Research

Impacts of sunlight exposure on physicochemical parameters, potential toxic metals, and microbial characteristics of sachet drinking water sold in Calabar metropolis, Nigeria

Nikita Elkanah Umoafia[1] · Uwem Okon Edet[1] · Akaninyene Paul Joseph[2,3] · Okoroiwu Uchechi Henshaw[4] ·
Elizabeth Nkagafel Mbim[5] · Honour Obeten[1] · Bassey Okon Edet[1] · Asanga Edet[6] · Oju Richard Ibor[7] ·
Nkoyo Nkang[8] · Hitler Louis[9] · Francisca O. Nwaokorie[10] · Ani Nkang[1]

Abstract

Poor handling of potable water is a well-known route of contamination. Despite this, Nigeria's most consumed packaged potable water called "sachet" or "pure water" is still poorly handled with long hours of exposure to sunlight which may affect its quality. This study examined the potential deterioration of the sachet water quality (potential toxic metals and bacteriological), and the health implication posed by long-time exposure to sunlight. Collected water samples were subjected to physiochemical analysis, metal analysis, risk assessment, and microbiological examination. Three sachet water brands (n = 4 bags per brand) exposed to sunlight daily for 14, 28, and 42 days were analysed for potentially toxic metals, and health risk assessments (Average daily dose-ADD, hazard quotient-HQ, hazard index-HI, and carcinogenic risk-CR). The physicochemical, and microbiological (total heterotrophic bacteria count-THBC, and total coliform count-TCC) parameters were equally analysed using the Kirby Bauer and pour plate technique. In all brands, the levels of physicochemical parameters (except pH), and potential toxic metals were comparatively higher than the control in an exposure time-dependent fashion. Furthermore, all the exposed samples failed to meet the safety limits of the WHO, and national standards (NAFDAC and NDSWQ) for drinking water. Among the metals, zinc was the most ingested metal in all the brands. The ADD, HQ, and HI evaluations revealed that consuming the exposed water could lead to higher accumulation of Cr, Cd, Pb, As, and Ni in the body following exposure. The CR of Cr, As, and Ni after exposure to sunlight were > than the safe value (10^{-4}), implying the likelihood of cancer after over 60 years. Microbial counts increased with the length of exposure, and all the isolates showed pathogenicity and multidrug resistance. The potential health risk inherent in sachet water after exposure to sunlight has significant health implications for consumers.

✉ Uwem Okon Edet, uwemedet27@gmail.com | [1]Department of Biological Sciences, Faculty of Natural and Applied Sciences, Arthur Jarvis University, Akpabuyo, Cross River State, Nigeria. [2]Department of Biosciences and Biotechnology, Faculty of Science, University of Medical Sciences, Ondo City, Ondo State, Nigeria. [3]Key Laboratory of Integrated Regulation and Resource Development On Shallow Lake, College of Environment, Hohai University, Nanjing 210098, People's Republic of China. [4]Department of Medical Laboratory Sciences, Faculty of Basic Medical Laboratory Sciences, Arthur Jarvis University, Akpabuyo, Cross River State, Nigeria. [5]Department of Public Health, Faculty of Basic Medical Laboratory Sciences, Arthur Jarvis University, Akpabuyo, Cross River State, Nigeria. [6]Department of Chemical Sciences, Faculty of Natural and Applied Sciences, Arthur Jarvis University, Akpabuyo, Cross River State, Nigeria. [7]Department of Zoology and Environmental Biology, Faculty of Biological Sciences, University of Calabar, Calabar, Cross River State, Nigeria. [8]Department of Science Laboratory Science, Faculty of Biological Sciences, University of Calabar, Calabar, Cross River State, Nigeria. [9]Department of Chemistry, Faculty of Physical Sciences, University of Calabar, Calabar, Cross River State, Nigeria. [10]Department of Medical Laboratory Sciences, College of Medicine, University of Lagos, Lagos, Lagos State, Nigeria.

Article highlights

• Concentration of potential toxic metal increased linearly with exposure time.
• Carcinogenic risk assessment showed potential cancer risk after 60 years.
• All the brands failed all bacteriological regulatory guidelines.

Keywords Potentially toxic metals · Water quality · Biological parameters · Sachet water · Cancer risk

Abbreviations

ANOVA	Analysis of variance
ADD	Average daily dose
APHA	American public health association
CR	Carcinogenic Risk
E and N	East and North
HI	Hazard Index
HNO_3	Nitric acid
MSM	Mineral Salt Medium
NAFDAC	National Agency for Food and Drug Administration and Control
PE	Polyethylene
TDS	Total dissolved solids
TCC	Total coliform count
THBC	Total heterotrophic bacteria count
HQ	Hazard Quotient
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

List of symbols

%	Percentage
Cfu/ml	Colony forming unit per ml
Cl	Centiliter
cm	Centimetre
°C	Degree centigrade
ft	Feet
Km	Kilometer
mm	Millimeter
g	Gram
ml	Mililiter
>	Greater than
µm	Micrometers
mg/L	Microgram per liter

1 Background of the study

Potable water is important for human health, and development [1]. Access to safe potable drinking water is a fundamental human right. However, this right is still elusive in most developing countries such as those in sub-Saharan Africa [2]. Ideally, water meant for human consumption should be free from biological, and chemical contaminants [3]. An estimate from the WHO [4] indicates that there is paucity of potable water, especially in sub-Saharan where it is estimated that approximately 1.1 billion persons, an equivalent of the entire population of Africa as of 2012 do not have access to safe drinking water [4, 5]. As a result of this inaccessibility, and the inability of some developing countries to provide potable water, alternative water sources are being explored. Notable among them is the sachet water, a widely consumed "potable" water not just in Nigeria but across other West Africa countries as well [6–10, 10, 11]. Yet, the risks (microbiology and chemical) that comes with its consumption is still not fully understood.

The sachet water is packaged using polythene (PE) plastic bags that are designed to hold approximately 60 cl of water, and is presumably treated using some mini-treatment plants [12]. Currently, sachet water is the most consumed form of potable water at homes, restaurants, bars, and social gatherings in Nigeria [13]. Therefore, as many as 60 million units of the packaged water are produced daily and sold within the country to meet its increasing demand [14]. Although the safety of the sachet or packaged water is regulated by the National Agency for Food and Drug Administration and Control (NAFDAC) [15], some sachet water brands still fail to meet the national, and international bacteriological standards for potable water [9, 13, 16]. This has been attributed to the poor handling or negligence of laid down procedures during the production, and supply of the water [17]. Within the Calabar metropolis, bags

of sachet water are often transported using motorized tricycles, and subsequently displayed in shops for sell to consumers under direct or indirect sunlight.

It has been shown that on poor storage, sachet water deteriorates in quality, and can act as a vehicle for the transmission of pathogenic organisms [18]. In addition, the polythene plastic used in packaging the water is usually susceptible to degradation under high temperature or direct sunlight condition [1, 3, 17, 19]. As such, ultraviolet rays from sunlight could have the potential to speed up degradation reactions that affects the photosensitive polyethene material used for packaging of the water [6]. More so, during the degradation process, potential toxic metals, microplastics and bacteriological activities are bound to affect the water quality [20, 21]. These potential toxic metals are highly persistent, and can bioaccumulate in the tissues of biological organisms, resulting in severe health issues in the process over time [22].

Several studies exist that have evaluated potential toxic metals, physicochemical parameters, and the bacteriological quality of various potable water samples (sachet water, and bottled water) exposed to sunlight [3, 6, 17, 18, 23–25]. However, none of these studies evaluated the health risks inherent in the sachet water before, and after exposure to sunlight during storage using risk assessment parameters such as the average daily dose (ADD) of potentially toxic metals ingested, hazard quotient (HQ), hazard index (HI), and carcinogenic risk (CR) used globally to evaluate health risks of chemicals in water [8, 26–30]. The main aim of this study was to evaluate the health risks that may be inherent in the consumption of sachet water exposed to sunlight via the evaluation of the average daily dose of potentially toxic metals ingestion, hazard quotient, hazard index, and carcinogenic risk in addition to its microbial quality.

2 Methods

2.1 Study area

This study lasting from November to December of 2021 was conducted in the Calabar metropolis. Calabar is the capital city of Cross River State, and is located in the south-south geopolitical zone of Nigeria (Fig. 1). The city is located on latitude 4° 58′ 32.5776″N and longitude 8° 20′ 30.1236″E. It has a total area of 406 km^2 (157 square miles), and an elevation of 32 m (105 ft) above sea level. It has characteristic dry and wet seasons atypical of a tropical climate, with a temperature range of 25–28 °C, and average annual rainfall of 3,000 mm [31, 32]. Calabar is one of the economic nerve centres of Cross River State. It is the hub for education, commerce, and tourism as far as the Niger Delta is concerned. Common occupations of inhabitants include farming, trading in various kinds

of businesses, civil and public servants (government workers), students, etc. There is a huge disparity in the income levels of the inhabitants of the city, and most of the inhabitants are classified as middle-class. The majority of the workforce is employed in the informal sector of the state's economy. The informal sector of the state is dominated by retailers of all kinds of services and service providers. As of the last official census conducted in 2006, its population stood at 371, 022 [32]. Since then, the population has witnessed tremendous growth [32]. According to City population, the population of Calabar metropolis is estimated to be 571,500 as of 2022, representing 2.60% annual growth rate. As expected, there is a high demand for potable sachet water for the teeming population to compliment the absence of potable pipe-borne water from the State Government.

2.2 Collection of sachet water samples

Purposively, three of the most produced, distributed, and consumed sachet water brands in the Calabar metropolis were selected for the study. These were coded as US, AN, and GP respectively to avoid conflict of interest. The sachet water samples were collected via by purchasing from each brand, four bags each containing a total of 20 sachet water samples. The purchase (sampling) was done directly from the companies within one hour of production, and packaged without any prior sunlight exposure. The water samples were obtained via simple random sampling. The samples were then transported in ice using a sterile cooler to the central biological sciences laboratory for further analysis.

