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Bioavailability of Plastic Contaminants and Their Effects on Plastic Bottled and Sachet Drinking Water Supplies

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Authors' contributions

This work was carried out in collaboration between all authors. The study idea was conceived by authors EIA and OMA performed the laboratory work and run literature review. The manuscript was written by author UU in complete agreement with all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study examined the bioavailability of plastic contaminants and their effects on plastic bottled and sachet drinking water supplies in Benin City.
Methodology: Plastic bottled water produced by Eva water supplies was collected in triplicates and three brands of sachet drinking water were collected from different pure water distributors within Benin City metropolis. All packaged water samples were stored at room temperature for four weeks. Analyses were conducted for week one week and four.
Results: The total viable bacterial counts ranged from 1.0×10^1 – 1.9×10^2 cfu/ml for the plastic bottled water and 1.3×10^2 – 2.3×10^2 cfu/ml, total coliforms (TC), faecal coliforms (FC) and *E. coli* were not found in all the plastic bottled water samples tested. The bacteria isolated from bottled water samples were *Bacillus subtilis*, *Micrococcus* spp. and *Staphylococcus aureus* while bacteria isolated from sachet water samples were *Klebsiella* spp., *Bacillus subtilis, Escherichia coli*,

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Acinetobacter spp., *Micrococcus* spp., *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The fungal isolates were *Aspergillus niger, Aspergillus flavus* and *Saccharomyces cerevisiae.* The Plastic bottled water sample appeared to have slightly better physico-chemical qualities than sachet water sample. At the end of four weeks storage period. Bisphenol A (BPA) congeners were found in the plastic bottled water samples and sachet water samples respectively. The concentration of BPA in plastic bottled water ranged from 0.001 mg/l in first week to 0.139 mg/l in fourth week and had higher concentration in sachet water samples (0.001 to 3.007mg/l) with vinyl chloride and methylene chloride having the highest peaks.

Conclusion: This study has shown that the bioavailability of BPA components, reduction of microbial load and physico-chemical qualities of bottled and sachet water appeared to start manifesting at the fourth week of storage.

Keywords: Bioavailability; plastic contaminants; sachet water; bottled water supplies.

1. INTRODUCTION

Bottled water is defined as any potable water that is bottled and distributed or offered for sale and specifically intended for human consumption. In many developing countries, availability of water has become a critical and urgent problem and it is a matter of a real concern to families and communities that depend on non-public water supply system [1]. Increase in the human population has exerted an enormous pressure on the provision of safe drinking water in developing countries [2]. To curb this health problem, bottled water was introduced, but only individuals who have good financial status can afford these products. Low income earners are left with no option but consume sachet packaged water that is cheaper. It is readily available and affordable; sachet water is sold in most road side vending stalls in Nigeria. Small nylon sachets which are electrically heated and sealed at both ends are used to package water 0.5 litres of water and these was introduced into the market in Nigeria. There are many different brands of sachet drinking water that are beautifully packaged, properly labeled and advertised. Although these products are popularly term “pure water”, they are usually not pure because the water supplies are often taken from boreholes which may have chemical or/and microbial contamination above the water safety limits [3,4,5]. Occasionally, contamination of sachet water may occur either during the processing, transportation or improper handling by hawkers. Moreover a greater proportion of the water that is used for production of sachet and plastic bottled water is obtained from borehole that are exposed to microbial contamination through rainfall runoffs and the fact that they are usually constructed very close to toilet systems [6,7].

