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(Research Article)

Evaluation of chemicals of potential concern and enzyme activity in stored sachet water produced in diobu Port Harcourt, Southern Nigeria

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Abstract

This study investigated water quality and the effect of prolonged storage on selected brands of sachet water produced in Diobu Port Harcourt, Southern Nigeria by estimating the levels of chemicals of potential concern (COPCs) and enzyme activity in the water samples. Five different brands of sachet water purchased from water factories in Diobu were labeled WS 1 - 5 and assessed for COPCs, toxic metals, physicochemical parameters and enzyme activity, following standard procedures. Results were compared with World Health Organization (WHO) standards for drinking water. Biochemical Oxygen Demand (BOD) in all five brands of sachet water ranged between 2.00±0.00mg/l and 17.51±0.14mg/l, indicating non-compliance with WHO permissible limits for BOD in drinking water. Similarly, Chemical Oxygen Demand (COD) showed exceedances in WS-5 when compared with regulatory standards, and ranged from 102.70 ± 0.67mg/l at day 0 to 207.30 ± 0.33 mg/l at day 7. WS-2 and WS-5 showed turbidity values above recommended limits at days 0, 7 and 14. Other physicochemical parameters analyzed were within WHO permissible limits. Also, values obtained for some COPCs and heavy metals were all within WHO guidelines for water potability. Assay for acid phosphatase, alkaline phosphatase and amylase enzymes in the water samples showed increasing enzyme activity between days 0 and 14. Overall, our results showed significant presence of microbial pollutants, progressive increase in enzyme activity and leaching of dichloromethane with prolonged storage of sachet water under direct sunlight. These findings indicate that the stored sachet water brands are not healthy for human consumption.

Keywords: Water Quality; COPCs; Microbial activity; Sachet water

1. Introduction

Clean water is essential for human existence as well as the health of ecosystems, communities, and economies [1]. Water plays a crucial role in body metabolism and proper functioning of cells. The quality of water is a major concern with reference to public health as health and well-being of human populace is closely tied to the quality of water available for consumption [2, 3]. Safe drinking water must meet certain physical, chemical and microbiological standards. Potable water must be clear, physically and chemically hygienic, neutral in smell and should have taste appeal [4, 5].

Further to demographic and socioeconomic factors, drinking water has been packaged as sachet water which has gained wide acceptability in recent times. Popularly known in Nigeria as “pure water”, sachet water packaged in low density polyethylene nylon is widely sold in public places such as motor parks, markets, event venues, and street corner shops. Sachet water has become the most affordable and readily available drinking water source particularly in urban centers [5]. Sachet water production has increased significantly and is popular among middle and low-income communities. However, there are concerns about the hygiene and storage conditions for sachet water. Preservation methods and improper vendor handling of sachet water have dual impact, affecting both the quality and safety of the product thereby leading to health problems [6, 7]. Previous water quality studies reported that sachet water contain bacteria such as *Pseudomonas sp*., *Klebsiella sp*., *Bacillus sp*., and *Streptococcus sp*., causing infectious disease such as typhoid, diarrhea,

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cholera and tuberculosis [8 -11]. Aroh et al. [12] earlier reported that continuous reliance on sachet water as a veritable potable water source may be responsible for the increasing level of water borne infections attested to by respondents during a survey in two cities covered in their study.

Physicochemical and biological (bacteriological) indices are two broad categories used to describe water quality index (WQI). The values of physicochemical characteristics of water samples serve as basis for monitoring water quality [13]. Chemicals of potential concern (COPCs) have been identified as critical chemical pollutants regularly detected in drinking water. COPCs were assigned to fractions specified in the EPA guidance [14]. Bisphenol A (BPA) compounds (dichloromethane, benzene, hexane, vinylchloride, perchloroethylene and toluene) have been well characterized as endocrine disruptors that can mimic the body's own hormones, potentially leading to reproductive defects, cancer, obesity and diabetes [15 - 17]. BPA is a key monomer and plasticizer in the production of epoxy resins and polycarbonate plastic. It was reported that drinking water and other beverages from plastic bottles made with BPA increased the urinary levels of the toxic chemical by nearly 70% [18]. Also, there are increasing concerns about the possibility of BPA migrating from the bottles under poor conditions such as high temperature and sun radiation [19].