2.3 Exposure of sachet water to sunlight

The experiment was designed by simply randomizing the water samples into two groups namely: the control, and the test groups. The control group (not exposed to sunlight) were stored indoor at 4 °C, while the test group (sachet water) from different brands were exposed to direct sunlight for six (6) h (10 am–4 pm) daily for 14, 28, and 42 days. At the end of each exposure window, the sachet water samples were analysed for toxic metals, physicochemical and biological parameters.

2.4 Physical analysis of the water sample

Physical parameters such as temperature, pH, conductivity, turbidity, total dissolved solids (TDS), and hardness were evaluated. Temperature (°C) was determined by dipping a mercury-in-glass thermometer into the water samples for three minutes. The pH of the water samples was measured using a hand-held pocket-size electronic pH meter (pH-1 model, Germany) by immersing the probe end of the
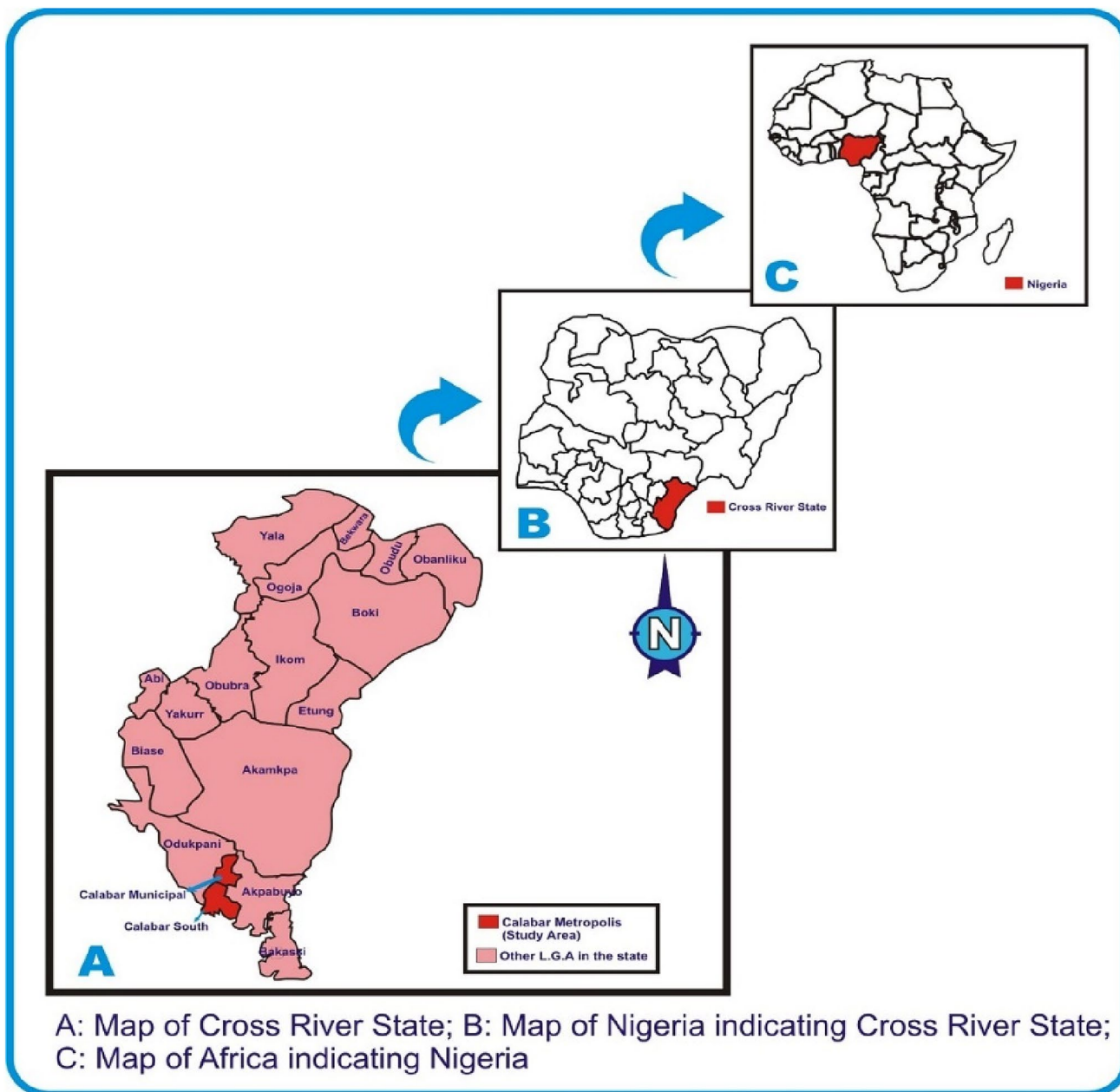
A: Map of Cross River State; B: Map of Nigeria indicating Cross River State; C: Map of Africa indicating Nigeria

Fig. 1 Map of Cross River State showing Calabar Metropolis [33]

meter into water samples for two min. Conductivity (µS/cm) was determined using a Hannah conductivity meter (BM-211model, Germany) dipped into the water for two minutes. Turbidity (N.T.U) was measured using a turbidity meter (BM-211model, Germany) dipped to the 2 cm level of the water from the surface. Total dissolved solids (TDS) (mg/l) were measured using a TDS meter (Extech meter model, Exstik EC 400) by inserting the probe into the water sample. The total hardness of the water samples was measured as described by Rice et al. [34] using a UV/VIS Spectrophotometer (model HACH 5000, Germany).

2.5 Chemical analysis of the water sample

At the end of each exposure window, the composite water samples were filtered through a 0.45 µm membrane to eliminate any remaining suspension, and 6 mL HNO_3 was used to prepare 25 mL aliquots from each sample [35]. The concentration of iron (Fe), manganese (Mn), chromium (Cr), aluminium (Al), cadmium (Cd), zinc (Zn), lead (Pb), arsenic (As), and nickel (Ni) in the samples before and after exposure to sunlight were analysed using an inductively coupled plasma optical emission spectrometer (ICP

OES, Varians-720-ES) to the nearest mg/L. The calibration curve technique was adopted for metal quantification. The standards of NF IN ISO 15587–2 (2002, AFNOR, France) were used to prepare the working standards (Merck, Germany) for each potential toxic metal. The working standards used for the calibrations were prepared from the certified standard solution (1000 mg/L) of each metal and milli-q water (Merck, Darmstadt, Germany) using a serial volume/volume dilution technique in a glass volumetric flask. The calibration curves were obtained from a plot of standard solution concentrations alongside the corresponding absorbance readings. To ensure the accuracy of the method used, potential toxic metals determined were compared with the internationally certified reference materials for water samples (SRM 1643e), and their recovery rates, and regression coefficients were calculated [35]. The limits of detection were 0.002 (Cr), 0.001 (Pb), 0.001 (Cd), 0.01 (Ni), 0.0026 (Fe), 0.0056 (Mn), 0.008 (Al), 0.11 (Zn), and 0.006 (As) mg/L. The recovery rates ranged from 88 to 105% while the regression coefficients ranged from 0.91 to 0.98.

2.6 Health risk assessment

The health implications from the ingestion of potential toxic metals leached from the sachet water before and after exposure to sunlight were evaluated using average daily dose (ADD), hazard quotient (HQ), hazard index (HI), and carcinogenic risk (CR) to predict the potential health risks to the consumers.

2.6.1 Average daily dose (ADD)

With the aid of Eq. 1 below, the average daily dose of potential toxic metals following exposure from the ingestion of the sachet water exposed to sunlight was estimated according to Tabassum et al. [36].

$$ADD = \frac{Cwater \times IR \times EF \times ED}{BW \times AT} \qquad (1)$$

where ADD = average daily dose of potential toxic metals exposure (mg/kg/day). C_{water} = concentration of potential toxic metals in water (mg/L). IR = water ingestion rate of adults: 2 L/day [28]. EF = exposure frequency: 365 days/year [36, 37]. ED = duration of adult exposure: 64.4 years [30]. BW = body weight of humans: 70 kg (adults) [36, 37]. AT = average time of adult exposure:365 days/year × 64.4 years (23, 506 days) [30].

2.6.2 Hazard quotient (HQ)

According to Tabassum et al. [38], the HQ due to the ingestion of potential toxic metals leached from the plastic package of the sachet water exposed to sunlight was determined as shown below in Eq. 2.

$$HQ = \frac{ADD}{RfD} \qquad (2)$$

where HQ = hazard quotient via ingestion of potential toxic metals. ADD = average daily dose. RfD = reference doses for ingestion of potential toxic metals (mg/kg/day).

The RfD_{inj} were 0.07, 0.14, 0.003, 7, 0.001, 0.3, 0.0035, 0.0003, and 0.02 mg/kg for Fe, Mn, Cr, Al, Cd, Zn, Pb, As, and Ni, respectively [8, 26, 39]. HQ values ˃1 indicate an impending health danger [40].

2.6.3 Hazard index (HI)

Furthermore, we evaluated the overall non-carcinogenic risk from the oral exposure of adults to potential toxic metals leached from the plastic package of the sachet water after exposure to sunlight using methods previously described [8, 26, 39] and shown in Eq. 3.

$$\begin{aligned} HI = {} & HQ\,(Fe) + HQ(Mn) + HQ\,(Cr) + HQ\,(Al) \\ & + HQ\,(Cd) + HQ\,(Zn) + HQ\,(Pb) + HQ\,(As) \\ & + HQ\,(Ni) \end{aligned} \qquad (3)$$

where HI = hazard index and HQ ˃1 shall be taken as an impending health worry [40].