However, the quality of the packaged plastic bottled water supplies cannot guarantee safety from contamination. The contamination may be entirely due to hazardous materials like bisphenol A and phthalates used in making polycarbonate plastic or the microbial contamination of the raw water and packaging materials [8]. Bisphenol A can be disseminated and leached into water from packaging materials. This can happen faster over time and in higher temperatures [9]. It has been proven that bisphenol A may affect estrogenic and estrogenic-related receptors in fish and insects [10]. Bisphenol A has been shown to have the capacity to transfer from pregnant mice to their embryos [11]. It has also been proved that bisphenol A is a component with multiple effects on many male and female tissues [12]. According to reports of European Food Safety Authority (EFSA), the specified tolerable daily intake (TDI) of bisphenol A is 4 µg/kg/day of bw/day [13,14]. As many health issues have been attributed to bisphenol A. Several studies have been performed on this chemical component on different scales. Some researchers have focused on the detection and measurement of bisphenol A and others on their effects on human and laboratory animals [15]. For example in the UK, [16] examined the ability of three methods to measure endocrine disrupting chemicals. A study in the United States has evaluated the presence of bisphenol A in packaged water [17]. In Iran, [18] and [19] investigated the presence of bisphenol A in canned foods, surface water and waste water respectively. However, it seems that not so much study has been done on the measurement of biphenol A in bottled and sachet water in Nigeria. This study examined the bioavailability of plastic contaminants and their effect on plastic bottled and sachet drinking water supplies in Benin City, Edo State.

2. MATERIALS AND METHODS

The plastic bottled water produced by Eva water supplies were collected in triplicate and three different brands of sachet water samples were also collected in triplicate at Ugbowo, Benin City. The plastic bottled and sachet water brands were the commonly consumed packaged water in most areas of Benin City. They were randomly purchased from local stores, supermarkets and "pure-water" factories within Benin metropolis. All the packaged water samples were stored at room temperature for four weeks, thus mimicking typical conditions in retail outlets, supermarkets and in homes. Sub-samples were drawn from the stock samples on weekly basis and within days of being purchased for microbiological and physico-chemical analysis, using WHO analytical methods [20]. Water samples for analysis of dissolved oxygen (DO) and biochemical oxygen demand (BOD) were collected in pre-sterilized brown bottle and fixed by adding 1.2ml of Winkler solution.

2.1 Microbiological Analysis of Water Samples

Total viable bacterial and fungal counts were determined by pour plate technique using standard methods [21]. Nutrient agar medium was used for the enumeration of viable aerobic bacteria while sabouraud dextrose agar was used for fungal count, MacConkey agar was used for coliform count while eosin methylene blue medium was used for fecal coliform and *E. coli* counts. The different potable water samples were serially diluted up to 10^{-3} dilution, then 0.1 ml of the appropriate dilutions were plated in nutrient agar, sabourand agar, MacConkey and Eosin methylene blue media. Nutrient and MacConkey agar plates were incubated at 37ºC for 24 hours, while sabouraud and Eosin methylene blue agar plates were incubated at room temperature for 72 hours and at 44.5ºC respectively. After incubation, the number of discrete colonies were counted and recorded in colony forming unit per milliliter (cfu/ml). The isolates were sub-cultured to obtain pure cultures. The pure cultures so obtained were transferred to agar slants by streaking and from there further biochemical tests were carried-out to identify the isolates. For the faecal coliform count that was determined using pour plate technique, organisms with greenish metallic sheen were taken as positive for *E. coli*. This was further confirmed by the ability of the organisms to ferment lactose at 44.4ºC.

2.2 Identification of Microbial Isolates

Aseptically purified representatives discrete colonies were obtained by streaking on their respective media plates. They were further stored in agar slants for further characterization. All the bacterial and fungal isolates were initially examined microscopically for morphological characterization followed by appropriate biochemical test for bacterial isolates (Gram staining, indole, catalase, motility, citrate utilization, urea production, oxidase, congulase and oxidative/fermentative utilization of lactose and glucose). The identification of bacterial isolates was done in accordance with criteria of Bergeys, Manual of Determinative Bacteriology [22]. The fungal isolates were identified microscopically using lactophenol cotton blue test. The identification was achieved by placing a drop of the stain on clean slide with the aid of a wire loop, where a small portion of the mycelium from the fungal cultures was removed and placed in a drop of lactophenol. The mycelium was spread on the slide with aid of wire loop. A cover slip was gently applied with little pressure to eliminate air bubbles. The slide was then mounted and observed with objective lens and identification done in accordance with [23].

