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BACTERIOLOGICAL AND PHYSICO-CHEMICAL ANALYSIS OF STORED BOREHOLE WATER IN BENIN CITY, EDO STATE, NIGERIA

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ABSTRACT

Water quality is of basic importance to human health, mostly in developing countries. Borehole water samples were collected from Ovia North-East, Oredo and Ikpoba Okha Local Government Areas of Edo State, Nigeria for physicochemical, bacteriological and Bisphenol A analysis for a period of 3 weeks. Bacterial load and physiochemical parameters were compared with the Nigeria Standard of Drinking Water Quality (NSDWQ) for potable water. Results showed that pH, turbidity, total suspended solids, biological oxygen demand (BOD) and conductivity reduced as storage increased. Magnesium and calcium for Oredo were found to have the highest value of 0.26 and 1.43 mg/l respectively. Total heterotrophic bacterial count (THBC) for the borehole water samples was found to be highest 9.53 $\pm$ 1.12 x 10^4 cfu/ml and the least 6.33 $\pm$ 0.57 x 10^4 cfu/ml after week 2 from Ikpoba Okha and Ovia North-East respectively. The total coliform count (TCC) ranged between 1.46 $\pm$ 0.15 x 10^4 to 8. 90 $\pm$ 0.82 x 10^4 cfu/ml. THBC and TCC were found to be higher than the NSDWQ Standard. The identified bacteral isolates from the borehole samples were Escherichia coli, Pseudomonas aeruginosa, Streptococcus faecalis, Bacillus cereus, Staphylococcus epidermidis and Serretia spp. Bisphenol A (BPA) detected after week 3 were methyl chloride, benzene and Dichlorobenzene and the highest values were 0.046 $\pm$ 0.003, 0.026 $\pm$ 0.003 and 0.053 $\pm$ 0.002 mg/l respectively. Storage of water for long periods should be discouraged as it's not microbiologically and chemically healthy.

1. INTRODUCTION

Good quality water is a basic factor in guaranteeing public health, protection of the environment and sustainable development (Rajini *et al*., 2010; Epundu *et al*., 2017). It is of basic importance to human physiology and man's continued existence depends very much on the availability of good quality water (FAO, 1997; Lamikara, 1999). By

2025, one-third of the population of the developing world will face severe water shortages due to pollution of water (Soto *et al*., 2005). Contaminated water is a global public health threat placing people at risk of a host of illness as well as chemical intoxication. The major risk to human health is faecal contamination of water supplies (Okonko *et al*., 2009; Eboh *et al*., 2017). A significant proportion of the world's population use potable water for drinking, cooking, personal and home hygiene (WHO, 2005). Before water can be described as potable, it has to comply with certain physical, chemical and bacteriological standards, which are designed to ensure that the water is safe for drinking (Tebutt, 1983; Epundu *et al*., 2017). The quality and quantity of available water have implication on the health status of a people. Over 50,000 people die daily due to water borne diseases (Marque *et al.,* 2003). About 2.3 billion people Worldwide have mortality and morbidity associated with water related ailment. In order to improve the bacteriological quality of water consumed by members of rural households, it is essential to address the quality of stored drinking water and the conditions under which the water supplies are stored (Aina *et al*., 2012). Storing of water in plastic containers for days or weeks increases bacteria load and leaching of Bisphenol A from the containers and as such reduces the quality of water (Jagals *et al*., 1999).

Bisphenol A (BPA) is widely used for mass production of plastic (polycarbonates) and epoxy resin (Staples *et al*., 1998; Makinwa and Uadia 2015). For many years, BPA was treated as a non-toxic compound, with no negative impact on humans and animals. BPA is act as intermediate in the production of products such as plastic cans, paints/lacquers, binding and filling-in materials (Staples *et al.,* 1998). BPA has been classified as a xenobiotic endocrine disruptor, disrupting the balance of the hormonal system of humans and animals research (Moriyama *et al*., 2002). However storage of water in BPA materials can lead to low concentration of the free chemical in water thus making the consumption a health problem (Makinwa and Uadia 2015). Stored water and Bisphenol A are like two components that go together when subjected to high temperature and stored over a long period of time (Wlodarczyk 2015). Therefore, auditing and monitoring of chemical and bacteria quality of drinking water is an essential aspect of water quality. Hence, this study focused on investigating the bacteriological quality and physico-chemical characteristics of borehole water stored over time.