2.6.4 Carcinogenic risk (CR)

Using a lifespan of 64.4 years, the CR was determined according to USEPA [41] as shown in Eq. 4.

$$CR = ADD \times CSF \qquad (4)$$

where CR = carcinogenic risk via ingestion of potential toxic metals. CSF = carcinogenic slope factor.

The carcinogenic slope factors were Pb (0.0085 mg/kg/day), Ni (1.7 mg/kg/day), As (1.5 mg/kg/day), and Cr (0.5 mg/kg/day) [29, 39, 42]. CR values above 10^{-6} to 10^{-4} indicate a possibility of having cancer over a 64.4 years lifetime.

2.6.5 Serial dilution and enumeration of total heterotrophic bacteria

From each of the samples (control, and the test samples from days 14, 28, and 42), 1 ml aliquot was used to perform

a ten-fold serial dilution as previously described [2]. From the stock solution, and the first dilution, the total heterotrophic bacteria count (THBC) was enumerated via pour plating using freshly prepared nutrient agar. The plates were incubated inverted at 37 °C for 24 h, and thereafter examined for the growth of distinct colonies as previously reported [2]. For the enumeration of the total coliform count (TCC), exactly 100 ml of the water samples from each exposure window was filtered via a vacuum pump, and the filter was placed on freshly prepared nutrient agar, and eosin methylene blue agar plates and then incubated at room temperature.

2.6.6 Cultural identification of the isolates

The resulting distinct colonies were purified twice via sub-culturing onto freshly prepared nutrient agar and then stocked in sterile Bijou sample bottles using nutrient agar slants. The resulting isolates were identified as previously described using a battery of biochemical, and cultural tests [43–46].

2.6.7 Primary screening for amylase and protease enzymes

The identified isolates were evaluated for their potential to elaborate amylase, and protease enzymes. Primary screening for amylase and protease enzymes was done using freshly prepared starch and milk agars. The starch agar was prepared by adding 1% soluble starch, and agar–agar powder (1.4 g/100 ml) to freshly prepared mineral salt medium (MSM) (100 ml of MSM). The MSM comprised 0.2 g $MgSO_4$, 1 g KH_2PO_4, 1 g K_2HPO4, 1 g NH_4NO_3, 0.02 g $CaCl_2$, and 0.05 g $FeCl_2$ dissolved in 1000 ml of sterile distilled water as previously described [47]. Using the same protocol, the milk agar was prepared, and used to evaluate protease elaboration among the isolates using 1 g of skimmed milk per 100 ml of MSM. Overnight cultures of the various isolates were transferred individually onto freshly prepared starch, and milk agar plates by streaking centrally to leave space around the streak line to allow for hydrolysis. The plates were then incubated inverted at 37 °C for 24 to 48 h, and then observed for halos around the line of streak that were measured and recorded. The starch agar plates were flooded gently with Gram's iodine solution (1 g of iodine and 2.0 g of potassium iodide in 100 ml of distilled water stored at room temperature), and observed for colour change. Unused starch reacts with the iodine to form the starch-iodine complex. Positive plates were indicated by the absence of a blue-black colouration around each isolate's line of streak. The zones were then described qualitatively, and quantitatively. The milk agar plates were observed for hydrolysis, measured, and recorded [48].

2.6.8 Antimicrobial sensitivity

The isolates were further screened to obtain their sensitivity patterns to various antibiotics using the disc diffusion method [49]. Briefly, overnight culture of the isolates was suspended in peptone water, and adjusted to MacFarland standard (approximately 10^8 cells/ml). Commercially available antibiotic discs containing varying concentrations of different antibiotics were placed at adequate distances on each of the seeded agar plates with the aid of sterile forceps under aseptic conditions. The antibiotics were OFX—Tarivid (10 µg), PEP – Reflacine (10 µg), CPX – Ciprofloxacin (10 µg), AU – Augmentin (30 µg), CN – Gentamycin (10 µg), SE – Streptomycin (30 µg), CEP – Ceporex (10 µg), NA – Nalidixic acid (30 µg), PN = Ampicillin (30 µg), and SXT – Septrin (30 µg). The plates were incubated for 24 h at 37 °C, and the resulting zones were measured, and interpreted as previously reported [49, 50].

2.7 Statistical analysis

The data obtained for potential toxic metals, and physicochemical parameters of sachet water before, and after exposure to sunlight passed the normality test. The potential toxic metals and physicochemical parameters data collected for the sachet were subjected to descriptive statistical analysis (Mean, standard deviation, and range). Analysis of variance (ANOVA) was used to determine the significant differences in the levels of potential toxic metals and physicochemical parameters of the sachet water between each duration of exposure compared to the control using Origin Lab Software (United State of America) at 0.05 level of significance, and their relevant degree of freedom. In addition, the microbial counts were transformed into a component bar chart. In addition, student t-test was also done to compare the mean values of the physicochemical parameters and that of the WHO limits for the control, and days (14, 28 and 42).

3 Results

3.1 Physicochemical parameters of sachet water

The summary of the physicochemical parameters of the sachet water before, and after exposure to sunlight is shown in Table 1. Temperature (°C) ranged from 25.50 to 25.83 for the control samples for the various brands, while after exposure, the temperatures for the various brands of sachet water changed, and these ranged from 27.17 to 29.17 for US for 14 to 42 days, while for AN and GP, the values were 26.67 to 28.33 °C. For pH, the control values were 6.65, 6.68, and 6.67 for US, AN, and GP sachet water

Table 1 Physicochemical parameter in sachet water before and after exposure to sunlight

Physico-chemical parameters	Brands	Control (0 days)	Exposure duration (days)			WHO [51]	NSDWQ [52]	NAFDAC [53]
			14	28	42			
Temperature (°C)	US	$25.83 \pm 0.75^{a*}$ (25.00–27.00)	$27.17 \pm 0.75^{b*}$ (26.00–28.00)	$28.17 \pm 0.75^{b*}$ (27.00–29.00)	$29.17 \pm 0.68^{b*}$ (28.00–30.00)			
	AN	25.50 ± 0.55^{a} (25.00–26.00)	26.50 ± 0.55^{b} (26.00–27.00)	27.67 ± 0.52^{b} (27.00–28.00)	28.33 ± 0.82^{b} (27.00–29.00)	< 40	NA	NA
	GP	25.83 ± 0.75^{a} (25.00–27.00)	26.67 ± 0.52^{a} (26.00–27.00)	27.67 ± 0.52^{b} (27.00–28.00)	28.33 ± 1.03^{b} (27.00–30.00)			
pH	US	6.65 ± 0.10^{a} (6.50–6.80)	6.41 ± 0.17^{a} (6.10–6.60)	5.82 ± 0.29^{b} (5.28–6.10)	5.45 ± 0.30^{b} (5.10–5.80)			
	AN	6.68 ± 0.16^{a} (6.50–6.90)	6.45 ± 0.29^{a} (6.00–6.80)	5.90 ± 0.24^{b} (5.60–6.20)	5.62 ± 0.29^{b} (5.20–6.00)	6.5–8.5	6.5–8.5	6.5–8.5
	GP	6.67 ± 0.16^{a} (6.50–6.90)	6.38 ± 0.16^{a} (6.20–6.60)	5.97 ± 0.30^{b} (5.40–6.20)	5.57 ± 0.34^{b} (5.00–5.90)			
Conductivity (µs/cm)	US	112.83 ± 28.72^{a} (90.00–170.00)	117.33 ± 27.14^{a} (98.00–72.00)	124.83 ± 29.98^{a} (102.00–185.00)	132.50 ± 28.43^{a} (115.00–190.00)			
	AN	56.70 ± 7.28^{a} (42.00–61.20)	63.83 ± 8.06^{a} (50.00–74.00)	67.83 ± 6.52^{b} (58.00–78.00)	71.16 ± 6.77^{b} (62.00–82.00)	250	NA	1000
	GP	83.58 ± 6.33^{a} (76.00–92.50)	87.50 ± 7.26^{a} (80.00–100.00)	92.17 ± 8.68^{a} (84.00–108.00)	98.67 ± 7.86^{b} (90.00–110.00)			
Turbidity (N.T.U)	US	0.89 ± 0.07^{a} (0.82–0.99)	1.30 ± 0.19^{b} (1.08–1.64)	1.43 ± 0.23^{b} (1.15–1.80)	1.85 ± 0.39^{b} (1.18–2.30)			
	AN	0.72 ± 0.06^{a} (0.62–0.79)	1.11 ± 0.11^{b} (1.02–1.26)	1.27 ± 0.14^{b} (1.10–1.46)	1.38 ± 0.19^{b} (1.15–1.64)	5.0	NA	NA
	GP	0.58 ± 0.11^{a} (0.41–0.71)	0.88 ± 0.23^{b} (0.68–1.32)	1.30 ± 0.10^{b} (1.20–1.45)	1.42 ± 0.15^{b} (1.26–1.61)			
Total dissolved solid (mg/l)	US	35.95 ± 6.39^{a} (31.60–48.60)	40.80 ± 5.78^{a} (33.40–50.20)	47.03 ± 4.85^{b} (40.00–51.20)	50.33 ± 4.63^{b} (44.00–56.00)			
	AN	33.60 ± 4.64^{a} (25.00–38.00)	40.73 ± 3.64^{b} (36.00–45.00)	44.67 ± 3.56^{b} (39.00–48.00)	51.17 ± 6.43^{b} (43.00–61.00)	500	500	500
	GP	51.02 ± 3.47^{a} (46.00–55.00)	56.77 ± 1.75^{b} (54.00–58.60)	62.83 ± 3.92^{b} (58.00–68.00)	67.83 ± 5.12^{b} (62.00–75.00)			
Hardness (mg/l)	US	14.60 ± 1.21^{a} (13.00–16.50)	17.17 ± 1.17^{a} (16.00–19.00)	20.03 ± 2.58^{b} (17.00–24.00)	26.17 ± 4.07^{b} (21.00–31.00)			
	AN	18.17 ± 4.26^{a} (12.00–24.00)	23.85 ± 5.47^{a} (17.10–31.00)	30.50 ± 9.27^{b} (19.00–46.00)	34.17 ± 9.60^{b} (20.00–48.00)	150	NA	500
	GP	19.00 ± 2.00^{a} (16.00–21.00)	21.95 ± 2.24^{a} (19.20–25.00)	24.67 ± 3.08^{b} (22.00–30.00)	30.00 ± 3.46^{b} (25.00–35.00)			

