



## Impact of Bisphenol A on the Physicochemical and Bacteriological Characteristics of Water in Storage Tanks from various Locations in Salem University Lokoja, Kogi State, Nigeria

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**ABSTRACT:** This study examined the impact of Bisphenol A (BPA) on the physicochemical and bacteriological characteristics of water in storage tanks in Salem University Lokoja. Borehole water samples were collected from three (3) locations within the University environment and stored in a jerry can for analysis. Total Heterotrophic Bacteria Count (THBC) in water samples ranged from  $1.00 \pm 0.30 \times 10^4$  at week 0 to  $8.95 \pm 1.00 \times 10^4$  cfu/ml at week 3, while the total coliform count (TCC) also ranged from  $1.30 \pm 0.15 \times 10^4$  to  $7.11 \pm 0.82 \times 10^4$  cfu/ml. TCC and THBC were found to be higher than the NSDWQ Standard. The identified isolates from the borehole samples were *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus faecalis*, *Bacillus cereus*, *Staphylococcus epidermidis* and *Serratia* spp. Bisphenol A (BPA) was not detected in week 0, after week 3, components of Bisphenol A detected were methyl chloride, Benzene and Dichlorobenzene and their highest values were  $0.054 \pm 0.033$ ,  $0.021 \pm 0.020$  and  $0.055 \pm 0.062$  mg/l respectively. The pH, Turbidity, Total suspended solids, BOD and conductivity reduced as storage increased. Magnesium and calcium for sample B were found to have the highest value of 0.31 and 1.73 mg/l respectively. Storage of water for a long period of time should be discouraged as it could trigger increased leaching of BPA into the water which will affect its physicochemical and microbiologically quality.

DOI: <https://dx.doi.org/10.4314/jasem.v24i2.5>

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**Dates:** Received: 16 November 2019; Revised: 11 January 2020; Accepted: 22 February 2020

**Keywords:** Bacteria, Bisphenol A, Storage Water Tanks, Drinking Water Quality.

Good quality water guarantees public health, protection of the environment and sustainable development (Ranjini *et al.*, 2010). Water of good quality is of basic importance to human physiology and man's continued existence depends very much on its availability (Lamikara, 1999; FAO, 1997). By 2025, one-third of the population of the developing world will face severe water shortage (Sota *et al.*, 2005). Contaminated water is a global public health threat placing people at risk of a host of diarrhoeal and other illness as well as chemical intoxication. The major risk to human health is faecal contamination of water supplies (Okonko *et al.*, 2009). 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 potable and safe for drinking (Atuanya *et al.*, 2016). 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. Storing of water in tanks for days

or weeks increases bacteria load and leaching of Bisphenol A and as such reduces the quality of water (Jagals *et al.*, 1999; Adeghe and Emejulu, 2016). According to (Eniola *et al.*, 2007), storage generally reduces the numbers of bacteria which is in contrast to (WHO, 2005) which identified water as a major components for bacteria growth. Several technologies for the treatment of household water in developing countries have been developed to improve the chemical and bacteriological quality of the water and to reduce waterborne diseases (Mintz *et al.*, 1995). These technologies include physical methods such as boiling, heating, sedimentation, filtration, exposure to ultraviolet radiation from sunlight and chemical disinfection with agents such as sodium hypochlorite, chlorine (Mintz *et al.*, 1995; Sobsey, 2002). Bacteria reactivate faster in dechlorinated water than in chlorinated water (Sobsey, 2002). BPA is widely used for mass production of plastic (polycarbonates) and epoxy resin (Morrissey *et al.*, 1987; Staples *et al.*, 1998). For many years, BPA was treated as a non-toxic compound with no negative impact on humans and animals. BPA-based products were commonly used to mention lacquers for cans and vessels for storage of food, water, and medicines (Staples *et al.*, 1998). Since the second half of the 90-ties, numerous reports have arrived stating a negative influence of BPA on human health (Biles *et al.*, 1997; Del-Olmo *et al.*, 1997). BPA

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has been classified as xenobiotic endocrine disruptor, disrupting the balance of the hormonal system (Moriyama et al., 2002). Stored water and Bisphenol A are like two components that go together when subjected to high temperature and over a long period of storage (Del-Olmo et al., 1997). Therefore, auditing and monitoring of chemical and bacteria quality of drinking water is an essential aspect of water quality. Therefore, this study examined the physicochemical and bacteriological characteristics of water in storage tanks from various locations in the Salem University Lokoja, Kogi State.

