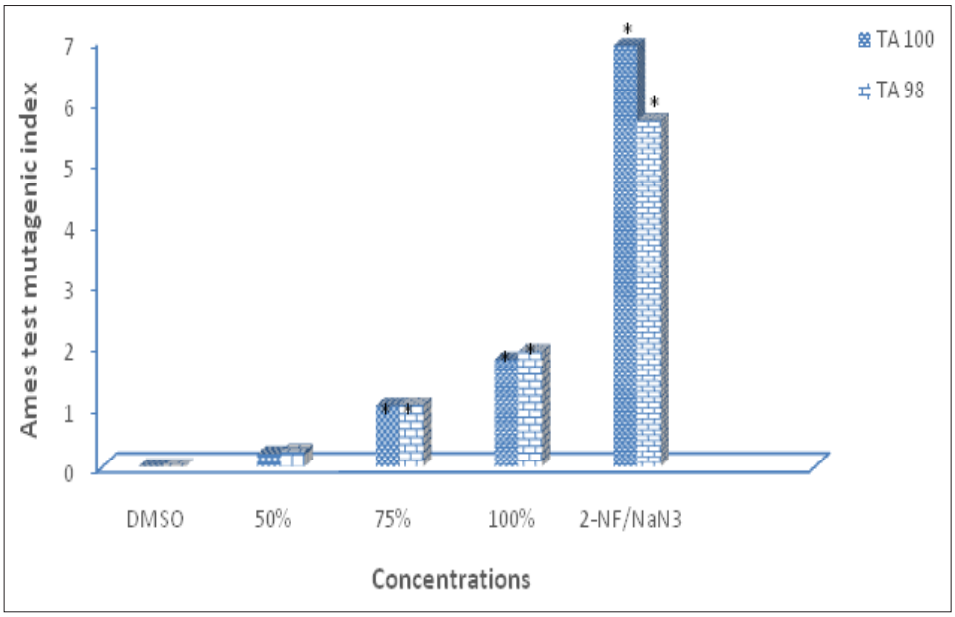

Figure 4. The mutagenic index observed in *Salmonella typhimurium* after treatment with various concentrations of sample 4 sachet water

Mutagenic index = number of sachet water's histone$^+$ revertants/number of negative control's histone$^+$ revertants. * data significant when compared with the negative control at 0.01.

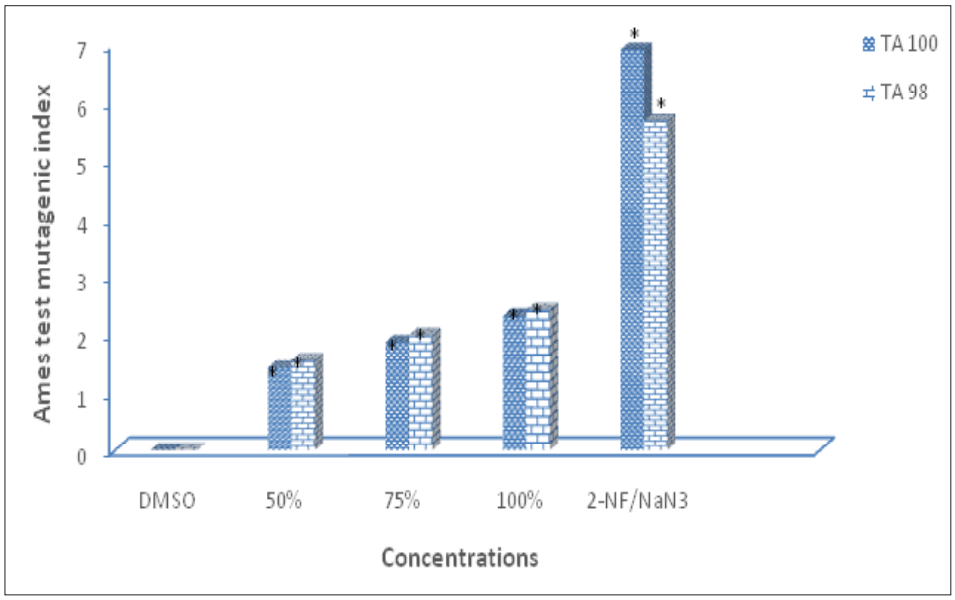

Figure 5. The mutagenic index observed in *Salmonella typhimurium* after treatment with various concentrations of sample 5 sachet water

Mutagenic index = number of sachet water's histone$^+$ revertants/number of negative control's histone$^+$ revertants. * data significant when compared with the negative control at 0.01.

