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Bioavailability and Health Effects of Plastic Contaminants in Borehole Water Stored in Plastic Containers

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Abstract

The aim of this study was to evaluate the bioavailability and health effects of plastic contaminants on borehole water stored in plastic containers. Three brands of plastic containers filled with borehole water were collected from homes in Ugbowo, Benin city. Physicochemical parameters were determined using standard methods. Total bacterial and coliform counts were determined using the pour plate technique. Conductivity, TDS, Chloride, Sulphate and Nitrate were within the recommended standards while turbidity and total iron were above recommended standards. The isolates identified include: Klebsiella sp, Bacillus sp, E. coli, Pseudomonas aeruginosa, Aspergillus flavus, Saccharomyces sp and Aspergillus niger. The total bacterial count in the water samples ranged from 1.4×103cfu/ml to1.8×103cfu/ml at week four while fungal counts was 1.3×102cfu/ml to 1.6×102cfu/ml. Bisphenol A was discovered to leach at detectable levels from the plastic containers as storage increased. The result of the BPA analysis revealed that BPA congeners ranged from 0.023mg/l within days of collection to 0.251ml/l at the fourth week of storage. This study has shown that storage of borehole water in plastic containers for prolonged period affects the bacteriological and chemical properties of the water, hence storage of borehole water in plastic for prolonged periods should be discouraged and discontinued.

Index terms— plastic contaminant, bioavailability, borehole water, bisphenol A.

1 I. Introduction

ater is a transparent, colourles, odourless and tasteless liquid that makes up the sea, lakes, rivers, rainfall as well as the liquid that makes up living organisms (Michael, 2000). Water is a compound of two elements; hydrogen and oxygen atoms with a chemical formula H 2 O and it is known to make up about 70 percent of the earth surface (Osei, 2005). Rivers, streams, wells and more recently boreholes, serve as the main source of drinking water and domestic use in developing countries like Nigeria, where most of the people reside in rural areas **??**Ibe and Okplenya, 2005). According to the World Health Organisation guidelines for drinking water underground water supplies are usually considered safe provided they are properly located, constructed and operated to WHO regulatory standards **??**WHO, 1971). Boreholes with hand pumps are commonly used by poor rural communities and this amounts to approximately 250,000 hand pumps in Africa. Studies have shown that water may become contaminated at any point between collection, storage and usage (Tambekar et al.,2006). Also, storing water in plastic containers and handling procedures of water at homes, hotels or restaurants causes water quality deterioration to such extent that it becomes potential risk of infection to consumers (Jagals et al., 1999).

Microorganisms associated with contaminated water includes Salmonella sp, Escherichia coli and Vibro cholera **??**Birmingham et al., 1997). Water borne diseases often arises when pathogenic microorganisms associated with contaminated water is consumed. Boreholes and wells are polluted either industrially, domestically and agriculturally. Industrial pollution may involve seepages of used water containing chemicals such as metals and

radioactive compounds while domestic pollution may involve seepage from broken septic tanks, pit latrines and privies. Runoff water after rainfall carrying pesticides, fertilizers, herbicides and faecal matter may contribute to agricultural pollution. However the pollution sources, the quality of packaging plastic bottle cannot guarantee safety from contamination. In the natural environment there are compound that have the potential to disturb equilibrium in living organisms and are mistakenly recognised by oestrogen receptors, treated the same as those naturally present in the organism. Substances of this type are known as Endocrine Disrupting chemicals. Bisphenol A is one of the highest volume chemicals produced world wide with more than 6million pounds produced each year (Burridge, 2003). It serves as a base line in the manufacturing of plastics and a major compound in the production of epoxy resins, printers ink, powdered paints, dental sealants and composites (Vandenberg et al., 2009;Markey et al., 2003). Bisphenol A can be released and leached into water from packaging materials. Hence, through consumption due to it use in packaging and storage containers, consumers are directly or chronically exposed to BPA (Brotons et al., 1995). Several health cases have been