## MATERIALS AND METHOD

**Sample collection and borehole water physicochemical analysis:** Borehole Water sample was collected from storage tank in three (3) different locations within the University premises: Hostel (Sample A), the Cafeteria (Sample B) and at the College of Natural and Applied Sciences (CNAS) (Sample C). The samples were spread into three different jerry can which represents our storage tank. The samples were kept outside and sealed to avoid contamination. A glass jar was used at the point of collection for analysis. The samples were analysed for their physicochemical, bacteriological and Bisphenol A composition. The physicochemical parameters analysed 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 (Aydin, 2007)

**Isolation and Identification of bacteria:** Nutrient agar was used in the isolation and enumeration of bacteria using the pour plate method. The pure culture was then transferred into nutrient agar slants for biochemical test. Nutrient and MacConkey agar were used to enumerate the bacteria in the water samples (Cheesbrough, 2000). Identification of isolates was based on cultural, morphological and biochemical characteristics following standard methods (Garrity et al., 2005; Holt et al., 2000).

**Analysis of Bisphenol A (BPA) in borehole water:** 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 0.25µm) 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 300°C. The transfer line and iron source temperature were 280°C and 200°C. The

column temperature was initially held at 40°C for 1 minute, raised at 120°C at the rate of 25°C/minute, then to 60°C at the rate of 10°C/minute, and finally to 300°C at the rate of 5°C/minute, held at the final temperature for 15 minute. Detector temperature was kept at 280°C. 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). Fifty ml (50± 0.01 ml) water was measured, and 100ml of dichloromethane (DCM) via separating funnel and shaken for 30mins for BPA extraction (Dean and Xiong 2000). This separating funnel was clamp and a mixture 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 (BPA) to DCM. All extracts were separated, and activated copper was added to the combine extract for desulphurization. After subsequent filter 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 5890 series II gas chromatograph with mass selective detection (GC-MS) (Dean and Xiong, 2000).

## RESULT AND DISCUSSION

The physicochemical analysis result is shown in (Table 1). The result showed that all physicochemical parameters for sample A were compliance with the Nigerian Standard of Drinking Water, while the ammonium ( $\text{NH}_3^+$ ) and calcium ( $\text{Ca}^{2+}$ ) content for sample B and C exceeded the maximum limit of the Nigerian Standard of Drinking Water. Also the iron ( $\text{Fe}^{2+}$ ) content for sample B exceeded the required limit. The pH value for sample B ( $6.6 \pm 0.06$  to  $6.47 \pm 0.97$ ) exceeded the maximum limit of the Nigerian Standard of Drinking Water. For total heterotrophic bacteria count, Sample B had the highest count which ranged from  $6.57 \pm 0.93$  to  $7.4 \pm 0.67$ cfu/ml between week 0 and week 1 while sample C had the highest count for week 2 and 3 which were  $8.95 \pm 1.00$ cfu/ml and  $4.26 \pm 0.42$ cfu/ml respectively (Table 2). The coliform bacteria count showed that Sample B recorded the highest count for week 0 which was  $4.01 \pm 0.57$ cfu/ml while sample C had the highest count for week 1, 2 and 3 which are  $6.16 \pm 0.61$ ,  $3.09 \pm 0.31$  and  $7.11 \pm 0.82$ cfu/ml (Table 3). Possible bacteria pathogens identified from the water sample were *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia* spp. and *Staphylococcus epidermidis* (Table 4). 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 *Serratia* spp. (11.1%) with least percentage occurrence (Table 5). The Bisphenol A contingents were identified from the water samples. It was observed that hexane, vinyl chloride, toluene and tetrachloroethylene were not detected in the water samples. Dichlorobenzene and methylene chloride had the highest value of  $0.055 \pm 0.062$  and  $0.054 \pm 0.033$  mg/l respectively while benzene had the lowest value of  $0.021 \pm 0.020$  mg/l (Table 6). The physiochemical parameters were critically analyzed for a period of 3 weeks. 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 to 6.6. The pH of Sample B is in line with Nigeria Standard of Drinking Water Quality (NSDWQ) of 6.5-8.5. This shows that as storage increases pH also increases this is in agreement with Aydin. (2007) who recorded increased pH as storage increases. The pH of Sample A and C for both week 0 and week 3 are below (NSDWQ). The pH of Sample B is in line with NSDWQ standard (NSDWQ, 2007). The pH of water samples at week 0 were suitable for bacteria proliferation, a neutral pH will support growth of a large number of bacteria (Madigan *et al.*, 2000). The turbidity of all water samples ranges from 0.07 to 0.6 ntu. There was decreased in turbidity as storage increased, low turbidity is often expected not to exceed 5 ntu (EPA, 2003).