Drinking water contaminated with heavy metals namely; arsenic, cadmium, nickel, mercury, chromium, zinc and lead is becoming a major health concern. The general mechanism involved in heavy metal-induced toxicity is recognized to be the production of reactive oxygen species resulting in oxidative damage and health-related adverse effects. Consumption of heavy metal-contaminated water has resulted in high morbidity and mortality rates all over the world [20].

Enzyme activity is readily measurable and has shown promise for monitoring and control during water quality evaluation. It has the advantage of quantifying biodegradation potentials for specific contaminants or indicating the presence of specific bacteria [21, 22].

Although it is recommended to store sachet water in a cool place and away from the sun and outdoors, in practice this is not always the case. Various processes are involved in water contamination by COCPs, such as leaching from the sachet due to photolytic formation or degradation of organic compounds that could take place during the storage of sachet water. BPA concentration released from bottles increases with storage time and under elevated temperature [23].

The present study was therefore designed to evaluate the quality of selected sachet water products after temporary storage by estimating the levels of chemicals of potential concern (COPCs) and enzyme activity in the water samples.

2. Material and methods

2.1. Study Area

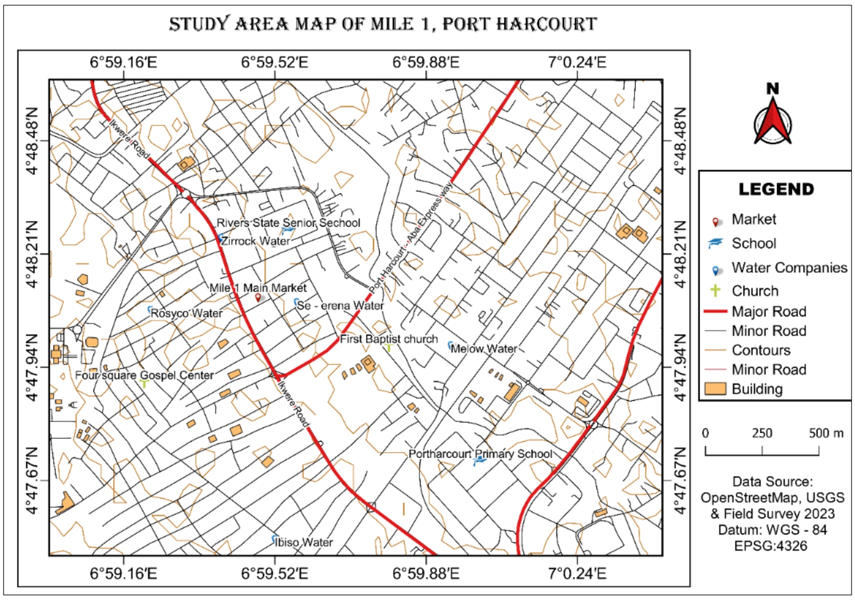

Figure 1 Map showing the study area (Diobu Port Harcourt)

Diobu is located in Port Harcourt City Local Government Area (PHALGA) of Rivers State in Nigeria. It is located on latitudes between latitudes between 40 47' 24" N and 40 49' 00" N; and longitudes between 60 59' 00" E and 70 01' 00"; with an elevation of 468m. Diobu is bordered by New G.R.A to the East, D/Line to the North-East, Rivers State University to the North-West, Old G.R.A to the East, Kidney Island to the South East and Eagle Island to the South-West. Diobu is a densely populated neighborhood consisting of Mile 1, Mile 2 and Mile 3 areas. It is the hub of commercial activities in Port Harcourt and houses two major markets in Port Harcourt; the Mile-1 and Mile-3 markets, as well as the famous Ikokwu motor spare parts market and the Mile-3 timber market. Diobu features a humid tropical climate with rainfall starting from the month of February through the month of November, while only the months of December and January truly qualifies as dry season months in the city. Rainfall is seasonal, variable, and heavy in Diobu. The mean annual temperature for Diobu is 26 °C.

2.2. Sample Collection

Five (5) different sachet water brands were purchased directly from sachet water factories located in the study area and labeled WS-1, WS-2, WS-3, WS-4 and WS-5. These five brands of sachet water selected for this study are produced, frequently supplied, most widely sold, consumed, and recognized in Diobu, located within Port Harcourt Metropolis. The sachet water brands have a dominant presence in the local market and are therefore considered representative of commonly available sachet water in the area. All samples were collected in 2L sterilized plastic bottles. The samples were kept in a mini cooler with ice packs and transported to the laboratory where sample analyses were carried out within 12 hours after collection.