Values are in mean ± standard deviation; ranges in parenthesis (); Values with different superscript *, a and b for each exposure duration compared to the control (0 days exposure to sun) is significantly different ($p < 0.05$)

NA not available; *NSDQW* Nigeria standard for drinking water; *NAFDAC* National Agency for Food and Drug Administration and Control

*Student t-test that showed no significance between the control, and days 14, 28 and 42 with the WHO limits. Superscript

brands, while on exposure for 14 to 42 days, the values changed and ranged from 5.45 to 6.41, 5.62 to 6.45, and 5.57 to 6.38 for the various brands of sachet water. Conductivity (µs/cm) values for the various 112.83, 56.70, and 83.55, respectively, for US, AN, and GP. On exposure, the conductivity values changed and ranged from 1177.33 to 132.50, 63.63 to 71.16, and 87.50 to 98.67. The turbidity (N.T.U.) for the control for US, AN, and GP was 0.89, 0.72, and 0.58. As observed for pH, temperature, and conductivity, the values changed from 1.30 to 1.85, 1.11 to 1.35, and

0.88 to 1.43, respectively, for US, AN, and GP, respectively. For TDS and hardness, the values for control were 35.95, 33.60, and 51.02 mg/l, and 14.80, 18.17, and 19.00 mg/l, respectively, for US, AN, and GP. On exposure, the values ranged from 40.80 to 50.33, 40.73 to 51.17, and 56.77 to 67.83 mg/l for US, AN, and GP. On the other hand, after exposure, the values ranged from 17.17 to 26.17, 23.85 to 34.17, and 21.95 to 30.00 mg/l.

The values of the physicochemical parameters of the different brands (US, AN, and GP) of sachet water exposed

to sunlight increased linearly with the duration of exposure except for pH. Temperature, conductivity, turbidity, and TDS significantly increased in all three brands except for conductivity in the US brand. The levels of conductivity, and total hardness in all the brands of sachet water were lower than those of WHO, and NAFDAC acceptable limits while levels of TDS were lower than the WHO, NAFDAC, and the Nigeria Standard for Water Quality (NSDWQ) acceptable limits. On the other hand, pH significantly decreased in all sachet water brands (US, AN, and GP) after exposure to sunlight for 42 days ($p < 0.05$) compared to the control. Furthermore, the mean pH in all the sachet water brands exposed to sunlight for 14, 28 and 42 days were not within the WHO, NAFDAC, and NSDWQ acceptable limits for drinking water (Table 1). Similarly, student t-test revealed no significant difference ($p > 0.05$) between the physicochemical parameters of the control and on the various days) and WHO limits.

3.2 Potential toxic metals concentration in sachet water

Table 2 shows the levels of potential toxic metals in three brands (US, AN, and GP) of sachet water before and after exposure to sunlight. For US, AN, and GP sachet water samples, the concentration of Fe was the same (0.01 mg/L each). However, after exposure for days 14, 28, and 42, the values were higher and ranged from 0.12 to 0.24, 0.26 to 0.46 and 0.13, and 0.26 and 0.46 mg/L for US, AN, and GP sachet water samples. For Mn, the concentrations of the control for US, AN, and GP sachet water brands were below detection level (BDL), 0.01 and 0.07 mg/l. However, after exposure for days 14, 28, and 42, the concentration ranged from 0.02 to 0.15, 0.11 to 0.38, and 0.11 to 0.20 mg/L, respectively, for US, AN, and GP sachet water brands. The concentration of Cr ranged from BDL to 0.003 mg/L for days 14 to 42, while that of the control was BDL (US and AN) and 0.03 mg/L for GP. For Al, the concentrations were 0.01, 0.003, and BDL for the respective brands, while on days 14, 28, and 42 following exposures, the concentrations ranged from 0.003 to 0.05, 0.02 to 0.04, and 0.13 to 0.30 mg/L. For Cd and Zn, the control concentrations ranged from BDL to 0.002 and 1.33 to 1.96 mg/L, respectively, for the various brands. However, on exposure, the concentrations for US, AN, and GP brands increased and ranged from 0.01 to 0.04, 0.01 to 0.05, and 0.02 to 0.05 mg/L, and 1.50 to 2.70, 3.01 to 5.21 mg/L, and 3.57 to 5.84 mg/L for Cd and Zn, respectively. For Pb and Ni, the concentration range for the controls ranged from BDL to 0.002 and BDL to 0.01, while for As, its concentration was BDL. On exposure, the concentrations were 0.01 to 0.14, 0.11 to 0.27, and 0.03 to 0.17 mg/L for Pb for US, AN, and GP water brands, while for As, after exposure, the

concentration ranged from 0.01 to 0.04, 0.02 to 0.12, and 0.03 to 0.16 mg/L for US, AN, and GP brands. For Ni, after exposure, the concentration ranged from 0.04 to 0.12, 0.11 to 1.22, and 0.03 to 1.80 mg/L. Our findings indicate that Fe, Mn, Cr, Al, Cd, Zn, Pb, As, and Ni had the highest significant increment in all brands of sachet water exposed to sunlight for 42 days ($p < 0.05$) compared to the control. The levels of Fe and Zn in AN and GP sachet water brands exposed for 42 days, the level of Cr in GP sachet water exposed for 14, 28, 42 days and that of AN brand exposed for 42 days, the Cd, Pb, and As concentrations in all sachet water brands exposed for 14 days, 28 days, and 42 days, the Ni of US sachet water brand exposed for 42 days, and Ni levels in AN and GP sachet water brands exposed for 14, 28, and 42 days were all above the WHO acceptable limits for drinking water (Table 2). Some of the toxic metals failed to meet WHO, NSDWQ, and NAFDAC acceptable limits for drinking water after exposure to sunlight. For the AN brand, following 42 days of exposure, Fe and Zn failed the standards. Similarly, the levels of Cd, As, Ni, and Pb after exposure for 14, 28, and 42 days as well as the level of Ni after for 14- and 28-days exposure all failed the regulatory limits. For the GP brand, the levels of Cd and Ni following exposure for 14, 28, and 42 days, as well as Fe and Zn following 42 days of exposure failed to meet the safety limits. In the US brands, Cd and Ni levels after exposure for 14, 28, and 42 days, as well as Cr after 42 days of exposure, and the levels of Pb and As after exposure for 28 and 42 days all failed to meet the regulatory limits.

3.3 Health risk assessment of sachet water before and after exposure to sunlight

3.3.1 Average daily dose (ADD) of sachet water

The summary of the ADD of sachet water before and after exposure to sunlight is shown in Table 3. Our findings indicate that Zn had the highest ADD values in the US (7.71×10^{-2} mg/kg/day), AN (1.49×10^{-1} mg/kg/day), and GP (1.67×10^{-1} mg/kg/day) sachet water samples after exposure for 42 days. This was followed by the ADD values of Fe in the US (6.88×10^{-3} mg/kg/day) sachet water, Ni in AN (3.47×10^{-2} mg/kg/day), and GP (5.13×10^{-2} mg/kg/day) sachet waters after exposure to sunlight for 42 days. The ADD values of Cr in GP sachet water brand after exposure for 14, 28, and 42 days were above the Rfd value of 0.003. The ADD values of Cd in all sachet water brands after exposure for 28 (except US and AN sachet waters), and 42 days were above the Rfd value of 0.001. The ADD values of Pb in all sachet water brands exposed for 28 (except US and GP sachet waters), and 42 days were above the Rfd value of 0.0035. The ADD values of As in all sachet water brands after exposure for 14, 28, and 42 days were above

Table 2 Potential toxic metals concentration in sachet water before and after exposure to sunlight