2. MATERIALS AND METHODS

2.1. Sample Collection

Borehole Water samples were collected from Ovia North East, Ikpoba-Okha and Oredo local government areas in Edo State. The samples were stored into three different plastic cans which represented storage tanks. The water samples are designated as Sample A water from Ovia North-East, Sample B from Oredo and Sample C from Ikpoba Okha. The samples were kept outside the Laboratory, to be exposed to sunlight thus mimicking a typical condition at homes, and well sealed to avoid contamination. A glass jar was used at the point of collection for analysis. The samples were analysed for physicochemical, bacteriological and Bisphenol A composition.

2.2. Physicochemical Analysis

The physicochemical parameters analyzed were pH, chloride, sodium, electricity conductivity, iron, total dissolved, biochemical oxygen demand, total suspended solid, calcium, manganese, zinc, copper, sulphide and phosphorus according to the method described by APHA (1998) and Aydin (2007).

2.3. Isolation and Identification of Bacteria

Total heterotrophic bacterial and coliform load were enumerated using pour plate method on Nutrient agar (NA) and MacConkey agar respectively. The water samples were diluted serially (10-fold) under aseptic condition. An aliquot

of 0.1 ml was transferred aseptically into the plates and incubated at 37 ºC for 24 h after which colonies were counted. The pure cultures were then transferred into nutrient agar slants for biochemical test (Cheesbrough, 2000). Identification of isolates was based on cultural, morphological and biochemical characteristics following standard methods of Holt *et al.* (2000) and Garrity *et al.* (2005).

2.4. Analysis of Bisphenol A (BPA)

Fifty millilitres (50 ml) of water samples from the various borehole were measured and added to 100 ml of dichloromethane (DCM) via separating funnel and vortexed for 30 mins for BPA extraction (Dean and Xiong 2000). This separating funnel was clamp and themixture was allowed to separate out. After separation, the DCM portion was collected. The process was repeated three times for complete extraction (FAO, 1997). Blanks were prepared following the same procedure without the sample. The standard sample used for quality control was prepared by adding standard solution of BPA to DCM. All extracts were separated, and activated copper was added to the combined extract for desulphurization. After subsequent filter over anhydrous sodium sulphate, the solution was concentrated to 1.0 ml using a rotary evaporator. An internal standard mixture (vinyl chloride) solution was run with the extract for quality control check using Hewlett Packard 5890 series II gas chromatograph with mass selective (GC-MS) detection (Dean and Xiong, 2000). Hewlett Packard 5890 series II gas chromatograph equipped with an Agilent 7683B injector (Agilent Technologies, Santa Clara, CA, USA), a 30m, 0.25mm i.d. HP-5MS capillary column (Hewlett–Packard, Palo Alto, CA, USA) coated with 5% phenyl-methylsiloxane (film thickness 0.25um) and an Agilent 5975 mass selective detector (MSD) was used to separate and qualify the (BPA) compounds. The samples were injected in the split less mode at an injection temperature of 300ºC. The transfer line and iron source temperature were 280 ºC and 200 ºC. The column temperature was initially held at 40 ºC for 1 minute, raised at 120 ºC at the rate of 25 ºC/minute, then to 60 ºC at the rate of 10 ºC/minute, and finally to 300 ºC at the rate of 5 ºC/minute, held at the final temperature for 15 minutes. Detector temperature was kept at 280 ºC. Helium was used as a carrier gas at a constant flow of 1ml/minute. Mass spectrometry was acquired using the electron ionization (EI) and selective ion monitoring (SIM).