2 W

Global Journal of attributed to bisphenol A and studies have been carried out on the chemical component at different scales. Some researches have focused on the detection and measurement of bisphenol A while others on the effects on humans and laboratory animals (Chang et al., 2009). For example a study in the United States have evaluated the presence of BPA in packaged water (Jin et al., 2004). In Iran, Raskari et al. (2011) and Jafari et al. (2006) investigated the presence of BPA in canned foods, surface water and waste water. Makinwa and Uadia (2015) carried out a survey on the levels of BPA in effluents, soil leachates, food samples, drinking water and consumer products in South Western Nigeria. However it seems that no study has been done on the measurement of BPA in borehole water filled into plastic containers in Nigeria. This study examined the bioavailability and health effects of plastic contaminants in borehole water filled into plastic containers.

3 II. Materials and Method

A total of three different brands of plastic containers filled with borehole water samples were collected from Ugbowo in Benin City. The 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 physicochemical analysis, using WHO analytical methods (WHO, 2011). 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.

4 a) Microbiological Analysis of Water Samples

Total viable bacterial and fungal counts were determined by pour plate technique using standard methods (APHA, 1998). 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 faecal coliform and E.coli counts. The different brands of borehole water samples were serially diluted upto 10 -3 dilution/ then 0.1ml 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 37 0 C for 24 hr, while Sabouraud and eosin methylene blue agar plates were incubated at room temperature for 72hr and at 44.5 0 C respectively. After incubation, the number of discrete colonies were counted and recorded in colony forming unit per milliter (cfu/ml).

The isolates were sub-cultured to obtain pure cultures. The pure cultures so obtained were transferred to agar slants by streaking and further biochemical tests were carried-out to identify the isolates. Faecal coliform count that was determined using pour plate technique, was recorded by the organisms ability to appear as greenish metallic sheen. This was taken as positive for E.coli. However further confirmatory test was carried out by the ability of the organisms to ferment lactose at 44.4 0 C.

5 b) Identification of Microbial Isolates

By streaking on their respective media plates, aseptically purified representatives of discrete colonies were obtained. 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 Determining Bacteria (Holt et al., 1994).

The fungal isolates were identified microscopically using lacto phenol 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 Barneth and Hunter (1982) criteria.

6 c) Physico-Chemical Analysis of borehole Water

Samples Physico-chemical parameters determined included: pH, temperature, conductivity, total dissolved solid (TDS), total suspendend solid (TSS), turbidity, alkalinity, total hardness, total iron, chloride, sulphate, phosphate, nitrate and biochemical oxygen demand (BOD). In carrying out this analysis 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 4 0 C. All the physico-chemical analysis were carried-out using standard method (APHA, 1998). Data collected were subjected to statistical analysis.

7 d) Sample Treatment and Analysis for Bisphenol a (BPA)

Bisphenol A was extracted from water samples using the modified procedure from Dean and Xion (2000). Fifty millitres (50ml) of water sample was measured into a separating funnel in which 100ml of dichloromethane (DCM) and shaked for 30min. The separating funnel was clamp and the mixture was allowed to separate-out. 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 diioned water. The standard sample used for quality control was prepared by adding the standard solution (Bisphenol A) to DCM. The extracts were separated, and activated copper was added to the combined extracts for desulphurization. After subsequent filtration over anhydrous sodium sulphate, the solution was concentrated to 1.0ml using a rotary evaporator, an internal standard mixture (Vinyl chloride) solution was run with the extract for quality control check using Hewlett Packard HP 5890 series II gas chromatograph with mass selective detection (GC-MS).

8 e) GC-MS Instrumentation and Conditions

Hewlett Packard HP 5890 series II Gas chromagraph equipped with an Agilent 7683B injector (Agilent Technologies Santa Clara, CA, USA), A 30m, 0.25mm i.d. HP-5MS capapillay column (Hewlett -Packard, Palo Alto, CA, USA) coated with 5% phenylmethylsiloxane (film thickness 0.25µm) and an Agilent 5975 mass selective detector (MSD) was used to separate and quantify the BPA compounds. The samples were injected in the splitless mode at an injection temperature of 300 0 C. The transfer line and ion source temperatures were 280 0 C and 200 0 C. The column temperature was initially held at 40 0 C for 1min, raised to 120 0 C at the rate of 25 0 C/min, then to 160 0 c at the rate of 10 0 C/min and finally to 300 0 C at 5 0 C/min, held at final temperature for 15min. Detector temperature was kept at 280 0 C. 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.