Potential toxic metal (mg/L)	Brands	Control (0 days)	Exposure duration (days)			WHO [51]	NSDWQ [52]	NAFDAC[53]
			14	28	42			
Fe	US	0.01.±0.004[a] (BDL – 0.01)	0.12±0.05[b] (0.06 – 0.21)	0.19±0.09[b] (0.09 – 0.31)	0.24±0.10[b] (0.12 – 0.38)	0.30	NA	0.30
	AN	0.01±0.001[a] (0.01 – 0.01)	0.26±0.15[b] (0.09 – 0.51)	0.29±0.17[b] (0.15 – 0.61)	0.46±0.19[b] (0.22 – 0.78)			
	GP	0.01±0.001[a] (0.01 – 0.01)	0.13±0.12[a] (0.04 – 0.39)	0.26±0.13[b] (0.12 – 0.48)	0.46±0.14[b] (0.29 – 0.66)			
Mn	US	BDL[a]	0.02±0.01[a] (0.01 – 0.02)	0.05±0.02[b] (0.02 – 0.08)	0.15±0.03[b] (0.12 – 0.19)	0.40	NA	NA
	AN	0.01±0.003[a] (BDL – 0.02)	0.11±0.04[a] (0.08 – 0.18)	0.29±0.14[b] (0.11 – 0.51)	0.38±0.18[b] (0.21 – 0.70)			
	GP	0.07±0.03[a] (0.01 – 0.09)	0.11±0.02[a] (0.09 – 0.15)	0.17±0.04[b] (0.12 – 0.21)	0.20±0.05[b] (0.13 – 0.25)			
Cr	US	BDL[a]	0.01±0.007[b] (BDL – 0.02)	0.08±0.01[b] (0.01 – 0.04)	0.04±0.01[b] (0.02 – 0.05)	0.05	NA	NA
	AN	BDL[a]	0.02±0.01[b] (0.01 – 0.03)	0.04±0.01[b] (0.02 – 0.04)	0.06±0.01[b] (0.04 – 0.08)			
	GP	0.003±0.001[a] (BDL – 0.01)	0.14±0.07[b] (0.08 – 0.28)	0.23±0.15[b] (0.11 – 0.50)	0.39±0.12[b] (0.22 – 0.58)			
Al	US	0.01±0.005[a] (BDL – 0.01)	0.03±0.01[b] (0.02 – 0.03)	0.05±0.01[b] (0.03 – 0.06)	0.05±0.01[b] (0.04 – 0.06)	1.0	NA	NA
	AN	0.003±0.001[a] (BDL – 0.01)	0.02±0.01[b] (0.01 – 0.02)	0.03±0.01[b] (0.02 – 0.04)	0.04±0.01[b] (0.03 – 0.05)			
	GP	BDL[a]	0.13±0.03[b] (0.08 – 0.16)	0.18±0.10[b] (0.09 – 0.37)	0.30±0.11[b] (0.16 – 0.46)			
Cd	US	BDL[a]	0.01±0.002[a] (0.001 – 0.01)	0.02±0.01[b] (0.01 – 0.03)	0.04±0.01[b] (0.003 – 0.05)	0.003	NA	0.03
	AN	BDL[a]	0.01±0.003[a] (0.003 – 0.01)	0.02±0.01[b] (0.01 – 0.04)	0.05±0.02[b] (0.04 – 0.06)			
	GP	0.002±0.001[a] (BDL – 0.01)	0.02±0.01[b] (0.01 – 0.03)	0.04±0.01[b] (0.03 – 0.05)	0.05±0.01[b] (0.04 – 0.06)			
Zn	US	1.33±0.02[a] (1.08 – 1.65)	1.50±0.22[a] (1.29 – 1.86)	2.18±0.14[b] (2.04 – 2.44)	2.70±0.50[b] (2.16 – 3.22)	5.0	NA	5.0
	AN	1.54±0.54[a] (1.02 – 2.32)	3.01±1.73[a] (1.33 – 6.21)	4.61±1.88[b] (2.01 – 7.02)	5.21±2.07[b] (3.18 – 8.66)			
	GP	1.96±0.75[a] (1.28 – 3.15)	3.57±1.49[b] (2.00 – 5.90)	4.93±1.20[b] (3.00 – 6.32)	5.84±1.18[b] (4.00 – 7.05)			
Pb	US	BDL[a]	0.01±0.005[a] (0.01 – 0.02)	0.04±0.01[b] (0.03 – 0.05)	0.14±0.06[b] (0.05 – 0.19)	0.01	0.01	0.002
	AN	0.002±0.001[a] (BDL – 0.01)	0.11±0.04[b] (0.08 – 0.16)	0.17±0.06[b] (0.10 – 0.24)	0.27±0.06[b] (0.18 – 0.33)			
	GP	BDL[a]	0.03±0.02[b] (0.01 – 0.06)	0.10±0.02[b] (0.08 – 0.14)	0.17±0.03[b] (0.12 – 0.21)			
As	US	BDL[a]	0.01±0.005[a] (0.01 – 0.02)	0.03±0.01[a] (0.01 – 0.04)	0.04±0.01[b] (0.03 – 0.05)	0.01	NA	NA
	AN	BDL[a]	0.02±0.004[a] (0.02 – 0.03)	0.04±0.01[b] (0.03 – 0.04)	0.12±0.09[b] (0.04 – 0.28)			
	GP	BDL[a]	0.03±0.01[a] (0.02 – 0.03)	0.06±0.02[b] (0.04 – 0.08)	0.16±0.06[b] (0.05 – 0.22)			
Ni	US	BDL[a]	0.04±0.01[a] (0.01 – 0.16)	0.07±0.06[b] (0.02 – 0.18)	0.12±0.07[b] (0.06 – 0.25)	0.07	NA	NA
	AN	0.01±0.002[a] (BDL – 0.01)	0.11±0.03[b] (0.09 – 0.16)	1.13±0.07[b] (1.06 – 1.22)	1.22±0.09[b] (1.12 – 1.38)			
	GP	0.01±0.005[a] (BDL – 0.04)	0.03±0.02[a] (0.09 – 1.08)	1.57±0.37[b] (1.20 – 2.00)	1.80±0.29[b] (1.40 – 2.13)			

Values are in mean±standard deviation; ranges in parenthesis (); Values with different superscript for compared to the control is significantly different (p < 0.05); BDL – below detectable limit

NA Not available; *NSDQW* Nigeria Standard for Drinking Water; *NAFDAC* National Agency for Food and Drug Administration and Control

Table 3 Average daily dose (ADD) of sachet water before and after exposure to sunlight

Potential toxic metal	Brands	Control (0 days)	Exposure duration (days)			Rfd
			14	28	42	
	US	1.43×10^{-4}	3.37×10^{-3}	5.43×10^{-3}	6.88×10^{-3}	
Fe	AN	2.86×10^{-4}	7.28×10^{-3}	8.37×10^{-3}	1.31×10^{-2}	0.07
	GP	2.86×10^{-4}	3.71×10^{-3}	7.43×10^{-3}	1.30×10^{-2}	
	US	–	4.28×10^{-4}	1.37×10^{-3}	4.34×10^{-3}	
Mn	AN	1.71×10^{-4}	3.17×10^{-3}	8.28×10^{-3}	1.09×10^{-2}	0.14
	GP	1.94×10^{-3}	3.20×10^{-3}	4.88×10^{-3}	5.66×10^{-3}	
	US	–	3.43×10^{-4}	7.71×10^{-4}	1.06×10^{-3}	
Cr	AN	–	5.14×10^{-4}	1.00×10^{-3}	1.66×10^{-3}	0.003
	GP	8.57×10^{-5}	$\mathbf{4.00 \times 10^{-3}}$	$\mathbf{6.66 \times 10^{-3}}$	$\mathbf{1.11 \times 10^{-2}}$	
	US	2.00×10^{-4}	7.14×10^{-4}	1.28×10^{-3}	1.43×10^{-3}	
Al	AN	8.57×10^{-5}	4.57×10^{-4}	9.14×10^{-4}	1.14×10^{-3}	7
	GP	–	3.57×10^{-3}	5.06×10^{-3}	8.66×10^{-3}	
	US	–	1.43×10^{-4}	5.71×10^{-4}	$\mathbf{1.23 \times 10^{-3}}$	
Cd	AN	–	2.00×10^{-4}	6.00×10^{-4}	$\mathbf{1.51 \times 10^{-3}}$	0.001
	GP	5.71×10^{-5}	5.14×10^{-4}	$\mathbf{1.06 \times 10^{-3}}$	$\mathbf{1.48 \times 10^{-3}}$	
	US	3.80×10^{-2}	4.28×10^{-2}	6.22×10^{-2}	7.71×10^{-2}	
Zn	AN	4.40×10^{-2}	8.59×10^{-2}	1.32×10^{-1}	1.49×10^{-1}	0.3
	GP	5.59×10^{-2}	1.02×10^{-1}	1.41×10^{-1}	1.67×10^{-1}	
	US	–	3.71×10^{-4}	1.14×10^{-3}	$\mathbf{3.86 \times 10^{-3}}$	
Pb	AN	5.71×10^{-5}	3.23×10^{-3}	$\mathbf{4.88 \times 10^{-3}}$	$\mathbf{7.71 \times 10^{-3}}$	0.0035
	GP	–	9.14×10^{-4}	2.91×10^{-3}	$\mathbf{4.77 \times 10^{-3}}$	
	US	–	$\mathbf{3.71 \times 10^{-4}}$	$\mathbf{7.71 \times 10^{-4}}$	$\mathbf{1.14 \times 10^{-3}}$	
As	AN	–	$\mathbf{6.28 \times 10^{-4}}$	$\mathbf{1.00 \times 10^{-3}}$	$\mathbf{3.40 \times 10^{-3}}$	0.0003
	GP	–	$\mathbf{7.14 \times 10^{-4}}$	$\mathbf{1.80 \times 10^{-3}}$	$\mathbf{4.60 \times 10^{-3}}$	
	US	–	1.08×10^{-3}	2.00×10^{-3}	3.28×10^{-3}	
Ni	AN	1.43×10^{-4}	3.20×10^{-3}	$\mathbf{3.23 \times 10^{-2}}$	$\mathbf{3.47 \times 10^{-2}}$	0.02
	GP	2.86×10^{-4}	7.94×10^{-3}	$\mathbf{4.48 \times 10^{-2}}$	$\mathbf{5.13 \times 10^{-2}}$	

Bold ADD values indicates health danger

the Rfd values of 0.0003. The ADD values of Ni in AN, and GP sachet water brands after exposure for 28 and 42 days were above the Rfd value of 0.02 (Table 3).

3.3.2 Hazard quotient (HQ) of sachet water

The summary of the HQ of sachet water before and after exposure to sunlight is shown in Table 4. The result indicates that As had the highest HQ values in the US (3.80×10^{0}), AN (11.33×10^{0}), and GP (15.33×10^{0}) sachet waters after exposure to sunlight for 42 days, followed by the HQ values of Cd in US (1.23×10^{0}) sachet water, Pb in AN (2.20×10^{0}), and GP (3.70×10^{0}) sachet waters after exposure to sunlight for 42 days. The HQ values of Cr in GP sachet water brand after exposure for 14, 28, and 42 days, HQ values of Cd in all sachet water brands after exposure for 28 (except US and AN sachet waters) and 42 days, HQ values of Pb in all sachet water brands after exposure for 28 days (except US, and GP sachet waters) were all greater than 1 (Table 4).