2.3 Physico-chemical Analysis of Packaged Potable Water Samples

Various sub-samples drawn from stock samples (stored) were taken to the laboratory in ice-packed coolers. Those that could not be analyzed the same day were stored in a refrigerator at a temperature of 4ºC. Physico-chemical parameters determined included: pH, temperature, conductivity, total dissolved solid (TDS), total suspended solid (TSS), turbidity, alkalinity, total hardness, total iron, chloride, sulphate, phosphate, nitrate and biochemical oxygen demand (BOD). All the physico-chemical analyses were carried-out using standard method [21]. Data collected were subjected to statistical analysis. The correlation coefficients between bacteriological and physico-chemical data were determined.

2.4 Sample Treatment and Analysis for Bisphenol A (BPA)

Bisphenol A was extracted from water samples using the modified procedure from [24]. Fifty milliliters (50 ml) of water sample was measured into a separating funnel in which 100 ml of dichloromethane (DCM) and shake for 30

minutes. The separating funnel was clamp and the mixture was allowed to separate. After separation, the DCM portion was collected. The process was repeated three times for complete extraction. Blanks were prepared following the same procedures without sample using deionized water. The standard sample used for quality control was prepared by adding the standard solution (Bisphenol A) to DCM. All extracts were separated, and activated copper was added to the combined extract for desulphurization. After subsequent filtration over an hydrous sodium sulphate, the solution was concentrated to 1.0 ml using a rotary evaporator, an internal standard mixture (Vinyl chloride) solution was analyzed with the extract for quality control check using Hewlett Packard HP 5890 series II gas chromatograph with mass selective detection (GC-MS).

2.5 Instrumentation and Conditions

Hewlett Packard HP 5890 series II Gas chromatograph equipped with an Agilent 7683B injector (Agilent Technologies Santa Clara, CA, USA), A 30 m, 0.25 mm i.d. HP-5MS capillary column (Hewlett – Packard, Palo Alto, CA, USA) coated with 5% phenyl-methylsiloxane (film thickness 0.25 µm) and an Agilent 5975 mass selective detector (MSD) was used to separate and quantify the BPA compounds. The samples were injected in the split less mode at an injection temperature of 300ºC. The transfer line and ion source temperatures were 280ºC and 200ºC. The column temperature was initially held at 40ºC for 1min, raised to 120ºC at the rate of 25ºC/min, then to 160ºC at the rate of 10ºC/min and finally to 300ºC at 5ºC/min, held at final temperature for 15 min. Detector temperature was kept at 280ºC. Helium was used as a carries gas at a constant flow rate of ml/min. Mass spectrometry was acquired using the electron ionization (EI) and selective ion monitoring (SIM) mode.

3. RESULTS AND DISCUSSION

The comparison of the bacteriological quality of sachet water and the plastic bottled water as shown in (Table 1), the total viable bacterial count was seen to increase with storage in plastic bottled water samples while there was no total coliform count in bottled water from the first week to fourth week. The total viable counts ranged from 1.0×10^1 to 1.9×10^2 cfu/ml and total coliform, faecal coliform and *E. coli* count were nil. The mean total viable count of sachet water ranged between 1.3×10^2 to 2.3×10^2 cfu/ml from the first week to fourth week. The result on Table 3 indicate the presence of *Bacillus subtilis, Staphylococcus aureus* and *Micrococcus* spp. in bottled and sachet water samples. Tables 3 and 4 showed the mean physico-chemical qualities of plastic bottled water samples and sachet water samples stored at room temperature for four weeks. The two tables revealed variations in values obtained in plastic bottled and sachet water respectively. Plastic bottled water samples appear to have better physico-chemical qualities than sachet water samples. Concentrations of a total of five bisphenol A (BPA) congeners were detected in the plastic bottled and sachet water samples under examination as shown on Tables 5, 6, and Figs. 1 to 4. The total concentration after four weeks of storage ranged between 0.001 mg/l and 0.139 mg/l for plastic bottled water and 0.001 mg/l and 3.007 mg/l for sachet water respectively.