9 III. Results

Table 4 shows the mean values of the total viable bacterial counts, faecal coliform counts, E. coli counts and fungal counts of water samples collected at intervals of within few days, one week and after four weeks of storage at room temperature. Total viable bacterial counts of the borehole samples had a range from 1.4 × 10 3 cfu/ml within day of collection to 1.8× 10 3 cfu/ml at the fourth week. Coliform counts of the borehole water samples also ranged from (4.7 × 10 2 cfu/ml) week 0, (4.4 × 10 2 cfu/ml) week 1 and (4.9 × 10 2 cfu/ml) week 4. The range of coliform count is from (4.4 × 10 2 cfu/ml -4.9 × 10 2 cfu/ml) with brand C having the highest counts. E. coli and fungal counts were; (6.0 × 10 1) week 0, to (7.0 × 10 1 cfu/ml) in week 4 and (1.3× 10 2 cfu/ml) week 0, to (1.6 × 10 2 cfu/ml) week 4 respectively.

10 IV. Discussion

The result of this study has revealed the effect of prolonged storage of borehole water samples stored at room temperature on the total heterotrophic bacterial count. Increase in storage of the samples led to a gradual increase in the total heterotrophic bacteria count as shown in table 4. This result is in line with (Atuanya et al., 2014) who revealed the effect of storage on the physicochemical and bacteriological qualities of potable water in Benin City. Total coliform count recorded ranged from 4.7× 10 2 cfu/ml in week 0 to 4.9×10 2 cfu/ml in week 4. This result is also in accordance with Rogbesan et al. (2002) who reported the presence of total coliform count above the range recommended by WHO. The observation from this result reveals that high heterotrophic bacteria count also reflected in total coliform count. E.coli and fungal counts recorded ranged from 6.0×10 1 cfu/ml to 7.0×10 1 cfu/ml and 1.3×10 2 cfu/ml to 1.6×10 2 cfu/ml respectively. The presence of high heterotrophic bacteria count, total coliform count, E.coli and fungal count could be as a result of the proximity of the borehole to a pit latrine at a distance less than 30meters that is recommended by WHO or as a result of the nature of the pipes used for the distribution of the water. They may be rusty, thus allowing seepage of microbial contamination into the borehole. The bacterial and fungal isolate identified in this study showed that the water is not wholesome, therefore not fit for consumption without additional treatment.

The result of the physicochemical analysis of the borehole water showed an increase in the pH from 6.6 in week 0 to 5.6 in week 4 as shown in table 3. Akinde et al. (2011) and Agbaje et al. (2012) obtained similar results for stored sachet and borehole water samples. A low pH encourages corrosion of pipes while a pH above 7 requires more chlorine and contact time for proper disinfection. Turbidity values ranged from 5.30 in week