3.3.3 Hazard index (HI) of sachet water

The summary of the HI of sachet water before and after exposure to sunlight is shown in Table 5. The result indicates that the HI of the US (1.85×10^{0}), AN (3.96×10^{0}), and GP (5.29×10^{0}) sachet water brands after exposure for 14 days, HI of the US (4.82×10^{0}), AN (7.88×10^{0}), and GP (12.96×10^{0}) sachet water brands after exposure for 28 days, and HI of US (7.03×10^{0}), AN (18.08×10^{0}), and GP (25.21×10^{0}) sachet waters after exposure for 42 days were greater than 1 (Table 5).

Table 4 Hazard quotient (HQ) of sachet water before and after exposure to sunlight

Potential toxic metals	Brands	Control	Exposure duration (days)		
			14	28	42
	US	2.04×10^{-3}	4.81×10^{-2}	7.76×10^{-1}	9.83×10^{-2}
Fe	AN	4.08×10^{-3}	1.04×10^{-1}	1.19×10^{-1}	1.87×10^{-1}
	GP	4.08×10^{-0}	5.30×10^{-2}	1.06×10^{-1}	1.86×10^{-1}
	US	–	3.06×10^{-3}	9.78×10^{-3}	3.10×10^{-2}
Mn	AN	1.22×10^{-3}	2.26×10^{-2}	5.91×10^{-2}	7.78×10^{-2}
	GP	1.38×10^{-2}	2.28×10^{-2}	3.48×10^{-2}	4.04×10^{-2}
	US	–	1.14×10^{-1}	2.57×10^{-1}	3.53×10^{-1}
Cr	AN	–	1.71×10^{-1}	3.33×10^{-1}	5.53×10^{-1}
	GP	2.86×10^{-2}	**1.33×10^{0}**	2.22×10^{0}	**3.70×10^{0}**
	US	2.86×10^{-5}	1.02×10^{-4}	1.83×10^{-4}	2.04×10^{-4}
Al	AN	1.22×10^{-5}	6.53×10^{-5}	1.30×10^{-4}	1.63×10^{-4}
	GP	–	5.10×10^{-4}	7.23×10^{-4}	1.24×10^{-3}
	US	–	1.43×10^{-1}	5.71×10^{-1}	**1.23×10^{0}**
Cd	AN	–	2.00×10^{-1}	6.00×10^{-1}	**1.51×10^{0}**
	GP	5.71×10^{-2}	5.14×10^{-1}	1.06×10^{0}	**1.48×10^{0}**
	US	1.27×10^{-1}	1.43×10^{-1}	2.07×10^{-1}	2.57×10^{-1}
Zn	AN	1.47×10^{-1}	2.86×10^{-1}	4.40×10^{-1}	4.97×10^{-1}
	GP	1.86×10^{-1}	3.40×10^{-1}	4.70×10^{-1}	5.57×10^{-1}
	US	–	1.06×10^{-1}	3.26×10^{-1}	**1.10×10^{0}**
Pb	AN	1.63×10^{-2}	9.23×10^{-1}	**1.39×10^{0}**	**2.20×10^{0}**
	GP	–	2.61×10^{-1}	8.31×10^{-1}	**1.36×10^{0}**
	US	–	**1.24×10^{0}**	**2.57×10^{0}**	**3.80×10^{0}**
As	AN	–	**2.09×10^{0}**	**3.33×10^{0}**	**11.33×10^{0}**
	GP	–	**2.38×10^{0}**	**6.00×10^{0}**	**15.33×10^{0}**
	US	–	5.40×10^{2}	1.00×10^{-1}	1.64×10^{-1}
Ni	AN	7.15×10^{-3}	1.60×10^{-1}	**1.61×10^{0}**	**1.73×10^{0}**
	GP	1.43×10^{-2}	3.97×10^{-1}	**2.24×10^{0}**	**2.56×10^{0}**

Bold THQ values indicate health danger

Table 5 Hazard index (HI) of sachet water before and after exposure to sunlight

Brands	Control (day 0)	Duration of exposure (days)		
		14	28	42
US	1.29×10^{-1}	**1.85×10^{0}**	**4.82×10^{0}**	**7.03×10^{0}**
AN	1.76×10^{-1}	**3.96×10^{0}**	**7.88×10^{0}**	**18.08×10^{0}**
GP	3.04×10^{-1}	**5.29×10^{0}**	**12.96×10^{0}**	**25.21×10^{0}**

Bold HI values indicates health danger

3.3.4 Carcinogenic risk (CR) of sachet water

The summary of the CR of the sachet water before and after exposure to sunlight is shown in Table 6. Ni had the highest CR values in the US (5.58×10^{-3}), AN (5.89×10^{-2}), and GP (8.79×10^{-2}) sachet water brands after exposure to sunlight for 42 days, followed by CR values of As in US

(1.71×10^{-3}), AN (5.10×10^{-3}), and GP (6.90×10^{-3}) sachet water brands after exposure to sunlight for 42 days.

3.3.5 Microbiological analyses

Figure 2 shows the results of the THBC, and TCC for the various samples. The result showed an increase in the THBC and TCC as days of exposure increased. For US sachet water, the THBC increased from 4 to 129 cfu/ml from before and after exposure to sunlight while for AN sachet water, the THBC ranged from 7 to 154 cfu/ml. For GP sachet water, the THBC. 5 to 123 cfu/ml before and after exposure to sunlight The TCC values were 8, 9 and 11 respectively for US, AN, and GP after exposure to sunlight. The isolates were further subjected to primary screening for amylase, and protease enzymes, and the result is presented in Table 7.

Table 7 shows the results of the screening of the various isolates for amylase, and protease enzymes. The zones

Table 6 Carcinogenic risk (CR) of sachet water before and after exposure to sunlight

Potential toxic metals	Brands	Control (Day 0)	Duration of exposure (days)		
			14	28	44
Cr	US	–	1.71×10^{-4}	3.85×10^{-4}	5.30×10^{-4}
	AN	–	2.57×10^{-4}	5.00×10^{-4}	8.30×10^{-4}
	GP	4.28×10^{-5}	2.00×10^{-3}	3.33×10^{-3}	5.55×10^{-3}
Pb	US	–	3.15×10^{-6}	9.69×10^{-6}	3.28×10^{-5}
	AN	4.85×10^{-7}	8.50×10^{-5}	4.15×10^{-5}	6.55×10^{-5}
	GP	–	7.77×10^{-6}	2.47×10^{-5}	4.05×10^{-5}
As	US	–	5.56×10^{-4}	1.16×10^{-3}	1.71×10^{-3}
	AN	–	9.42×10^{-4}	1.50×10^{-3}	5.10×10^{-3}
	GP	–	1.07×10^{-3}	2.70×10^{-3}	6.90×10^{-3}
Ni	US	–	1.84×10^{-3}	3.40×10^{-3}	5.58×10^{-3}
	AN	2.43×10^{-5}	5.44×10^{-3}	5.49×10^{-2}	5.89×10^{-2}
	GP	4.86×10^{-5}	1.35×10^{-2}	7.62×10^{-2}	8.72×10^{-2}

Bold CR values indicates health danger
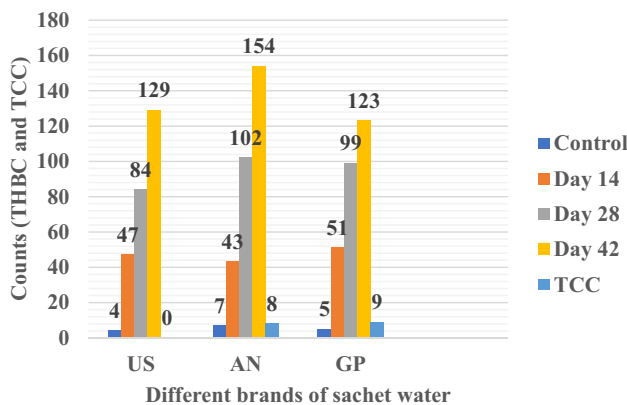
Fig. 2 Total heterotrophic bacteria count (THBC) and TCC from the various sachet water samples

Table 7 Primary screening of the isolates for amylase and protease

Probable isolates	Amylase		Protease	
	Qualitative	Quantitative (mm)	Qualitative	Quantitative (mm)
Providencia spp (n = 2)	+	14.0	+	15.0
Pseudomonas spp	–	–	+	16.5
Citrobacter fruendi	+	13.5	+	15.0
Escherichia coli	–	–	+	16.0
Staphylococcus aureus	+	12.0	+	13.5
Enterobacteria faecalis	–	–	+	14.0
Salmonella spp	+	14.5	+	15.0
Providencia spp	+	14.0	+	17.0
Shigella spp	+	13.0	+	14.0

+ under amylase = positive result after 24 h; + under protease = positive isolates 48 h of incubation

of hydrolysis for the various isolates for amylase ranged from 12.5 to 14.5 mm after 24 h of incubation. Not all the isolates were amylase positive, in contrast to the protease that were positive after 48 h of incubation (at a range of 13.5 to 17.0 mm). The isolates were *Providencia* spp (n = 2), *Pseudomonas* spp, *Citrobacter fruendi*, *Esherichia coli*, *Staphylococcus aureus*, *Enterobacter faecalis*, *Salmonella* spp, and *Shigella* spp. Table 8 shows the result of the antibiotic sensitivity of the test organisms. All the isolates showed multidrug resistance, that is, resistance to at least two antibiotics except for *S. aureus*.