2.3. Water Quality Analysis

2.3.1. Determination of Physicochemical parameters

Physicochemical parameters - pH, electrical conductivity (EC), turbidity, total suspended solid (TSS), total dissolved solid (TDS) were measured in situ using a portable digital HANNA multi-purpose meter (Model: HI9813-6). Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) were determined using the APHA 5210 and APHA 5220C methods respectively [24].

2.3.2. Determination of COPCs in water samples

A Varian CP3800 GC with Flame Ionization and PTV detectors was used for determination of COPCs in water samples. Isolation and determination method including a VF-1ms capillary column (30 m x 0.33 mm x 0.25 µm) was used to examine different injections for COCPs (Bisphenol A components). During FID, the temperature was maintained at 280 °C. The research used nitrogen as a component of both the make-up gas and the carrier gas. The maximum air and hydrogen gas fluxes that the flame detector could manage are 300 and 30 milliliters per minute, respectively. Using external standards, Bisphenol A components were quantified. On three separate occasions, concentrations of 10, 25, and 50µg/L were used for the calibration.

2.3.3. Determination of heavy metals in water samples

Metals in water (Lead, Mercury, Chromium, Cadmium and Arsenic) were determined using Atomic Absorption Spectrophotometer method [25].

2.3.4. Assay for Enzyme activity in water samples

Assay for acid phosphatase, alkaline phosphatase and amylase enzymes in the water samples were carried out using standard procedures [26 - 28].

2.4. Statistical Analysis

Statistical Analysis Results in this study are expressed as Means ± Standard Error Mean (SEM) while one-way ANOVA was used to test for differences between treatment groups using SPSS version 20. The results were considered significant at pvalues of less than 0.05, that is, at 95% confidence level ($P < 0.05$).

3. Results and discussion

Results for physicochemical evaluation of sachet water samples collected from the study area are presented in Table 1. Analytical results for experimental samples were compared with World Health Organization (WHO) permissible limits. Biochemical Oxygen Demand (BOD) in all five brands of sachet water ranged between 2.00±0.00mg/l and 17.51±0.14mg/l, indicating non-compliance with WHO permissible limits for BOD in drinking water. Similarly, Chemical

Oxygen Demand (COD) showed exceedances in WS-5 when compared with regulatory standards, ranging from 102.70 ± 0.67mg/l at day 0 to 207.30 ± 0.33 mg/l at day 7. WS-2 and WS-5 showed turbidity values above recommended limits at days 0, 7 and 14, and varied from 1.10 ± 0.10 to 1.330 ± 0.06 NTU. Other physicochemical parameters analyzed were within WHO permissible limits. Table 2 shows the mean levels of chemicals of potential concern (COPCs) in sampled sachet water brands while analytical results for heavy metals are presented in Table 3. The activity of selected enzymes in sampled sachet water is shown in Table 4. Results obtained for some COPCs and heavy metals in the water samples were all within WHO guidelines for water potability. Assay for acid phosphatase, alkaline phosphatase and amylase enzymes in the water samples showed increasing enzyme activity between days 0 and 14.

Water quality index (WQI) is a rating that reflects the composite influence of different quality parameters on the overall quality of water. WQI is frequently used for the identification and evaluation of water pollution. To evaluate the water pollutants, a variety of scientific techniques and instruments are deployed [29]. In these methods, many factors, including pH, turbidity, temperature, dissolved oxygen, and alkalinity, among others are analyzed. If these factors have values that are greater than the safe limits established by the World Health Organization (WHO) and other regulatory agencies, the drinking water quality may be impacted [30]. Utmost satisfaction is attained from water utilization when it is within the accepted quality standards. However, where there are deviations away from the set standards in the physiochemical and heavy metals parameters, it is pertinent that the water samples go through necessary processes to boost quality before consumption, especially for drinking, household, and agricultural purposes [31].