SOS Chromo Test

The result of the SOS Chromo assay of the five sachet waters in the present study is shown in Table 6. The result was considered genotoxic if the IF was ≥ 1.1. The result obtained confirmed the initiation of SOS response in *E. coli* PQ37 after exposure to the sachet waters. The tested samples caused a statistically significant ($p < 0.05$), concentration-dependent genotoxicity. Sample 3 caused the highest genotoxicity in a similar fashion to the data of the Ames fluctuation assay, followed by samples 1, 2, 5 and the least was sample 4, with the 100% concentration of each sample inducing the highest genotoxicity. The IF ranged from 0.47±0.01 in 50% of sample 4 to as high as 2.06±0.02 in the 100% of sample 3.

Table 6. Induction Factor induced by the different concentrations of five sachet waters in SOS Chromo test using *E. coli* PQ37

Sample	Concentrations (µg/mL)	IF=Mean±SD	Genotoxicity
Positive(4-Nitro-Quionoline Oxide)	0.31	0.18±0.27	-
	0.63	0.29±0.04	-
	1.25	0.59±0.17	-
	2.50	0.75±0.50	-
	5.00	1.24±0.47	+
	10.00	1.52±0.07	+
Sample 1	50	0.73±0.06	-
	75	1.16±0.21	+
	100	1.65±0.50	+
Sample 2	50	0.57±0.02	-
	75	1.10±0.11	+
	100	1.25±0.08	+
Sample 3	50	0.93±0.01	-
	75	1.26±0.10	+
	100	2.06±0.02	+
Sample 4	50	0.47±0.01	-
	75	0.87±0.04	-
	100	1.15±0.05	+
Sample 5	50	0.48±0.20	-
	75	0.91±0.08	-
	100	1.20±0.03	+

IF = Induction factor; IF ≥1.1 is considered genotoxic; + = positive for genotoxicity.

DISCUSSION

The provision of adequate, affordable, and safe drinking water plays an essential part in reducing disease burden and health promotion. The Sustainable Development Goal target 6.1 aims for everyone to have fair and universal access to affordable and safe drinking water by 2030. Nonetheless, consuming water tainted with heavy metals has become a worldwide public health issue. To properly evaluate water quality, it is essential to identify possible human health impacts of contaminants in drinking water. In the present study, analysis of heavy metal concentrations in five commercially available sachet water in Nigeria was investigated. Also, the health risks, mutagenicity and genotoxicity were investigated.

The data revealed that the sachet waters contained certain heavy metals at level higher than the acceptable maximum limit by standard organization for drinking water. One of the common and major drinking water pollutants is heavy metal (Don et al., 2022), with characteristics such as persistence, bioaccumulation, and biotoxicity (Liu et al., 2020). The pollution of water sources by heavy metals like Cd, Pb, Cr, and As is through anthropogenic activities like industrial discharge, improper waste disposal, and mining or natural geological processes, causing significant health effects to global populations (Mirzabeygi et al., 2017; Abbasnia et al., 2019).

The metals detected at high concentrations in the sachet waters include As, Cd, and Cr. Cd, As, and Cr are heavy metals with carcinogenic potential at low concentrations that could cause health risks. Of these, USEPA has identified As as the only human carcinogen through drinking water (Murphy and Guo, 2003). It is true that certain metals are categorized as essential elements in humans because of their roles in some of the physiological activities in the biological system at low concentrations, however, their high concentrations can become detrimental to human health (Ukah et al., 2019). For example, Zn and Cu which were reported at high concentrations in the present study play essential function at low concentrations in the metabolic activities within the biological systems but their high concentrations have been reported to be detrimental to the same biological system. High concentrations of Mn, Pb, Cr, and Cr are regarded as highly toxic to aquatic and human life (Ouyang et al., 2002), causing adverse effects such as kidney and liver, genotoxicity and carcinogenicity (Gambrell, 1994; Knight et al., 1997). Indeed, exposure to Cr, Cd, and As through drinking water for a long period has been consistently associated with different types of cancers such as kidney, lung, and skin cancers (Noh et al., 2020). USEPA and IARC have reported that exposure to heavy metals from drinking water is a major concern essentially because of their non-carcinogenic and carcinogenic effects in exposed individuals. In fact, in more than thirty countries of the world, reports have shown that drinking water contaminated with As, Cd, and Cr pose a serious human health concern. Evidence has shown that drinking Cr (8.3–51 μgL^{-1}) and As (50 μgL^{-1}) in water at 1 L/day over one's lifetime can induce liver, lung, bladder

and kidney cancer, and drinking As (0.0012 mg/kg/day) in water increased occurrence of respiratory disorders and skin damages in human (Dawoud et al., 1996; Chowdhury et al., 2016). Long term exposure to Cd can cause osteoporosis, hypertension, cardiovascular diseases, anemia, anosmia, and chronic renal failure (Chowdhury et al., 2016). When these metals bioaccumulate in humans, there is disruption of normal cellular functions and chronic toxicity thereby weakening the immune system, increasing disease susceptibility and causing organ damage (Alidadi et al., 2019). It is however, very important to recognize that factors like cumulative effects over time, duration of exposure, and individual susceptibility can affect the detrimental effects of exposure to heavy metals. Regions where drinking water is contaminated will experience serious public health challenges and there is therefore an urgent need to prevent continuous exposure to these detrimental toxic metals thereby safeguarding overall public health (Radfard et al., 2019; Sener et al., 2023).