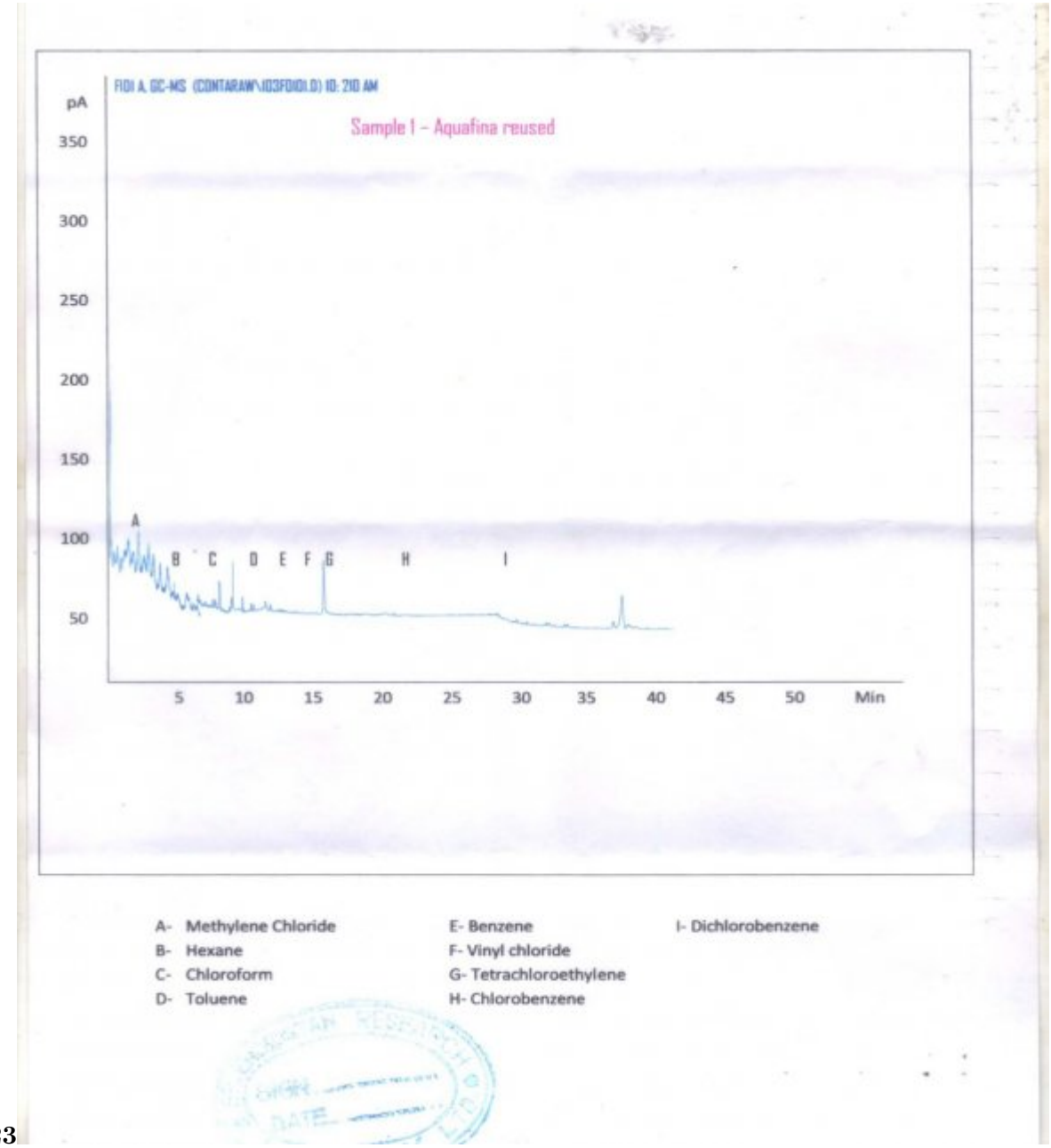
0 to 5.80 NTU in week4. These values were above the maximum acceptable limit of 5NTU recommended by WHO. This high levels of turbidity in plastic bottle filled with borehole water is a source of concern because the particles forming the turbidity could habour and shield pathogenic microorganisms and hence escape the action of disinfection **??**EPA, 2001). The total iron also increase from 1.031mg/l to 1.051mg/l in week 4 and exceeded the WHO recommended standard. This element is present in ground water in the soluble ferrous form (Fe 2+). It is easily oxidized to the insoluble ferric (Fe 3+) upon exposure to air.

Bisphenol A (BPA) is predominantly an intermediate to the production of other products. Its main use include binding, plasticizing and hardening functions in plastic products, paints/lacquers, binding materials and filling-in materials (Makinwa and Uadia 2015). However, exposure to BPA occurs primarily via hydrolysis of polycarbonate plastics and epoxy resins resulting in low concentration of free BPA in food and liquids thus making dietary consumption the major mode of human exposure **??**Wilson et al., 2007). As shown in table 5, the borehole water samples refilled into plastic containers contained high levels of BPA congeners ranging from 0.023mg/l to 0.251mg/l at week 4. This result is in tandem with **??**tuanya et al. (2016) who investigated the bioavailability of plastic contaminants and their effects on plastic bottled and sachet drinking water supplies. A progressive increase in the concentration of BPA congeners as storage increased was recorded. Although there was a significant higher levels, this result has shown that there was a gradual release of BPA congeners that increased with storage period