4 Discussion

4.1 Physicochemical parameters of sachet water exposed to sunlight

There was an increase in physicochemical parameters of the sachet water after exposure to sunlight compared to before exposure (control). Increase in temperature, conductivity, turbidity, TDS, hardness, and not pH level was observed in all three brands of the sachet water (US, AN, and GP). The increase was dependent on the duration of exposure to sunlight. Similar findings were recorded by Muhamad et al. [28], Janan et al. [54],

Table 8 Antibiotic sensitivity profile of test organisms

Isolates	Antibiotics/interpretations of zones									
	OFX	NA	PEF	CN	AU	CPX	SXT	SE	PN	CEP
Providencia spp	S	R	S	S	S	S	S	R	R	S
Pseudomonas spp	S	S	S	R	R	R	S	S	S	S
Citrobacter fruendi	S	S	S	S	S	R	R	S	S	R
Escherichia coli	R	S	S	R	S	S	R	S	S	S
Staphylococcus aureus	S	S	S	S	R	S	S	S	S	S
Enterobacteria faecalis	R	S	R	S	R	S	S	R	S	R
Salmonella spp	R	S	R	S	S	S	S	S	S	R
Providencia spp	S	S	R	S	S	S	R	S	S	S
Shigella spp	S	S	S	R	R	S	S	S	S	S

R Resistant, *S* Sensitive, *OFX* Tarivid, *NA* Nalidixic acid, *PEF* Reflacine, *CN* Gentamycin, *AU* Augmentin, *CPX* Ciprofloxacin, *SXT* Septrin, *SE* Streptomycin, *PN* Ampicillin, *CEP* Ceporex

Chinenye and Amos [18] and Danopoulos et al. [20] who all reported an increase in physicochemical parameters of sachet water after exposure to sunlight over time. The increased levels of conductivity, turbidity, TDS, and hardness in sachet water expose to sunlight could be attributed to the leaching of the chemical components of the plastic package material into the water [6, 19, 20, 25]. Furthermore, temperature, conductivity, turbidity, TDS, and hardness had the highest significant increment in all brands (except for conductivity in the US brand), while pH had the highest significant decrease in all sachet water brands (US, AN, and GP) after exposure to sunlight for 42 days (p < 0.05) compared to the control. This denotes that the duration of exposure of the sachet water influenced the quantity and number of chemicals being leached into the water, thereby making the sachet water after exposure for 42 days to record the highest significant increase in temperature, conductivity, turbidity, TDS, hardness, and not the pH level [18]. Also, the increase in temperature of the three brands of sachet water after exposure to sunlight have been linked to increased microbial load of the water, thereby increasing turbidity of the sachet water [18]. Increased turbidity level of this study caused by sunlight reduces the amount of light penetrating the water, possibly making solar disinfection ineffective [54].

Meanwhile, the level of the pH of all sachet water brands after exposure to sunlight were not within the WHO [51], NAFDAC [53], and NSDWQ [53] acceptable limits for drinking water. More so, the pH observed for the three brand of sachet water decreased with duration of exposure probably due to the plastic (polyethene) package containing acidic chemical components which potentially lowered the pH and acidity of the water. The levels of pH in the sachet water (US, AN, GP) after exposure to sunlight in the present study were lower than the findings reported by Chinenye and Amos [18] for the effect of

sunlight exposure on water quality, Onosakponame [19] for the effect of sunlight on sachet water quality, Okeola et al. [55] for the stability of packaged water produced in Ilorin (Nigeria), Adedire et al. [17] for the effect of direct sunlight on the chemical properties of sachet water, and Bolawa and Adelusi [7] for the water quality of sachet water obtained from Markets in Lagos State, Nigeria.

The levels of temperature in all sachet water brands exposed to sunlight were lower than the findings of Okeola et al. [55]. The mean conductivity in all sachet water brands after exposure to sunlight was lower than the findings of Akharame et al. [1] and Okeola et al. [55] for the effect of sunlight exposure on the quality of water parameters in bottled water. The mean turbidity in all brands of the after exposure to sunlight sachet water in the present study was lower than the findings of Chinenye and Amos [18] and Okeola et al. [55]. The mean TDS in all brands of the exposed sachet water in the present study was higher than the findings of Chinenye and Amos [18], Okeola et al. [55] and Adedire et al. [17]. The mean hardness in the present study was lower than the findings of Chinenye and Amos [18] and Okeola et al. [54], but higher than the findings of Adedire et al. [17]. The differences in the mean of the physicochemical parameters for all sachet water brands with the other studies compared could be due to the differences in the duration of exposure to sunlight, geographical region, temperature, the water quality of the water before exposure to sunlight, level of photochemical reactions induced by sunlight [6, 17], level of fragmentation of polyethene materials of the plastic pack [25], and the leaching level of the chemical components of the sachet water pack into the water [1, 3, 17, 19].

4.2 Potential toxic metals concentration of sachet water exposed to sunlight

With the long-term effect of drinking contaminated water already well known around the world [1, 3, 8], our study provides evidence regarding the contamination of sachet water by potential toxic metals leached from its plastic pack and potentially leading to serious health issues to consumers over time [19]. This potential health disaster is an issue of immense public health concern, especially in developing countries where sachet water is considered the cheapest, and most accessible source of potable water [12]. To ensure that the United Nation's sustainable development goal of good health and well-being is maintained, our study investigated for the first time, the possible health implications (ADD, HQ, HI, and CR) of ingesting potential toxic metals leached from the plastics package materials of sachet water into the water when exposed to sunlight.

In all brands of the sachet water (US, AN, and GP) after exposure to sunlight, the mean potential toxic metals concentration (Fe, Mn, Cr, Al, Cd, Zn, Pb, As, and Ni) increased with duration of exposure to sunlight when compared with the unexposed group. This was in line with the observations of Akharame et al. [1], Adedire et al. [17], and Okeola et al. [55], who reported increased levels of toxic metals in packed water when exposed to sunlight. The increased toxic metal levels in the sachet waters exposed to sunlight compared to the unexposed group (control) could be due to the fragmentation of polyethene materials of the sachet water, thereby leaching its chemical components such as potential toxic metals into the sachet water [25]. Our study revealed that the levels of potential toxic metals in the different brands (US, AN, and GP) of sachet water exposed to sunlight increased with level of exposure. Precisely, Fe, Mn, Cr, Al, Cd, Zn, Pb, As, and Ni had the highest significant increase in all brands of sachet water exposed to sunlight for 42 days ($p < 0.05$) compared with the control. This implies that exposure duration to sunlight influenced the fragmentation of the plastics, and subsequent leaching rate of potential toxic metals into the water, thus resulting in increased potential toxic metals concentration in the sachet water group after exposure for 42 days, and this conformed with the findings of Akharame et al. [1], Adedire et al. [17], and Okeola et al. [55].

The mean Fe concentration in sachet water of all exposed brands was higher than the findings of Adedire et al. [17] for the effect of sunlight on the chemical properties of sachet water, and Bolawa and Adelusi [7] for potential toxic metal levels in sachet water collected from Lagos Markets (Nigeria), but lower than the findings of Okeola et al. [55] for the stability of packaged water in Ilorin (Nigeria). The mean Mn concentration in the present study was lower than the findings of Bolawa and

Adelusi [7]. The mean concentration of Cr in the sachet water exposed to sunlight in the present study was lower than the findings of Okeola et al. [55], but higher than the findings of Bolawa and Adelusi [7]. The mean concentrations of Al and Cd were higher, while the mean concentration of Zn was lower than the findings of Bolawa and Adelusi [7]. The mean concentration of Pb in the exposed sachet water was lower than the report of Okeola et al. [55], and higher than the values reported by Bolawa and Adelusi [7]. The mean As concentration in the sachet water exposed to sunlight was higher than the values reported by Akharame et al. [1] in the impact of sunlight exposure on the potential toxic metal levels of plastic packaged water. Finally, the mean concentration of potential toxic metals in sachet water of all brands exposed to sunlight was higher than the findings of Bolawa and Adelusi [7]. The variances in the mean levels of potential toxic metals in all sachet water brands exposed to sunlight compared to the aforementioned studies could be due to the differences in the duration of exposure to sunlight, the temperature of the region, geographical area, quality of the water before sunlight exposure, level of the sunlight induced photochemical reactions [6, 17], level of fragmentation of polyethene materials of the plastic package [25], and the leaching level of the chemical components of the sachet water pack into the water [1, 3, 17, 19].

As noted earlier, some of the toxic metals failed to meet the WHO, NSDWQ, and NAFDAC acceptable limits for drinking water following various durations of exposure. This denotes that the exposure of the sachet water of all brands to sunlight for the aforementioned exposure duration played a significant role in the leaching of the chemical components of the plastic package of the sachet water into the water, thereby contaminating the water, and making it unsafe for human consumption. With the importance of towing the United Nation's goal of good health and well-being, the exposure of sachet water to sunlight is an issue of global concern, especially as it is well documented that the water for human consumption must be free from organisms and chemical substances that may affect human health [1, 3]. As mentioned previously, sachet water has a specific temperature with which it must be kept and failure to do so could cause contamination and subsequent health challenges such as diarrhoea, cholera, dysentery, typhoid fever, legionnaire's disease, and parasitic diseases [18]. This could turn out to be the reality of consumers of sachet water poorly handled via exposure to sunlight over time. The ingestion of unsafe concentrations of Fe from drinking water after exposure to sunlight over time could cause stomach and intestinal side effects such as nausea, and vomiting, while Zn poisoning could result in indigestion, diarrhoea, headache, nausea, and vomiting [56]. The ingestion of unsafe levels of Cr

could cause nose and skin irritations, nose ulcer, running nose, asthma, cough, liver, and kidney damage among the consuming cohort. Also, Cd poisoning could result in cardiovascular diseases, arthritis, osteoporosis, growth impairment, cancer, osteomalacia, pneumonitis, and learning disorder [56]. Poisoning from excessive Pb ingestion could cause neuro-developmental effects, impaired renal function, cardiovascular disease, hypertension, impaired fertility, and anaemia while Ni poisoning could cause dermatitis, encephalopathy, and reduced sperm count [56]. Poisoning from excessive As ingestion could cause cancer, skin lesions, cardiovascular disease, and diabetes [56]. However, although safe concentrations of Mn, and Al were recorded in all brands of sachet water exposed to sunlight, it could still build up over time, and possibly cause health concerns to the consuming public. As a result, it is pertinent that the practice of local stores leaving sachet water under sunlight before being sold should be avoided to ensure, and preserve the quality of sachet water for human consumption.