The results obtained from this study showed that the plastic bottled and sachet drinking water sold in Benin City metropolis, exhibited variable characteristics in terms of their microbiological and physico-chemical qualities. The levels of bacterial population in stored plastic bottled water and sachet drinking water samples increased to maximum levels after four weeks of unrefrigerated shelf-life. Plastic bottled water samples appear to be slightly better in bacteriological and physico-chemical qualities than sachet drinking water samples at the end of four weeks storage period. The plastic bottled water samples had lower conductivity, turbidity, hardness and chloride contents than sachet drinking water samples; the bottled water with pH of 6.05±0.72 at week four is no longer suitable for drinking (Tables 3 and 4). Similar results were obtained by [25] and [7] who reported the effects of storage on bacteriological and physicochemical qualities of packaged potable water supplies in Port Harcourt and Benin City metropolis respectively. During the period of study, total coliform, faecal coliform and *E. coli* counts were not detected from any of the plastic bottled brands of water samples tested. The number of these indicator bacteria in water and food should be zero cfu/100 ml [26]. The absence of faecal indicator bacteria in all brands of plastic bottled and sachet drinking water may be attributed to better hygienic practices observed in the industry. These include the use of protective sealed caps on bottles, improved and automated hygienic filling system. This result is in accordance with the report of [27]. The

presence of *Staphylococcus aureus, Bacillus subtilis* and *Micrococcus* spp. from bottled and sachet drinking water samples (Table 2) may be attributed to contamination during packaging or handling since some of the organisms are normal flora of the human skin [28]. However, the presence of *Staphylococcus aureus* in drinking water is of public health importance because it is usually responsible for Staphylococcal food poisoning [29].

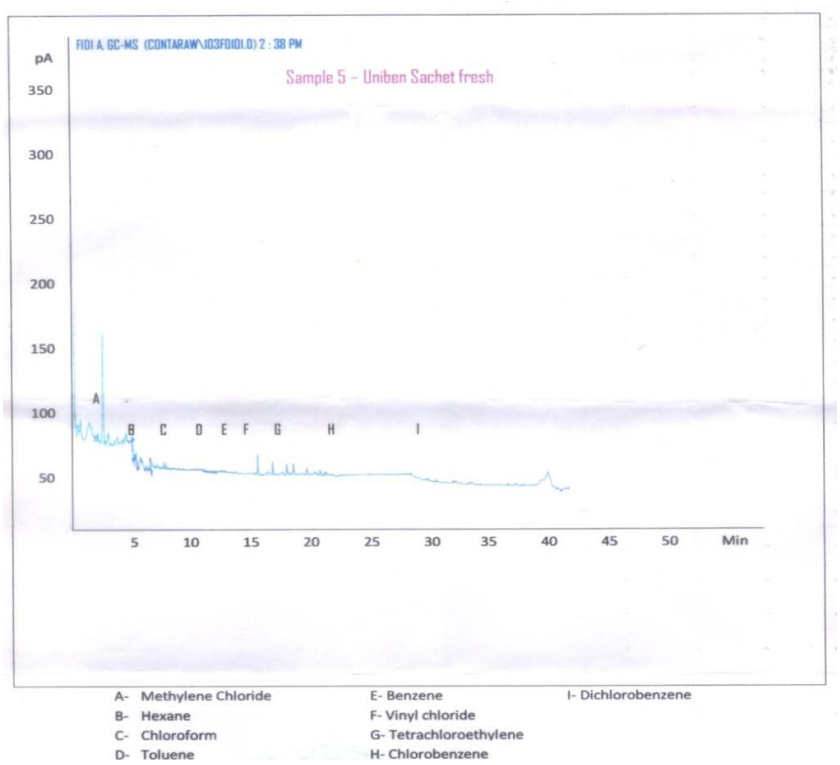

Fig. 1. G.C.-MS peaks of sachet water for week 1

Table 1. Microbial counts of the sachet and plastic bottle filled with water

Microbial counts (cfu/ml)	Sachet water		Plastic bottled water	
	Week 1	**Week 4**	**Week 1**	**Week 4**
Total viable bacterial counts	1.3×10^2	2.3×10^2	1.0×10^1	1.9×10^2
Coliform counts	Nil	Nil	Nil	Nil
E. coli counts	Nil	Nil	Nil	Nil
Fungi counts	Nil	Nil	Nil	Nil