3. RESULTS AND DISCUSSION

In this study, borehole water samples collected from Ovia North-East (Sample A), Oredo (Sample B) and Ikpoba Okha (Sample C) were analyzed for physicochemical and bacteriological quality. The physicochemical analysis result is presented in Table 1. The result showed that all physicochemical parameters for sample A were in compliance with the Nigerian Standard of Drinking Water, while the ammonium (NH_3^+) and calcium (Ca^{2+}) content for samples B and C exceeded the maximum limit of the Nigerian Standard of Drinking Water. Also, the iron (Fe^{2+}) content for sample B exceeded the required limit. The pH value for sample B (6.6 ± 0.06 to 6.47 ± 0.97) exceeded the maximum limit of the Nigerian Standard of Drinking Water. The physiochemical parameters were critically analyzed for a period of 3weeks. There was gradual increase in the pH of water samples from week 0 to week 3, the pH of all samples ranged from 5.33-6.6 and in line with Nigeria Standard of Drinking Water Quality (NSDWQ) of 6.5-8.5. This shows that as storage time increases, there is an increase in pH. This is in agreement with Aydin (2007) who recorded increased pH as storage increases. The increased pH values could be due to the microbial activities or their death resulting in the release of inorganic substances like ammonia (Bisi-Johnson *et al.*, 2017). The pH of water samples at week 0 are not good for bacteria proliferation, a neutral pH will support growth of large numbers of bacteria (Madigan *et al.,* 2000). The turbidity of all water samples ranges from 0.07-0.6 ntu. There was decrease in turbidity as storage time increased. Low turbidity is often associated with low level of disease causing bacteria. Turbidity is a measure of cloudiness of water. At no time can turbidity go above 5NTU (USEPA, 2002). The total dissolved solids in Sample A and C reduced as storage time increased. Total dissolved solids (TDS) of all the samples were lower than NSDWQ Standard of 500mg/L. Total dissolved solids in drinking water has been associated with sewage urban runoff, natural sources, industrial waste water (NSDWQ, 2007). Total suspended solid (TSS) ranged from 0.25-2.68 mg/l. There was a

decrease in TSS as storage time increased. Biological oxygen demand (BOD) serves as an indicator of the biological quality of water. The biological oxygen demand (BOD) reduced as storage time increased and this could be as a result of the concentration of organic matter and the resident flora (Bisi-Johnson *et al.*, 2017). Chromium and Iron compositions increased at week 3 and the highest values (0.27 ± 0.01 and 0.74 ± 0.03 mg/l respectively) were detected in Sample B. High quantity of chromium and iron in water are toxic to humans. This water quality may be compromised through the mineralization of the soil particles that may be present in water (Ngah *et al.*, 2016)

The presence of sodium, calcium and magnesium salts in water helps in reducing incidence of cardiac disease (Mintz *et al.*, 1995). The iron content of the water samples in this study is in line with NSDWQ standard of 0.3 mg/l (NSDWQ, 2007).

Table 1: Physicochemical parameters of borehole water sample During Storage over a period of 3 weeks