Table 1 Physicochemical parameters of sampled sachet water

		pH	EC (µs/cm)	TURBIDITY (NTU)	TDS (mg/l)	TSS (mg/l)	BOD (mg/l)	COD (mg/l)
WS-1	DAY 0	7.53 ± 0.02	422.70 ± 0.58	0.00 ± 0.00[a]	211.00 ± 2.00[a]	3.19 ± 0.01[a]	17.51 ± 0.14[a]	7.88 ± 0.07[b]
	DAY 7	7.52 ± 0.01	423.30 ± 1.53	0.00 ± 0.00[a]	212.30 ± 1.15[a]	3.22 ± 0.01[a]	17.32 ± 0.89[a]	7.74 ± 0.25[b]
	DAY 14	7.50 ± 0.01	423.30 ± 0.58	0.33 ± 0.06[a]	212.00 ± 0.00[a]	3.41 ± 0.01[a]	17.36 ± 1.12[a]	7.80 ± 0.32[b]
WS-2	DAY 0	7.86 ± 0.04	102.00 ± 0.00[a]	1.10 ± 0.10[a]	51.00 ± 0.00[a]	2.23 ± 0.00[a]	12.50 ± 0.10[a]	5.25 ± 0.32
	DAY 7	7.73 ± 0.04	102.00 ± 0.00[a]	1.00 ± 0.00[a]	51.00 ± 0.00[a]	2.42 ± 0.07[a]	12.81 ± 0.21[a]	5.62 ± 0.10
	DAY 14	7.78 ± 0.01	102.00 ± 0.00[a]	1.33 ± 0.06[a]	51.00 ± 0.00[a]	2.55 ± 0.02[a]	13.65 ± 0.17[a]	6.11 ± 0.09
WS-3	DAY 0	7.49 ± 0.01	13.50 ± 0.50[a]	0.00 ± 0.00[a]	6.50 ± 0.50[a]	0.07 ± 0.01[a]	10.48 ± 0.09[a]	5.00 ± 0.02
	DAY 7	7.41 ± 0.01	15.50 ± 0.50[a]	0.00 ± 0.00[a]	6.33 ± 0.58[a]	0.06 ± 0.01[a]	10.76 ± 0.07[a]	4.88 ± 0.05
	DAY 14	7.78 ± 0.01	102.00 ± 0.0[a]	0.00 ± 0.00[a]	7.00 ± 1.00[a]	0.07 ± 0.00[a]	10.91 ± 0.03[a]	5.07 ± 0.07
WS-4	DAY 0	7.24 ± 0.01	38.00 ± 0.00[a]	0.00 ± 0.00[a]	19.00 ± 0.00[a]	0.10 ± 0.00[a]	11.83 ± 0.07[a]	5.48 ± 0.14
	DAY 7	7.21 ± 0.02	39.33 ± 0.58[a]	0.00 ± 0.00[a]	19.33 ± 0.58[a]	0.11 ± 0.00[a]	11.96 ± 0.03[a]	5.76 ± 0.25
	DAY 14	7.19 ± 0.01	40.00 ± 0.00[a]	0.20 ± 0.00[a]	20.00 ± 0.00[a]	0.12 ± 0.00[a]	12.02 ± 0.01[a]	5.67 ± 0.05
WS-5	DAY 0	7.91 ± 0.04	213.70 ± 1.53	1.30 ± 0.01	14.97 ± 0.21[bc]	6.27 ± 0.15[bc]	2.10 ± 0.00[b]	102.70 ± 0.67[bc]

	DAY 7	7.84 ± 0.02	214.70 ± 0.58	1.23 ± 0.06	19.57 ± 0.25[ac]	8.50 ± 0.10[a]	2.00 ± 0.00[a]	207.30 ± 0.33[ac]
	DAY 14	7.81 ± 0.01	213.70 ± 2.31	1.20 ± 0.10	18.17 ± 0.12[ab]	8.27 ± 0.06[a]	2.07 ± 0.059	203.30 ± 0.88[ab]
WHO (Permissible limit)		6.5 -8.5	400	1	300	50	1 - 2	10

Values are Mean ± Standard Deviation. Data with the same alphabets (a,b,c) as superscript shows non-significant differences (p≥0.05), while that with different alphabets as superscript shows significant differences (p≤0.05).

The pH values for the sachet water samples under study were within WHO permissible limits, but showed non-significant reduction between days 0 and 7, varying from 7.21 ± 0.02 to 7.91 ± 0.04 throughout the investigation period. This finding is at variance with a previous study on storage effects on the quality of sachet water by Akinde et al. [32] where pH was non-compliant with WHO limits in all 10 samples analyzed at the onset of the investigation (Week 0) while an increase in pH was observed in all samples up to week 8, with about 40 to 100% falling within WHO limit , and followed by a decline between weeks 8 and 16. It has been established that pH plays a significant role in determining the bacterial population growth and diversity in sachet water. Prescott et al. [33] noted that microorganisms frequently change the pH of their own habitat by producing acidic or basic metabolic waste products. Reduction in pH of sachet water samples as observed in this study could be attributed to the production of metabolic waste products by increasing bacterial population.