Table 2. Distribution of the bacterial isolates in the bottled and sachet water samples

Bacterial isolates	Bottled water	Sachet water
Klebsiella spp.	-	-
Bacillus subtilis	+	+
Escherichia coli	-	-
Acinetobacter spp.	-	-
Micrococcus sp.	+	+
Staphylococcus aureus	+	+
Pseudomonas aeruginosa	-	-
Fungal isolates		
Aspergillus niger	-	-
Aspergilhis flavus	-	-
Saccharomyces cerevisiae	-	-

Key: += Present, - = Absent

Table 3. Physico-chemical properties of bottled water

Parameters	Week 1	Week 4	WHO..(2011) water standards	SON (2007) water standard
PH	7. 15±0.39	6.05±0.72	6.5-8.5	6.5-8.5
Temperature (℃)	31.63±1.03	33.32±1.20	Ambient	Ambi ent
Conductivity (us/cm)	19.10±1.26	17.43±1.72	900	1000
TDS (mg/L)	0.00±0.00	0.00±0.00	1000	1500
TSS (mg/L)	0.00±0.00	0.00±0.00		
Turbidity (NTU)	0.20±0.09	0.20±0.09	5.0	5.0
Alkalinity (mg/L)	3.50±2.84	3.50±2.84	150	150
Total hardness (mg/L)	0.25±0.11	0.25±0.11	150	150
Total iron (mg/L)	0.01±0.01	0.01±0.01	0.30	0.30
Chloride (mg/L)	0.39±0.27	0.39±0.27	250	300
Sulphate (mg/L)	0.04±0.04	0.02±0.04	400	100
Phosphate (mg/L)	0.00±0.00	0.00±0.00		
Nitrate (mg/L)	0.00±0.00	0.00±0.00	70	50-70
BOD (mg/L)	0.52±0.85	0.52±0.85		

Key: NTU=Nephelometric turbidity unit

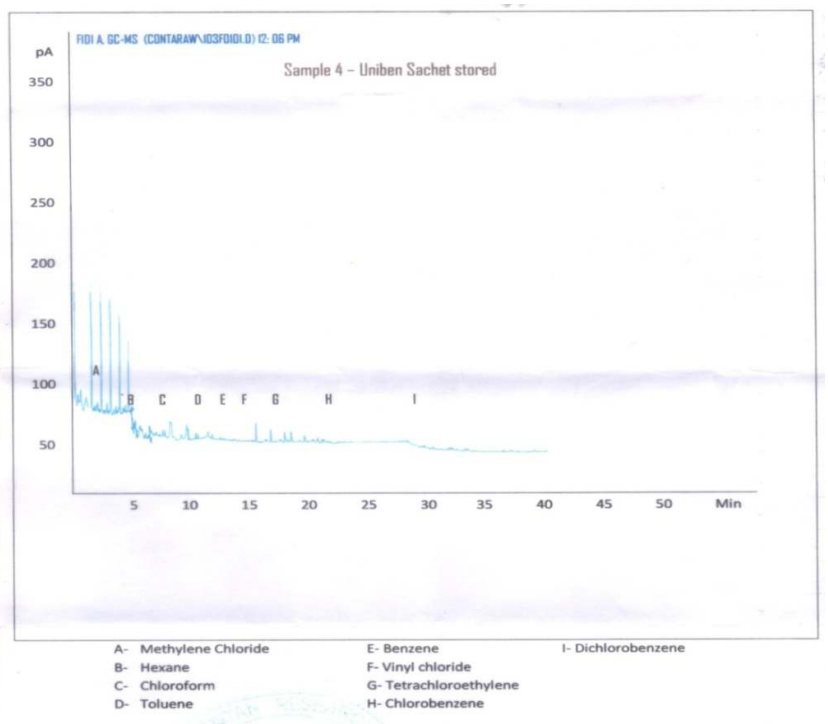

Fig. 2. G.C.-MS peaks of sachet water for week 4

The total bisphenol A concentration of the two groups of packaged drinking water samples under study that were stored for four weeks ranged between 0.001 mg/l and 0.139 mg/l for plastic bottled water and 0.001 mg/l to 3.007 mg/l for sachet samples respectively (Table 4, 5 and Figs. 1-4). The results indicate the presence of lower amount of Bisphenol A in plastic bottled drinking water than in the sachet drinking water samples with vinyl chloride and methylene chloride congeners having the highest peaks. Human exposure to BPA occurs primarily via hydrolysis of polycarbonate plastics and epoxy resins, resulting in low concentrations of free BPA in food and liquids. This makes dietary consumption the major mode of human exposure [30]. BPA may enter the environment through physical and chemical breakdown during