Parameters	Sample A (week)		Sample B (week)		Sample C (week)		NSDWQ Maximum Standard
	0	3	0	3	0	3	
pH	5.45 ± 0.04	5.33 ± 0.03	6.6 ± 0.06	6.47 ± 0.97	5.51 ± 0.05	5.59 ± 10	6.5 - 8.5
EC (us/cm)	18.33 ± 2.52	26.66 ± 3.51	57.67 ± 2.65	67.00 ± 23.43	12.67 ± 2.08	20.66 ± 2.52	1000
Cl (mg/l)	6.23 ± 1.36	9.06 ± 1.19	19.68 ± 0.90	22.78 ± 0.86	4.31 ± 0.71	7.02 ± 0.86	250
TSS (mg/l)	0.37 ± 0.10	1.06 ± 0.07	1.15 ± 0.11	2.68 ± 0.05	0.25 ± 0.08	0.82 ± 0.05	NS
TDS (mg/l)	9.16 ± 1.26	13.33 ± 1.76	98.83 ± 1.32	33.5 ± 1.26	6.33 ± 1.04	10.33 ± 1.26	500
Turbidity (NTU)	0.11 ± 0.02	0.24 ± 0.02	0.36 ± 0.02	0.60 ± 0.02	0.07 ± 0.02	0.18 ± 0.02	5
BOD (mg/l)	1.24 ± 0.20	2.12 ± 0.70	2.51 ± 0.21	5.35 ± 0.11	0.59 ± 0.1	1.65 ± 0.05	NS
SO_4^{2-} (mg/l)	4.93 ± 0.73	7.73 ± 0.95	15.5 ± 0.77	19.43 ± 0.27	1.72 ± 0.00	5.99 ± 0.68	100
NO_3^- (mg/l)	3.3 ± 50	5.33 ± 0.63	10.38 ± 0.53	13.4 ± 0.45	3.42 ± 0.42	4.13 ± 0.45	50
PO_3^- (mg/l)	1.1 ± 0.23	2.4 ± 0.21	3.46 ± 0.24	6.03 ± 0.15	2.28 ± 0.19	1.86 ± 0.15	50
NH_3^+ (mg/l)	0.06 ± 0.20	0.18 ± 0.01	0.19 ± 0.02	0.45 ± 0.01	0.76 ± 0.02	0.41 ± 0.01	0.2
Ca^{2+} (mg/l)	0.44 ± 0.07	0.72 ± 0.85	1.43 ± 0.07	1.81 ± 0.06	0.04 ± 0.06	0.55 ± 0.06	0.4
Mg^{2+} (mg/l)	0.04 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.26 ± 0.01	0.03 ± 0.01	0.08 ± 0.01	0.2
Na^{2+} (mg/l)	1.61 ± 0.45	48 ± 0.31	5.07 ± 0.48	17.06 ± 0.22	0.32 ± 0.37	3.72 ± 0.22	200
Zn^{2+} (mg/l)	0.21 ± 0.04	0.42 ± 0.04	0.65± 0.04	1.07 ± 0.03	0.14 ± 0.04	0.33 ± 0.03	3
Cu^{2+} (mg/l)	0.18 ± 0.03	0.26 ± 0.04	0.60 ± 0.03	0.67 ± 0.03	0.15 ± 0.02	0.20 ± 0.03	1
Cr^{2+} (mg/l)	0.07 ± 0.01	0.11± 0.02	0.23 ± 0.01	0.27 ± 0.01	0.06 ± 0.01	0.08 ± 0.01	0.05
Fe^{2+} (mg/l)	0.20 ± 0.03	0.29± 0.04	0.61 ± 0.03	0.74 ± 0.03	0.15 ± 0.02	0.22 ± 0.03	0.3

The total heterotrophic bacteria count of borehole water from different locations are presented in Table 2. Sample C had the highest count for week 2 and 3 which were $9.53 \pm 1.12 \times 10^4$ and $4.26 \pm 0.42 \times 10^4$ cfu/ml respectively. The least bacterial count in week 2 was $6.33 \pm 0.57 \times 10^4$ cfu/ml from sample A. All the values for bacterial loads were found to be higher than NSDWQ maximum standard 1.00×10^2 cfu/ml. The result for coliform bacterial count revealed an increase in number during storage of water in plastic cans (Table 3). Sample C recorded the highest count for week 2 ($8.90 \pm 0.82 \times 10^4$ cfu/ml). The identified bacterial isolates in the borehole water samples (Table 4) were found to *Escherichia coli, Pseudomonas aeruginosa, Serretia* spp. and *Staphylococcus epidermidis.* The distribution of bacterial isolate in borehole water samples was investigated, and it was observed that *Escherichia coli* had the highest percentage occurrence with 30.5% and *Serretia* spp. had 11.1% with least percentage occurrence (Table 5).