4.3 Health risk assessment from drinking sachet water before and after exposure to sunlight

Health risk assessment is a reliable tool for exploring the potential health risk of ingesting potential toxic metals from drinking water. This reliable method was effectively utilized in this study to investigate the possible health risk of ingesting the potential toxic metals leached from sachet water exposed to sunlight over time. Globally, these health risk evaluation approaches have been used to great effect by several researchers to reveal the health risks inherent in drinking contaminated water [8, 26–30]. The present study revealed that Zn had the highest ADD values in all the brands of sachet waters exposed to sunlight for 42 days, implying that Zn was the most ingested toxic metal when drinking water exposed to sunlight for 42 days, though Zn did not pose any health dangers due to its high Rfd value. Furthermore, the ADD values of Cr in GP sachet water brand after exposure for 14, 28, and 42 days were above the Rfd value of 0.003, ADD values of Cd in all sachet water brands exposed for 28 (except US, and AN brands), and 42 days were above the Rfd value of 0.001, ADD values of Pb in all sachet water brands after exposure for 28 (except US and GP sachet waters) and 42 days were above the Rfd value of 0.0035, ADD values of As in all sachet water brands after exposure for 14, 28, and 42 days were above the Rfd values of 0.0003, and ADD values of Ni in AN and GP sachet water brands following exposure for 28 and 42 days were above the Rfd value of 0.02. This denotes that individuals drinking the sachet water exposed to sunlight for different aforementioned durations could be exposed to Cr, Cd, Pb,

As, and Ni poisoning over time, depending on the respective water brand, and exposure duration.

According to the HQ evaluation, As had the highest HQ values in US, AN, and GP sachet waters exposed to sunlight for 42 days, indicating that As posed the highest risk in all sachet water brands exposed to sunlight for 42 days. Furthermore, the HQ values of Cr in GP sachet water brand after exposure for 14, 28, and 42 days, HQ values of Cd in all sachet water brands exposed for 28 days (except US, and AN sachet waters) and 42 days, HQ values of Pb in all sachet water brands exposed for 28 days (except US and GP sachet waters) were all greater than 1. This collectively resulted in the HI values of US, AN, and GP sachet waters exposed for 14 days, HI of US, AN, and GP sachet water after exposure for 28 days, and HI of US, AN, and GP sachet waters exposed for 42 days being greater than 1. This denotes that the consumption of all sachet water following exposure to sunlight for 14, 28, and 48 days could cause Cr, Cd, Pb, As, and Ni toxicity in the long-term. Furthermore, Ni had the highest CR values in US, AN, and GP sachet water brands after exposure to sunlight for 42 days, which is an indication that Ni was the major carcinogenic source in all brands of sachet water following exposure to sunlight for 42 days. Furthermore, the CR of Cr, As, and Ni in all sachet water brands after exposure to sunlight for 14, 28, and 42 days were above the safe value of 10^{-4}, denoting a likelihood of the population having cancer after over 60 years of exposure.

The higher ADD, HQ, HI, and CR values in all brands of sachet water after exposure to sunlight compared to the unexposed group (control), indicate possible health consequences from unsafe ingestion of Cr, Cd, Pb, As, and Ni, and the impending carcinogenic dangers of Ni (as shown by its CR values) in sachet water stemmed from the leaching of potential toxic metals from plastic packs of the sachet water into the water. Although the ADD, HQ, HI, and CR values of Fe, Zn, Mn, and Al in the exposed sachet water were deemed inconsequential and safe, they could build over time and generate cumulative long-term health worries to humans.

4.4 Microbiological quality of the potable sachet water before and after exposure to sunlight

According to the WHO [4], water meant for consumption irrespective of the form should be free of coliforms, and the total number of total heterotrophic bacteria in packaged water should not exceed 50 cfu/ml. According to Udoh et al. [57], sachet water is widely consumed in Nigeria, and the whole of West Africa, and about 18% of households in Nigeria utilize sachet water as their primary source of drinking water. Microbial contamination of sachet water has been reported not just in Nigeria, but

across West Africa [57–60]. We evaluated three brands of sachet water for various microbiological parameters, and antimicrobial sensitivity profiling of the resulting isolates. Our findings indicate that all the sachet water brands failed to meet the WHO guidelines, and this is in line with several reports [57–60].

In an earlier study, several microbial species were obtained from ten (10) sachet water brands sold on a university campus in Enugu State [58]. These isolates were *E. coli, Streptococcus, Micrococcus sp., Bacillus sp., Klebsiella sp.* And *Pseudomonas aeruginosa* among others. In line with our findings, *E. coli*, and *Pseudomonas* were also isolated in our study. Mosi et al. [59] evaluated sachet water from 41 communities in Ghana, and they observed that a total of 24 samples were positive for total coliforms out of which 7 were positive for faecal *E. coli*. Compared to our sachet water sample, the presence of *E. coli*, and total coliform were also observed in our study. Our finding was also consistent with those of Ohanu et al. [60] for sachet water, where *E. coli*, and *S. faecalis* were isolated with colony forming units (CFU) ranging from 7 to > 500. Furthermore, our findings also corroborate those of Udoh et al. [57] who reported a microbial prevalence rate of 53.27% (n = 52) for their samples indicative of microbial contamination. Among the fecal contaminants reported by Udoh et al. [57], *E. coli* was the most prevalent with a prevalence rate of 13.30% and other microbial contaminants were *P. aeruginosa*, *Klebsiella sp.*, and *Enterococcus faecalis*. The observed presence of total coliform in our study and the aforementioned studies is an indication of one thing, and that is, the failure of the treatment processes utilised in the manufacture of sachet water in the various study areas. This position is in line with Mosi et al. [59] and Adesakin et al. [61], who posited that contamination observed in their study could be a result of the poor sanitary conditions, and non-adherence to good manufacturing practices during the production process. The failure of our control samples to meet the TCC and THBC safety limits confirms the fact that good manufacturing protocols were not strictly adhered to in the production processes. Also, on handling the sachet water samples, the TCC as well as the THBC counts increased across all samples from days 0 to 42. This suggests that poor handling as often the case with vendors that sell sachet water is also responsible for the increased microbial load observed in our study, and this explain the diversity of microbial species observed in sachet water samples sold across various regions and States in Nigeria.

From a public health point of view, the non-pathogens observed in the various studies, and our study, may not constitute much of a concern like the regular pathogens, however, for immunocompromised persons, this could to opportunistic infections [61, 62]. Furthermore, the isolates obtained in our study were largely Gram-negative and these included *E. coli, Salmonella* spp, *Shigella* spp and *Enterococcus* spp among others. These isolates have been implicated in various gastrointestinal illnesses [63]. We also evaluated the pathogenic ability of the isolates via the evaluation of extracellular enzymes (amylase, and protease). A total of 6 out of 9 isolates were positive for amylase while all the isolates were positive for protease. Amylase and protease enzymes elaboration have been established as crude means of establishing the pathogenicity of an isolate [64–66]. As an enzyme, protease elaboration from pathogens have been linked to increased pathogenicity, and increased skin colonization, especially when its integrity is compromised [67]. On the other hand, extracellular amylase has been linked to the formation of biofilm on various surfaces [64], an important vehicle for sharing of resistance genes [65]. Our isolates were further evaluated for their sensitivity towards commonly used antibiotics in the management of bacterial infections. All the isolates showed resistance to the antibiotics used in this study. The presence of multidrug resistant isolates in the sachet water samples is worrisome as they can complicate clinical outcomes, and constitute a significant public health challenge [66, 68].

5 Conclusion

The findings from this study raise concerns over the impending health dangers of orally ingesting potential toxic metals, and other chemicals leached from the plastic packaged materials of sachet water into the water, microorganisms. According to our findings, it can be concluded that exposure of the sachet water to sunlight enabled the leaching of potential toxic metals and other chemicals from the plastic packs into the water, thereby increasing the physicochemical parameters (except pH), and potential toxic metals content to levels unsafe for human consumption. The study also suggests that temperature could be the catalyst behind the leaching of the chemical components of the plastics into drinking water. Furthermore, the leaching of the plastic packs of the sachet water was dependent on the duration of exposure to sunlight. The ADD, HQ, and HI values of the bottle, and sachet water exposed to sunlight revealed that the ingestion of Cr, Cd, Pb, As, and Ni could result in severe non-carcinogenic health issues over time. The HQ values further disclosed that As posed the highest non-carcinogenic risk in all sachet water brands after 42 days of exposure to sunlight. Ni was the major carcinogenic source to consumers of the exposed sachet water, although Cr, As, and Pb also raised carcinogenic concerns, but to a lesser extent. With the potential of consumers facing severe health challenges

through the drinking of packaged potable water exposed to sunlight in the future, the act of local store owners displaying this plastic packaged water outside to aid faster purchase needs to be stopped, to preserve the health of the populace. The various brands of sachet water failed regulatory microbiological standards with contaminating isolates showed multidrug resistance, and pathogenic potentials. Our results suggest the urgent need for improved regulatory oversight, and monitoring of the physical, chemical, and biological parameters of sachet water across the state, and country at large to avert any impending health crisis in the future. More similar studies analysing other types of contaminants in packaged water exposed to sunlight needs to be carried out around the world.

Data availability All the data generated in the study are captured in this manuscript.

Declarations

Competing interests Authors do not have any competing interest to declare.

Ethical approval Ethical approval for this study was sought and obtained from the research and ethics committee of Arthur Jarvis University with reference number REC09/10/22O04.

Consent for publication Not applicable.

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