disposal and recycling operation process [31]. Physical parameters such as pH could be problematic if the water is too soft resulting in water that may not be acceptable to consumers in terms of taste and scale deposition. Storage temperature plays a major role as it can impact on the acceptability of other inorganic constituents and enhance the growth of microorganisms resulting in offensive tastes and odour [32].

Table 4. Physico-chemical properties of sachet water

Parameters	Week 1	Week 4	WHO..(2011) water standards	SON (2007) water standard
PH	7.60±0.55	7.40±0.43	6.5-8.5	6.5-8.5
Temperature (℃)	29.20±0.56	28.90±0.57	Ambient	Ambi ent
Conductivity (us/cm)	40.30±0.53	40.10±0.49	900	1000
TDS (mg/L)	0.00±0.00	0.00 ±0.00	1000	1500
TSS (mg/L)	0.00±0.00	0.00 ±0.00		
Turbidity (NTU)	0.27±0.00	0.27±0.00	5.0	5.0
Alkalinity (mg/L)	1.00±0.00	1.00±0.00	150	150
Total hardness (mg/L)	29.20±0.06	28.90±0.04	150	150
Total iron (mg/L)	0.004±0.00	0.007±0.00	0.30	0.30
Chloride (mg/L)	0.44±0.00	0.53±0.00	250	300
Sulphate(mg/L)	0.326±0.00	0.306±0.00	400	100
Phosphate (mg/L)	BDL	BDL		
Nitrate (mg/L)	BDL	BDL	70	50-70
BOD (mg/L)	BDL	BDL		