Electrical Conductivity (EC) in the sachet water samples ranged from 13.50 ± 0.50µS/cm – 423.30 ± 1.53µS/cm; all the water samples but WS-1 were within WHO recommended limit (400 µS/cm). This partly corroborate findings by Ojukwu and Nwankwoala [31] of EC values higher values than WHO limits for water samples collected from different water stations in Port Harcourt Nigeria, including Diobu. High EC value is an indication of high levels of contamination due to dissolved ions [34]. It mirrors the amount of total dissolved salts (TDS) present in the water, as conductivity is directly proportional to TDS, which increases generally as corrosivity of water increases. TDS content of water derives from both natural and anthropogenic sources [35]. Water containing more than 300 mg/L of TDS is considered unhealthy for drinking [36]. TDS of the sampled sachet water sources ranged from 6.33 ± 0.58 to 212.30 ± 1.15 mg/L, all within the acceptable limit of WHO.

Table 2 Chemicals of potential concern (COPCs) in sampled sachet water

WATER SAMPLE		**DCM (CH_2Cl_2) (µg/L)**	**BENZENE (C_6H_6) (µg/L)**	**HEXANE (C_6H_{14}) (µg/L)**	**VCM (*$H_2C{=}CHCl$*) (µg/L)**	**PCE (C_2Cl_4) (µg/L)**	**TOLUENE ($C_6H_5CH_3$) (µg/L)**
WS-1	DAY 0	0.032 ± 0.000[b]	0.098 ±0.000	ND	ND	ND	ND
	DAY 7	0.052 ± 0.000[b]	0.073 ±0.000	ND	ND	ND	ND
	DAY 14	0.073 ± 0.000[b]	0.000 ± 0.00	ND	ND	ND	ND
WS-2	DAY 0	0.010 ± 0.001[b]	0.009 ± 0.000	ND	ND	ND	ND
	DAY 7	0.012 ± 0.000[b]	0.011 ± 0.001	ND	ND	ND	ND
	DAY 14	0.015 ± 0.000[b]	0.014 ± 0.000	ND	ND	ND	ND
WS-3	DAY 0	0.001 ± 0.00	0.001 ± 0.000[a]	ND	ND	ND	ND
	DAY 7	0.001 ± 0.000	0.002 ± 0.000[a]	ND	ND	ND	ND
	DAY 14	0.001 ± 0.000	0.002 ± 0.000[a]	ND	ND	ND	ND
WS-4	DAY 0	0.001 ± 0.000	0.003 ± 0.000[a]	ND	ND	ND	ND
	DAY 7	0.002 ± 0.000	0.00 ± 0.00[a]	ND	ND	ND	ND
	DAY 14	0.002 ± 0.000	0.00 ± 0.00[a]	ND	ND	ND	ND
	DAY 0	0.001 ± 0.000	0.003 ± 0.000[a]	ND	ND	ND	ND

WS-5	DAY 7	0.002 ± 0.000	0.000 ± 0.000[a]	ND	ND	ND	ND
	DAY 14	0.002 ± 0.000	0.000± 0.000[a]	ND	ND	ND	ND
WHO (Permissible limit)		5	5	-	2	5	1000

*Dichloromethane – DCM; Vinylchloride – VCM; Perchloroethylene – PCE; *Values are Mean ± Standard Deviation. Data with the same alphabets (a,b,c) as superscript shows non-significant differences (p≥0.05), while that with different alphabets as superscript shows significant differences (p≤0.05).*

Table 3 Selected metals in sampled sachet water

		Pb (µg/L)	Hg (µg/L)	Cr (µg/L)	Cd (µg/L)	As (µg/L)
WS-1	DAY 0	ND	ND	ND	ND	ND
	DAY 7	ND	ND	ND	ND	ND
	DAY 14	ND	ND	ND	ND	ND
WS-2	DAY 0	ND	ND	ND	ND	ND
	DAY 7	ND	ND	ND	ND	ND
	DAY 14	ND	ND	ND	ND	ND
WS-3	DAY 0	ND	ND	ND	ND	ND
	DAY 7	ND	ND	ND	ND	ND
	DAY 14	ND	ND	ND	ND	ND
WS-4	DAY 0	ND	ND	ND	0.001 ± 0.001	ND
	DAY 7	ND	ND	ND	0.001 ± 0.00	ND
	DAY 14	ND	ND	ND	0.001 ± 0.00	ND
WS-5	DAY 0	ND	ND	ND	ND	ND
	DAY 7	ND	ND	ND	ND	ND
	DAY 14	ND	ND	ND	ND	ND
WHO (Permissible limit)		0.00	1	100	5	10

Values are Mean ± Standard Deviation. Data with the same alphabets (a,b,c) as superscript shows non-significant differences (p≥0.05), while that with different alphabets as superscript shows significant differences (p≤0.05).