The total dissolved solids in Sample A and C reduced as storage increased, an increase was observed in Sample B. 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 and industrial waste water (NSDWQ, 2007). This finding is contrary to the observation of (Ballester and Sunyer, 2000) whose TDS were in line with NSDWQ standard. Total suspended solid (TSS) ranged from 0.25-2.68 mg/l which decreased as storage increased. The biological oxygen demand (BOD) reduced also followed suit as there is no standard limit to BOD. Cadmium and lead were not detected in the water samples at week zero, but at week 3 components of cadmium and lead were detected (Table 1). 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). The chlorine content of the stored water ranges from  $4.31 \pm 0.71$  to  $19.68 \pm 0.90$  mg/l and  $7.02 \pm 0.86$  to  $22.78 \pm 0.86$  mg/l for week 0 and week 3 respectively.

Chlorine content in Sample A and B reduced as storage increased which was the same for Sample C which increased with time. Conductivity, sulphates, nitrates and phosphates reduced as storage increased in all the samples. The physiochemical parameters were compared to Nigeria Standard of Drinking Water Quality. The total bacterial counts for all the water samples were generally high and exceeded the limit of  $1.0 \times 10^2$  cfu/ml which is the standard limit of heterotrophic count for drinking water (NSDWQ, 2007).

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

Parameters	Sample A		Sample B		Sample C		NSDWQ Maximum Standard
	week 0	week 3	week 0	week 3	week 0	week 3	
pH	$5.45 \pm 0.04$	$5.33 \pm 0.03$	$6.6 \pm 0.06$	$6.47 \pm 0.97$	$5.51 \pm 0.05$	$5.59 \pm 10$	6.5 - 8.5
EC, us/cm	$18.33 \pm 2.52$	$26.66 \pm 3.51$	$57.67 \pm 2.65$	$67.00 \pm 23.43$	$12.67 \pm 2.08$	$20.66 \pm 2.52$	1000
Cl, mg/l	$6.23 \pm 1.36$	$9.06 \pm 1.19$	$19.68 \pm 0.90$	$22.78 \pm 0.86$	$4.31 \pm 0.71$	$7.02 \pm 0.86$	250
TSS, mg/l	$0.37 \pm 0.10$	$1.06 \pm 0.07$	$1.15 \pm 0.11$	$2.68 \pm 0.05$	$0.25 \pm 0.08$	$0.82 \pm 0.05$	NS
TDS, mg/l	$9.16 \pm 1.26$	$13.33 \pm 1.76$	$98.83 \pm 1.32$	$33.5 \pm 1.26$	$6.33 \pm 1.04$	$10.33 \pm 1.26$	500
Turbidity, ntu	$0.11 \pm 0.02$	$0.24 \pm 0.02$	$0.36 \pm 0.02$	$0.60 \pm 0.02$	$0.07 \pm 0.02$	$0.18 \pm 0.02$	5
BOD, mg/l	$1.24 \pm 0.20$	$2.12 \pm 0.70$	$2.51 \pm 0.21$	$5.35 \pm 0.11$	$0.59 \pm 0.1$	$1.65 \pm 0.05$	NS
SO <sub>4</sub> <sup>2-</sup> , mg/l	$4.93 \pm 0.73$	$7.73 \pm 0.95$	$15.5 \pm 0.77$	$19.43 \pm 0.27$	$1.72 \pm 0.00$	$5.99 \pm 0.68$	100