11 V. Conclusion

The result of this study has shown that the levels of bacterial population in borehole water stored at room temperature increased to maximum levels at the fourth week of water storage. It also showed that the bioavailability of bisphenol A components in borehole water, appeared to start manifesting at the fourth week of water storage. Storage temperature for long period plays a major role, creating impact on the acceptability of other organic constituents and enhancing the growth of microorganisms. Finally it is obvious from this study that BPA leaching from plastic containers into water can be affected by storage temperature and time, hence proper awareness should be created on the emergence of such an endocrine disrupting chemical.

12 Volume XVI Issue III Version I



123

Figure 1: Figure 1 :Figure 2 :Figure 3 :

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Water Samples

Figure 2: Table 1 :

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Figure 3: Table 2 :

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Parameters	Week 0	Week 1	Week 4	WHO (2011) water standards
pH	6.6	5,6	5.6	6.5-S.5
Temperature (°C)	32.9	32.2	32,5	
Conductivity (us/cm)	40.6	29.0	29.5	900
TDS (mg/L)	15.5	7.8	9.5	1000
TSS (mg/L)	7.5	6.0	3.5	
Turbidity	5.30	5.80	5.50	5
Alkalinity (mg/L)	8.0	12.0	8.0	
Total hardness(mg/L)	42.90	44.60	43.10	
Total iron(mg/L)	1.031	1.051	1.001	0.30
Chloride (mg/L)	10.6	11.5	9.7	250
Sulphate (mg/L)	8^22	8.23	8.21	400
Nitrate (mg/L)	0.22	0.24	0.22	50
Phosphate(mg/L)	102.0	115.2	96.8	6.5
BOD(mg/L)	26.4	36.0	33.6	

Figure 4: Table 3 :

4

Plastic bottle filled with borehole water		
	Week 0	Week 1
Total viable bacteria count	1.4 x 10 3	1.6 x 10 3 1.8 x 10 3
Total Coliform count	4.7 x 10 2	4.4 x 10 2 4.9 x 10 2
E. coli count	6.0 x 10 1	5.0 x 10 1 7.0 x 10 1
Fungal count	1.3 x 10 2	1.0 x 10 2 1.6 x 10 2

After subsequent cultural characteristics, morphology as well as biochemical tests, eight isolates were identified. Three of the isolates were fungal species. The isolates include: Klesiella sp., Escherichia coli, Acinetobacter sp., Bacillus sp., Pseudomonas aeruginosa, Aspergillus niger, Aspergillus flavus and Saccharomyces cerevisiae as shown in table 1 and 2

Figure 5: Table 4 :

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Characteristics	F1	F2	F3
Cultural characteristics	Greenish yellow colony with reverse side yellow	Medium creamy colony with convex elevation and entire margin	Black fluffy colony with reverse side yellow
Microscopic characteristics			
Nature of hyphae	Septate	Pseudohyphae	Septate
Colour of spore	Yellow	Cream Brown	
Type of spore	Conidiophores	Chlamydospore	Conidiophores
Appearance of special structure	Foot cells	Budding Foot cells	
Possible isolates	Aspergillus flavus	Saccharomyces sp.	Aspergillus niger

Parameter	Week 0	Week 1	Week 4	WHO Standard	Global Journal of
Methylene chloride	0.015	0.031	0.065		1.0
Hexane	<0.001	<0.001	<0.001		1.0
Chloroform	<0.001	0.001	0.001		1.0
Toluene	<0.001	<0.001	<0.001		1.0
Benzene	<0.001	<0.001	<0.001		1.0
Vinyl chloride	0.008	0.067	0.164		5.0
Tetrachloroethylene	<0.001	0.015	0.021		1.0
Chlorobenzene	<0.001	<0.001	<0.001		1.0
Dichlorobenzene	<0.001	<0.001	<0.001		1.0
Total (mg/l)	0.023	0.114	0.251		

Figure 6: Table 5 :

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