Table 4 Activity of selected enzymes in sampled sachet water

		ACP (µL)	ALP (µL)	AMY (µL)
WS-1	DAY 0	0.31 ± 0.01	41.3 ± 0.58	0.82 ± 0.02
	DAY 7	0.42 ± 0.02	42.3 ± 0.58	0.91 ± 0.01
	DAY 14	0.45 ± 0.01	45.7 ± 1.15	0.97 ± 0.01
WS-2	DAY 0	0.10 ± 0.01	27.3 ± 1.15	0.12 ± 0.02
	DAY 7	0.13 ± 0.015	33.3 ± 2.08	0.41 ± 0.02
	DAY 14	0.21 ± 0.021	41.7 ± 1.53	0.61 ± 0.03
	DAY 0	0.19 ± 0.08	22.0 ± 1.00	0.60 ± 0.02

WS-3	DAY 7	0.62 ± 0.03	34.7 ± 3.51	0.76 ± 0.13
	DAY 14	0.71 ± 0.03	41.0 ± 2.00	1.13 ± 0.02
WS-4	DAY 0	0.21 ± 0.02	26.7 ± 1.53	0.71 ± 0.02
	DAY 7	0.35 ± 0.03	40.0 ± 1.00	0.81 ± 0.01
	DAY 14	0.47 ± 0.02	47.0 ± 2.00	0.97 ± 0.01
WS-5	DAY 0	38.3 ± 1.03[bc]	0.42 ± 0.02[bc]	0.69 ± 0.21[c]
	DAY 7	43.3 ± 0.88[a]	0.57 ± 0.04[a]	0.97 ± 0.12[c]
	DAY 14	46.7 ± 0.33[a]	0.64 ± 0.01[a]	1.90 ± 0.15[ab]

ACP - acid phosphatase; ALP - alkaline phosphatase; AMY – Amylase

Values are Mean ± Standard Deviation. Data with the same alphabets (a,b,c) as superscript shows non-significant differences (p≥0.05), while that with different alphabets as superscript shows significant differences (p≤0.05).

In the present study, BOD values were higher than permissible limits in the entire sachet water samples studied, while COD values showed exceedances in WS-5. High BOD has been established to be due to the presence of microorganisms (high bacteria count) which is an indication of water contamination. When the BOD value of water is in the range 3 - 5 ppm, the water is moderately clean. Polluted water has a BOD value in the range of 6 - 9 ppm. In polluted water, organic waste is present [37].

Values obtained for some COPCs (Bisphenol-A compounds) and heavy metals were all within WHO guidelines for water potability. Bisphenol A is a chemical building block used primarily to make epoxy resins and polycarbonate plastic. Our findings for COPCs agrees with a previous investigation by Shao et al. [38] which did not detect BPA in different plastic containers for beverages, including drinking water. However, our results showed progressive leaching of dichloromethane with prolonged storage of sachet water under direct sunlight. This could be attributed to the migration of plasticizers from the water sachet to the water since sachet quality may vary depending on the raw material and the technology used in production [39]. Although storage of sachet water in a cool place and away from the sun and outdoors is recommended, in practice this is not always the case. Various processes are involved in water contamination by BPA, such as leaching from the container due to photolytic formation or degradation of organic compounds that could take place during the storage of bottled water. BPA concentration released from bottles increases with storage time and under elevated temperature [39]. Between day 0 and 14 of the present study, there was progressive increase in enzyme activity indicating significant microbial load. The ultimate goal in qualitative assessment of water is to ensure that water is free from any form of contaminating agent and pathogenic microorganisms likely to affect human health. However, increasing enzyme activity in the current study is an indication that the sachet water samples are contaminated.

4. Conclusion

Results obtained in this investigation showed that the sachet water samples examined were contaminated, as evidenced by high turbidity, BOD and COD values, signifying high bacteria count and organic matter content. The study also revealed that sachet water, when stored for a long period, can show increase in microbial activity and BPA concentration to levels that may be harmful to human health.

Compliance with ethical standards

Disclosure of conflict of interest

Authors have declared that no competing interests exist.

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