NO <sub>3</sub> <sup>-</sup> , mg/l	$3.3 \pm 50$	$5.33 \pm 0.63$	$10.38 \pm 0.53$	$13.4 \pm 0.45$	$3.42 \pm 0.42$	$4.13 \pm 0.45$	50
PO <sub>4</sub> <sup>3-</sup> , mg/l	$1.1 \pm 0.23$	$2.4 \pm 0.21$	$3.46 \pm 0.24$	$6.03 \pm 0.15$	$2.28 \pm 0.19$	$1.86 \pm 0.15$	50
NH <sub>4</sub> <sup>+</sup> , mg/l	$0.06 \pm 0.20$	$0.18 \pm 0.01$	$0.19 \pm 0.02$	$0.45 \pm 0.01$	$0.76 \pm 0.02$	$0.41 \pm 0.01$	0.2
Ca <sup>2+</sup> , mg/l	$0.44 \pm 0.07$	$0.72 \pm 0.85$	$1.43 \pm 0.07$	$1.73 \pm 0.06$	$0.04 \pm 0.06$	$0.55 \pm 0.06$	0.4
Mg <sup>2+</sup> , mg/l	$0.04 \pm 0.01$	$0.11 \pm 0.01$	$0.12 \pm 0.01$	$0.31 \pm 0.01$	$0.03 \pm 0.01$	$0.08 \pm 0.01$	0.2
Na <sup>+</sup> , mg/l	$1.61 \pm 0.45$	$48 \pm 0.31$	$5.07 \pm 0.48$	$17.06 \pm 0.22$	$0.32 \pm 0.37$	$3.72 \pm 0.22$	200
Zn <sup>2+</sup> , mg/l	$0.21 \pm 0.04$	$0.42 \pm 0.04$	$0.65 \pm 0.04$	$1.07 \pm 0.03$	$0.14 \pm 0.04$	$0.33 \pm 0.03$	3
Cu <sup>2+</sup> , mg/l	$0.18 \pm 0.03$	$0.26 \pm 0.04$	$0.60 \pm 0.03$	$0.67 \pm 0.03$	$0.15 \pm 0.02$	$0.20 \pm 0.03$	1
Cr <sup>2+</sup> , mg/l	$0.07 \pm 0.01$	$0.11 \pm 0.02$	$0.23 \pm 0.01$	$0.27 \pm 0.01$	$0.06 \pm 0.01$	$0.08 \pm 0.01$	0.05
Fe <sup>2+</sup> , mg/l	$0.20 \pm 0.03$	$0.29 \pm 0.04$	$0.61 \pm 0.03$	$0.74 \pm 0.03$	$0.15 \pm 0.02$	$0.22 \pm 0.03$	0.3

Note: NTU=Nephelometric Units, NS = No Standard, NSDWQ = Nigerian Standard of Drinking Water

**Table 2:** Total Heterotrophic Bacterial Count in Borehole Water during Storage in Jerry can ( $10^4$  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	4.7 ± 0.46	6.16 ± 0.61
2	4.36 ± 0.42	6.76 ± 0.61	7.11 ± 0.82
3	1.30 ± 0.15	2.33 ± 0.21	3.09 ± 0.31

Table 3: Total Coliform bacteria Count in Borehole Water During Storage in Jerry Can (10<sup>4</sup> cfu/ml).

Week	Sample A	Sample B	Sample C
0	1.00 ± 0.30	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	8.95 ± 1.00
3	2.13 ± 0.21	3.63 ± 0.31	4.26 ± 0.42

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

Test	Org 1	Org 2	Org 3	Org 5	Org 6
Shape	Circular	Round	Round	Round	Round
Color	Milky	Creamy	Cream	Cream	Orange
Margin	Entire	Entire	Entire	Red	Lobate
Opaque	Opaque	translucent	Opaque	Transparent	Opaque
Elevation	Flat	Flat	Flat	Flat	Flat
Wet/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	+	-	-	-	+
<b>Fermentation</b>					
Lactose	+	+	+	+	+
Sucrose	+	-	+	-	+
Sorbitol	-	-	-	-	+
Glucose	+	+	+	+	+
Manitol	-	-	+	-	+
<b>Probable Isolate</b>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus epidermidis</i>	<i>Escherichia coli</i>	<i>Serratia spp.</i>	<i>Bacillus cereus</i>