The total heterotrophic bacteria count (THBC) measures a range of bacteria that are naturally present in the environment. The total bacterial counts for all the water samples were generally high exceeding the limit of 1.0×10^2 cfu/ml which is the standard limit of heterotrophic count for drinking water (NSDWQ, 2007). The high total heterotrophic count is indicative of the presence of high organic and dissolved salts in the water resulting to microbial growth (Erah *et al.*, 2002). Possible sources of contamination could be improper handling and contamination of water by organisms harboured in the connecting pipes during pumping of water from the borehole (Mgbakor *et al.*, 2011). Technically speaking, underground such as borehole is supposed to be the purest form of water (Olatunji *et al.*, 2015), due to soil purification properties. However, contamination of the water sample maybe improper construction,

proximity to toilet facilities, sewage, refuse dump-site and various human activities around the borehole sit (Ehiowemwenguan *et al*., 2014; Atuanya *et al*., 2016). The total coliform count for all samples were exceedingly higher than the NSDWQ of maximum contamination level (MCL) for coliform bacteria in drinking water of 0.02 x10^2 cfu/ml of water (NSDWQ, 2007). The high coliform count obtained in the samples may be an indication that the water sources are faecally contaminated (Ehiowemwenguan *et al*., 2014; Eboh *et al*., 2017). None of the water samples complied with NSDWQ standard for coliform in water. The presence of coliform indicates that all water samples are not fit for drinking and the observation in this study suggest that high heterotrophic count in water reflects high coliform count

Table 2: Total heterotrophic bacterial count in borehole water during storage (10^4 cfu/ml)

Week	Sample A	Sample B	Sample C
0	1.10 ± 0.60	6.57 ± 0.93	6.13 ± 0.12
1	3.30 ± 0.40	7.43 ± 0.67	2.61 ± 0.82
2	6.33 ± 0.57	8.70 ± 1.01	9.53 ± 1.12
3	2.13 ± 0.21	3.63 ± 0.31	4.26 ± 0.42

CFU = Colony Forming unit, A = Ovia North-East, B = Oredo, C = Ikpoba Okha

Table 3: Total coliform bacteria count in borehole water during storage (10^4 cfu/ml)

Week	Sample A	Sample B	Sample C
0	2.16 ± 0.37	4.01 ± 0.57	3.74 ± 0.12
1	1.66 ± 0.31	4.70 ± 0.46	6.16 ± 0.61
2	4.36 ± 0.42	6.76 ± 0.61	8.90 ± 0.82
3	1.46 ± 0.15	2.33 ± 0.21	3.09 ± 0.31

Table 4: Cultural, morphological and biochemical characterization of bacteria isolates in borehole water samples

Test	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5
Shape	Circular	Round	Round	Round	Round
Color	Milky	Creamy	Cream	Cream	Orange
Margin	Entire	Entire	Entire	Red	Lobate
Opaque	Opaque	transluscent	Opaque	Transparent	Opaque
Elevation	Flat	Flat	Flat	Flat	Flat
Wey/dry	Wet	Wet	Wet	Wet	Wet
Gram reaction	-	+	-	-	+
Shape	Rod	Cocci	Baccilli	Cocci	Baccilli
Arrangement	Single	In clusters	Single	Chains	Single
Catalase	+	+	+	+	+
Oxidation	-	-	-	-	+
Indole		-	+	-	+
Urease	-	-	-	-	-
Citrate	-	-	-	-	-
Coagulase		+	-	-	-
Spore	+	-	-	-	+
Fermentation					
Lactose	+	+	+	+	+
Sucrose	+	-	+	-	+
Sorbitol	-	-	-	-	+
Glucose	+	+	+	+	+
Manitol	-	-	+	-	+
Probable Isolate	*Pseudomonas*	*Staphyloccus*	*Escherichia*	*Serretia* spp.	*Bacillius*