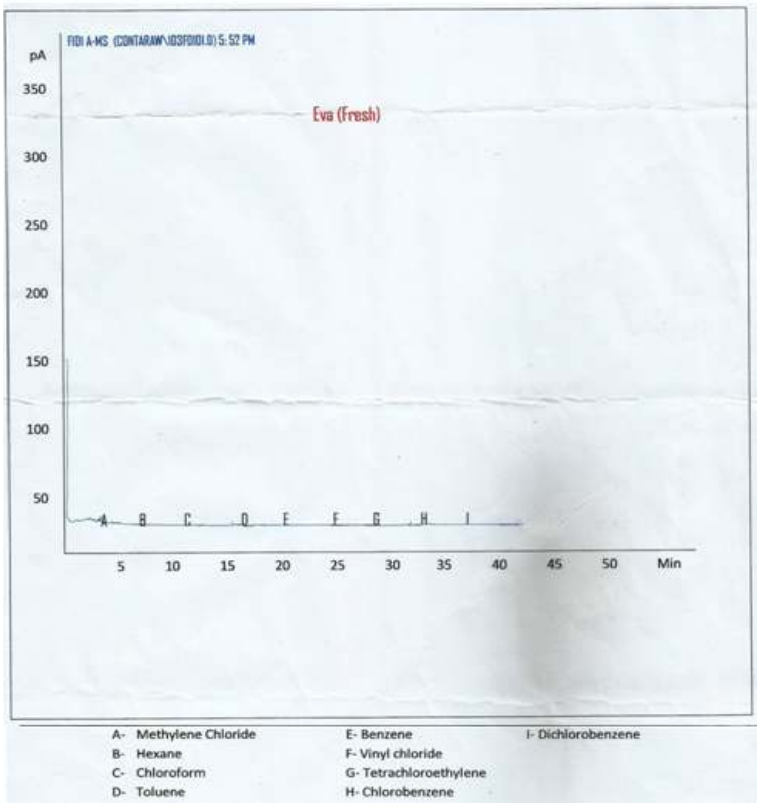

Fig. 3. G.C.-MS peak of bottled water (Eva) for week 1

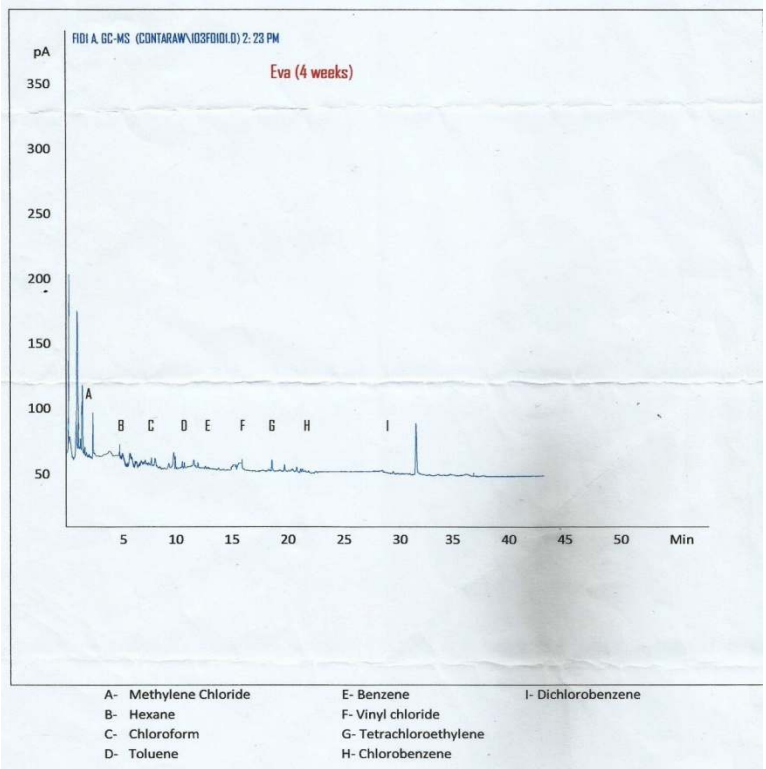

Fig. 4. G.C.-MS peaks of bottled water (Eva) for week 4

Table 5. Concentration of bisphenol A in sachet water samples

Parameter	Week 1	Week 4
Methylene chloride	0.005	3.007
Hexane	0.001	0.013
Chloroform	0.001	<0.001
Toluene	0.001	<0.001
Benzene	<0.001	0.001
Vinyl chloride	0.024	0.062
Tetrachloroethylene	0.001	0.003
Chlorobenzene	<0.001	0.002
Dichlorobenzene	<0.001	0.001
Total (mg/1)	0.029	0.087

Table 6. Concentration of bisphenol A in bottled water

Parameter	Week 1	Week 4
Methylene chloride	0.009	0.055
Hexane	< 0.001	< 0.001
Chloroform	<0.001	0.001
Toluene	<0.001	0.001
Benzene	0.001	<0.001
Vinyl chloride	0.024	0.139
Tetrachloroethylene	<0.001	0.018
Chlorobenzene	<0.001	0.001
Dichlorobenzene	<0.001	<0.001
Total (mg/1)	0.033	0.214

4. CONCLUSION

Finally, this study has shown that the bioavailability of bisphenol A components triggers increase in microbial load and reduction of the physico-chemical qualities of packaged drinking water which appeared to start manifesting at the fourth week of water storage. Hence, packaged drinking water stored at room temperature for prolonged periods can result in high heterotrophic bacterial population through biofilm formation and can cause health problems to individuals, especially among the sensitive population. Plastic chemical concentrations such as BPA have been found to increase with time of storage. Plastic bottle water shelf-life is not of major concern provided the water is refrigerated and expiring date should not exceed four weeks. Based on results obtained from this study, the followings have been recommended. The practice of continuous refilling of borehole water into plastic containers for storage without refrigeration should be discouraged and discontinued, more regular monitoring of plastic bottled and sachet water producing factories should be enforced and public health awareness should be created on the emergence of

endocrine disrupting chemical in our food and drinks.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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