Table 5: Distribution of Bacterial isolate in Borehole Water Samples

Bacteria isolates	Frequency of occurrence	Percentage of occurrence (%)
<i>Bacillus cereus</i>	5	13.8
<i>Escherichia coli</i>	11	30.5
<i>Pseudomonas aeruginosa</i>	7	19.4
<i>Staphylococcus epidermidis</i>	9	25
<i>Serratia spp.</i>	4	11.1

Table 6: Bisphenol A Composition in Borehole Water samples over time

BPA Composition (mg/L)	Sample A		Sample B		Sample C	
	week 0	week 3	week 0	week 3	week 0	week 3
Methylene Chloride	0	0.026±0.027	0	0.024±0.027	0	0.054±0.033
Benzene	0	0.016±0.004	0	0.021±0.020	0	0
Dichlorobenzene	0	0.053±0.022	0	0.046±0.031	0	0.055±0.062
Hexane	0	0	0	0	0	0
Vinyl Chloride	0	0	0	0	0	0
Tetrachloroethylene	0	0	0	0	0	0
Toluene	0	0	0	0	0	0

The count is indicative of the presence of organic and dissolved salts in the water. From the result above, it was observed that in all water samples the mean total heterotrophic bacteria count ranges from  $1.00 \pm 0.30 \times 10^4$  to  $2.13 \pm 0.21 \times 10^4$  cfu/ml in Sample A,  $3.63 \pm 0.31 \times 10^4$  to  $8.70 \pm 1.01 \times 10^4$  cfu/ml in Sample B and  $2.61 \pm 0.82 \times 10^4$  to  $8.95 \pm 1.00 \times 10^4$  cfu/ml in Sample C which had the highest count (Table 2). The total coliform counts for all samples were exceedingly higher than the NSDWQ of maximum contamination level (MCL) for coliform bacteria in drinking water (NSDWQ, 2007). The high coliform count obtained in the samples may be an indication that the water sources are faecally contaminated (Obi and Okocha, 2007). None of the water samples complies with NSDWQ standard for coliform in water. Bacteria isolated from all water samples include *Bacillus cereus*, *Serratia* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus faecalis*, and *Staphylococcus epidermidis* which are also of public health significance. *Staphylococcus epidermidis* is known to produce enterotoxin (Aydin, 2007). 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. Presence of high coliform count in borehole water samples could be attributed to the proximity of the borehole near to a septic tank at a distance less than the 30m recommended by NSDWQ. The results above shows that the coliform population increased with time. There was alternate increase and decrease of Bacteria count in samples C as storage progresses this is in contrast to Orji *et al.* (2006) who recorded a direct increase of Bacteria count. Bisphenol A analysis at week zero shows that no BPA contingents were detected in all the water samples, but at week 3 there were detectable quantities of BPA components. This indicates that temperature and storage duration is a major factor in Bisphenol A degradation in storage tanks (Table 6). The composition of Bisphenol A found in week 3 is enough to cause serious health hazards when accumulated in the body. The BPA detected in this research work is in line with Vandenberg *et al.* (2007) who recorded increase in Bisphenol A contingents as storage time increases. The presence of potential pathogens such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia* spp. and *Staphylococcus epidermidis* (Table 4) in the water samples indicates the absence of sanitation on the part of the users.

**Conclusion:** This study shows that storage of water overtime leads to increased leaching of BPA which will encourage the proliferation of bacteria and also affect its physicochemical properties. In comparison to NSDWQ standard, all water samples studied were not

fit for drinking. High temperature and a prolonged storage have been implicated in the increased release of BPA in water tanks. Hence plastic borehole overhead tanks should not be directly exposed to the atmosphere as direct rays of the sun leads to fast leaching of toxic chemicals. This research 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 public awareness programmes to educate the public on the possible health implications of drinking water which has been stored for a prolonged time.

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