Table 5: Percentage frequency of bacterial isolates in borehole water samples

Bacterial isolates	% frequency of occurrence
Bacillus cereus	13.80
Escherichia coli	30.50
Pseudomonas aeruginosa	19.40
Staphylococcus epidermidis	25.00
Serretia spp.	11.10

The presence of bacterial isolates from all water samples such as *Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Streptococcus faecalis,* and *Staphylococcus epidermidis* are of public health significance (Eboh *et al*., 2017). Faecal coliform presence such as *E. coli* indicate pollution of water by animal/humans faecal wastes and sewage (EPA, 2002; Eboh *et al*., 2017). *Staphylococcus epidermidis* is known to produce enterotoxin (Aydin, 2007). The presence of these bacterial species may be as a result of improper handling and poor sanitary conditions (Mgbakor *et al*., 2011). Isolation of *P. aeroginosa*, a pathogen known for its high resistance to antibiotics and a nosocomial infection is a source of concern (Mgbakor *et al*., 2011; Shittu *et al*., 2014). The isolation of pathogens in the water is an indication that sanitary measures should be put in place in order to avoid possible outbreak of bacterial infections (Epundu *et al*., 2017).

The Bisphenol A (BPA) composition in borehole water samples over time is presented in Table 6. From the result, it showed that there was an increase in BPA concentrations in the borehole water during storage. It was observed that methylene chloride and dichlorobenzene had the highest value of 0.046 ± 0.003 and 0.053 ± 0.002 mg/l respectively while benzene had the lowest value of 0.020 ± 0.002 mg/l while hexane, toluene and tetrachloroethylene had zero concentrations.

Bisphenol A (BPA) as a building block chemical, is used in epoxy resins, polycarbonate plastics and other industrial products (Elobeid *et al*., 2012). Trace amount of BPA was detected in the borehole water stored in the Jerry cans. It showed that BPA was liberated in to the stored cans and migrated into water samples. Storage of water in plastic containers is been recommended and kept in cool places away from Sun and outdoors, but not always the case. Water contamination by BPA can happen through leaching from containers due to organic compounds degradation taking place during storage (Elobeid *et al*., 2012) and the concentration of BPA released from storage can increased with time. Amiridou and Voutse (2011) reported that increase in BPA from water is due to photolysis under sun radiation. Essentially, the concentration of BPA that will leached from the containers to the water samples depends on the temperature and size of containers during storage (Wlodarczyk 2015). The BPA detected in this research work is in line with Vandenberg *et al.* (2007) who recorded increase in Bisphenol A composition as storage time increased.

Table 6: Bisphenol A composition (mg/l) in borehole water samples over time

BPA Composition	Sample A (weeks)		Sample B (weeks)		Sample C (weeks)	
	0	3	0	3	0	3
Methylene Chloride (mg/l)	0	0.026±0.003	0	0.024±0.003	0	0.046±0.003
Benzene (mg/l)	0	0.016±0.004	0	0.020±0.002	0	0
Dichlorobenzene (mg/l)	0	0.053±0.002	0	0.046±0.003	0	0.053±0.002
Hexane (mg/l)	0	0	0	0	0	0
Tetrachloroethylene (mg/l)	0	0	0	0	0	0
Toluene (mg/l)	0	0	0	0	0	0

A = Ovia North-East, B = Oredo, C = Ikpoba Okha

4. CONCLUSION

The investigation discovered that all borehole water is not microbiologically and chemically fit for drinking when stored overtime due to proliferation of bacteria and during photolytic formation of Bisphenol A components from the container. This research work has shown that there is need for an improvement in disinfection and cleaning of storage tanks, hence drinking water should be stored and used within days. There is also need for awareness programmes to be put in place to educate people on the possible health implications of drinking water which has been stored for a long time.

5. ACKNOWLEDGMENT

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6. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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