Drinking water contaminated with heavy metals reported in this study is similar to reports from other parts of the world such as Thailand, Saudi Arabia, China, Chile, Bangladesh, India, Mexico, and Iran (WHO, 2013; Rajeshkumar et al., 2018). High concentration of Hg, Pb, Cu, As, and Cd is present about 43% of the tested drinking water from wells and storage tanks in Sonora, Mexico (WHO, 2013). Similarly, in many cities in Saudi Arabia, concentrations of drinking water's Cu, Pb, and Cd were higher than the permissible limit (Chowdhury et al., 2016). In the last 10 years, India also documented the presence of Zn, Ni, As, Cd, Mn, and Pb in drinking water at concentrations greater than the guideline value due to geo-genic contamination and wastes from pesticide, fertilizer, paint, and pharmaceutical industries (Chowdhury et al., 2016; Bajwa et al., 2017). About 42.1% of drinking water in Bangladesh has been documented to contain above 50 µg/L of As in the last 14 years (EPA 2012), and in Thailand (Wongsasuluk et al., 2014; Li et al., 2016) and some Iranian metropolitan cities (Mosaferi et al., 2008; Savari et al., 2008; Hadiani et al., 2015), their drinking water contained an average levels of Zn, Ni, Cr, and Pb higher than the guidelines value as a result of the poor domestic treatment and pipeline corrosion.

Direct comparison of the concentrations of pollutants in drinking water with maximum permissible limit was the traditional method deployed for the evaluation of health effects, however, this is insufficient to identify all the contaminants of health concerns and understand their detailed hazard levels. Health risk assessment has become an important tool that can be used to estimate the potential human health effects posed by different contaminants (Sany et al., 2015; USEPA, 2019). Indeed, this assessment has been used and documented to be sensitive in evaluating the potential adverse health effects posed by contaminants in drinking water (Hartley et al., 1999; Sun et al., 2007; Kavacar et al., 2009). Therefore, the present study calculated the carcinogenic and non-carcinogenic risks linked with drinking the sachet waters.

In Nigeria, there is a lack of information regarding the non-carcinogenic impacts of heavy metals in sachet water; thus, the chronic daily intake (CDI) via the consumption of As, Cd, Cr, and Fe was assessed in the sachet waters. The findings from this study indicated that consuming sachet water may be an important pathway for exposure to heavy metals. Alidadi et al. (2019) have also recorded this pathway of exposure to heavy metals. Furthermore, according to the data recorded for the average value of total CDI, children are at least two times more exposed to drinking water contaminated with heavy metals when compared with the values obtained for the adults. This observation agrees with reports of other studies where children recorded significantly higher total heavy metal intake than adults from drinking water. For instance, studies from Thailand and Australia regarding the average total daily intake of heavy metals from drinking water indicated that it was about 2.5 and 1.7 times greater in children compared to adults, respectively (Wongsasuluk et al., 2014; Saha et al., 2017).

Furthermore, in adults and children, Cr demonstrated the greatest average contribution of THQ elements. Considering the non-carcinogenic risk posed by drinking contaminated sachet water, Cr seems to be the most hazardous element. Moreover, the elevated THQ values for children in this study indicated that they are more vulnerable to the non-carcinogenic risks associated with the toxic metals than adults. This outcome aligns with findings from research conducted in Hong Kong (Rajeshkumar et al., 2018), Australia (Saha et al., 2017), and Iran (Alidadi et al., 2019). The study's average cancer risk values for children and adults highlighted the possible cancer risks for both groups if they are exposed to carcinogenic elements (Cr, Cd, and As) throughout their lives by consuming sachet water in Nigeria. Furthermore, present data showed that in comparison to As and Cd, Cr contributed the highest to the average TCR values, suggesting Cr as the most potent carcinogen in this carcinogenic risk assessment. Similar to the data obtained for the non-carcinogenic risk, the data obtained for TCR in this study was higher for children than for adults, an indication that children will be more susceptible to cancer risk from the heavy metal-contaminated sachet water. This is most likely because children drink more water in proportion to their weight than adults, hence, making them more vulnerable especially since their nervous, reproductive, immune, and digestive systems are still developing (Alidadi et al., 2019). At this early stage of their development, heavy metal exposure can cause irreversible damage (Peek et al., 2018). Furthermore, other subpopulations besides children including people with pre-existing health challenges and pregnant women could be more vulnerable to the toxic effects of heavy metals causing a higher disease burden. The report of this study showed that sachet water production in Nigeria requires

some control measures, remediation and intervention so as to ensure that the presence of carcinogenic metals in the sachet water is within permissible range. There is need for the implementation of appropriate improved purification programs, proper monitoring, and mitigation measures for drinking water to protect the health of the citizens. Further study and monitoring efforts on the exposed citizens are essential to have a comprehensive understanding of the health implications and the extent of adverse effects already induced by the consumption of the heavy metal-contaminated sachet water in these areas. Besides, the comparison of the present data with previous studies globally showed that drinking water contamination by heavy metals is a major health concern worldwide. Report (Banerjee et al., 2023) has shown that in different regions there is increased levels of carcinogenic heavy metals in drinking water, laying emphases on the urgent need to carry out comprehensive risk assessments and execute appropriate strategies to mitigate this dangerous trend.

The data obtained in this report highlight the possible detrimental health effects that can occur by exposure to heavy metals from sachet water and why it is important to implement good measures that can reduce the levels of these deleterious metals in this type of drinking water. There is need for further studies on the probable sources of contamination of the sachet water by heavy metal. Although the present study did not identify the specific sources of contamination, however, it is imperative to carry out investigations on potential contributors. Understanding these sources might help in the implementation of targeted interventions which can mitigate against sachet water contamination by heavy metals.

To further confirm the toxicity of the sachet water, biological assays were used. This study further assessed the genotoxicity and mutagenicity of sachet water. Since no single assay exists for the detection of the full spectrum of various mutagenic and genotoxic end points (Dearfield et al., 2002; Alabi et al., 2019), hence, this study used two test systems: SOS chromo and Ames Salmonella fluctuation assays. These assays indicated that the sachet waters studied can cause mutation and are genotoxic. The Ames Salmonella fluctuation assay showed that the sachet water induced mutation through atleast two separate molecular mechanisms which are nucleotide deletion or insertion leading to frameshift mutation as shown in TA98 strain and base pair substitution mutation in TA100 strain (Alabi and Adeoluwa, 2021). Owing to its heightened sensitivity relative to the traditional Ames test, the Ames Salmonella fluctuation assay employed in this study is especially more suitable for identifying mutagens in water samples (Monarca et al., 1985). Furthermore, the assay permits the incorporation of a greater volume of the samples which can facilitate the detection of small concentration of mutagenic compounds without using any concentrating method. As there is no concentration technique capable of retrieving all significant substances in equal amounts from the sample (Stahl, 1991; Alabi, 2022), the Ames Salmonella fluctuation assay shows the advantage of a method of concentration devoid of the drawbacks. This sachet water mutagenicity report agrees with previous reports where sachet water was reported to induce reproductive toxicity in mice (Alabi et al., 2024). The genotoxicity was further confirmed using the SOS chromo assay. The test employs a sophisticated regulatory framework of the E. coli PQ37's error-prone DNA repair mechanism, known as the SOS response, triggered by genotoxic substances (Walker, 1987). Primary agents capable of damaging the DNA are easily detectable by the SOS chromo assay in *E. coli*. Observed genotoxicity of the sachet water in this assay could be as a result of the presence of high concentrations of toxic metals. Alabi and Bakare (2011) suggested that toxicant's DNA damage in the biological system could be as a result of heavy metal's presence and subsequent interactions with DNA. Ability of heavy metals to induce DNA damage has been documented (DeFlora et al., 1990) to be due to the formation of cross-links between DNA–protein and DNA–DNA (DeFlora et al., 1990). The heavy metals in the sachet water in this report can cause mutation and possibly cancer in the biological systems. Their interaction in the biological systems might result in more harmful synergistic chemical combinations compared to the individual effects. Furthermore, other pollutants not analyzed in this study might also be contributors to the reported genotoxicity and mutagenicity.

CONCLUSION

In this study, heavy metal analysis and its health risk assessment was carried out using daily intake and ingestion route for sachet water in two populations of adults and children. Assessment of health risk showed that the non-carcinogenic and carcinogenic risks of ingestion of sachet water are higher than USEPA's safety levels, hence, sachet water consumers in this vicinity might be at risk of developing cancer and other diseases from its long term consumption. The data further showed that children compared to adults are more likely to experience these carcinogenic and non-carcinogenic effects. The sachet water further showed potential mutagenic and genotoxic effects in microbial assays. Therefore, constant monitoring and determination of the levels of heavy metal in commercially available drinking water are necessary to safeguard the long-term wellbeing and public health in the affected communities. In addition, more investigations and epidemiological studies are suggested to determine any potential long-term or subtle health risks from chronic exposure to low concentrations of these toxic metals from sachet water. These findings should equip the necessary Nigerian government agencies to make informed decision on the implementation of appropriate strategies to mitigate against the presence of toxic metals in sachet water and establish guidelines that can minimize the risks posed by these toxic metals.

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