



Effect of Solar Water Disinfection on Microbial Load and Concentration of Bisphenol-A in Rivers State, Nigeria

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ABSTRACT: This research was aimed at determining the efficacy of solar radiation as a good source of reducing microbial load in water and the determination of Bisphenol- A concentration in bottled water and sachet water. The parameters evaluated are: Total bacterial count, Total fungi count, Salmonella Shigella, Coliform, Bisphenol A, pH, Dissolve oxygen, Total dissolve solid, Turbidity, total hardness. The bacteria genera isolated are; *Staphylococcus*, *Pseudomonas* sp, *Echerichia coli*, *Klebsiella* sp, *Salmonella* sp, *Bacillus* sp, *Micrococcus* sp, *Proteus* sp, *Corynebacterium* sp. The fungi genera isolated are *Penicillium* sp, *Aspergillus niger*, *Mucor* sp, *Cadida* sp, *Fusarium* sp. These organisms reduce as the number of hours of exposure increases. The Bisphenol A concentration increases as the exposure time increases. The study revealed that solar radiation (sunlight) is a good source of water disinfection. The study however recommends the use of PET Polyethylene Terephthalate bottle in water purification using sunlight since PET bottles contains much less additives than bottles made from Poly Vinyl Chloride (PVC)

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Water borne diseases occur mostly in the rural area of the world because the water sources are often contaminated by pathogenic microorganisms (Caslake, *et al.*, 2004). Water purification is essential to better health and for those in the rural area with low financial income it is difficult to apply advance water purification technologies. Solar disinfection of water (SODIS) is a practical technique that uses solar energy (combining heat and ultraviolet UV radiation) to inactivate and destroys microorganisms present in water (Burgess 2002). Convectional techniques for water disinfection include; ozonation, chlorination, distillation, reverse osmosis and electro dialysis (Ugwuoko, *et al* 2017). Chlorination which is the application of chloride in gas or diluted to water is effective against viruses and bacteria. However, the techniques have the disadvantage of leaving residual by product which is toxic to humans and also required skilled individuals for application. Boiling of water can be used at emergencies, requiring the supplied of

fuel wood as energy. Boiling techniques have the disadvantage of deforestation as well as non-application when toxic metal and chemicals such as lead, mercury asbestos, pesticides or nitrate contaminate the water source (Mcloughlin, *et al.*, 2004). The solar disinfection technique is cheap, efficient, useful renewable, environmental friendly and very simple for skilled and unskilled individual. It is a prefer technique that can be applied at household level (Narain, *et al.*, 2012) also for countries with adequate sunlight but lack safe water (Ciochetti and Metcalf, 1984). SODIS has a few limitations; such as weather dependent and also leaching of some chemicals into the water at high temperature (Omer *et al.*, 2016). Bisphenol A (BPA) is an organic chemical that is mainly used for the production of plastics (Omer *et al.*, 2016). BPA enhances plastic durability and heat resistance. Bisphenol A residue has been identified in plastic containers. While SODIS reduces the microbial load of water; what effect does it have

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on the Bisphenol-A concentration? The study is aimed at (1) To determine the microbial quality of water after Solar disinfecting. (2) Determination of bisphenol-A concentration in water samples exposed to direct sun light.

MATERIALS AND METHODS

Collection of Sample: Water sample (bottled and sachet water) were obtained from Uniport Bottling Company located in Unipark, Abuja, University of Port Harcourt, and water sample from boreholes obtained from Aluu and Choba community in Rivers State. The water samples collected were sent to the laboratory and used for the analysis.

Analysis: pH: The pH is measured using a veneer pH meter. The pH is determined using the procedure prescribed by APHA (1998).

Electrical conductivity: The electrical conductivity is done using a digital conductivity meter. [Standardization of the meter is prescribed using 0.1N KCl at 250C].

Turbidity measurement: This is conducted using a digital turbidity meter. The turbidity of each water sample is measured and recorded.

Total hardness: Determination of total hardness is conducted by measuring 10cm³ of water pipetted in a conical flask 1cm³ of buffer solution NH₄Cl with P H 10 and 3 drops of Erichrome black T indicator is added to the flask. The mixture is titrated with 0.01M Ethyl Diamintetra Acetic Acid (EDTA) until the colour changes from wine red to blue. The procedure is repeated two more times to obtain the average titre value.

Dissolved oxygen: The Alsterberg (Azide) modification of Winkler method is adopted to determine dissolve oxygen (DO).

Total dissolved solids: This is determined using a conductivity meter. The conductivity meter is switched to total dissolved solid 100cm³ of the sample is measured into a beaker and the electrode is introduced into the sample. The result of total dissolved solid is displayed and recorded.

Total Viable Cell Count: The purpose of this technique was to determine the total number of viable cells present in samples under examination. The pour plate method was used for this technique. MacCartney bottles containing 9 ml normal saline (0.09 % w/v NaCl) were arranged in three's and labelled accordingly 1:10, 1:100, and 1:1000). A 1 ml volume of the prepared water sample was aseptically

transferred into the 1:10 labelled MacCartney bottle. A three ten-fold serial dilution was carried out through the rest of the bottles in the sequence of 1:100 to 1:1000. A 0.1 ml inoculum of the diluted sample of the various concentration of 1:10, 1:100 and 1:1000 were aseptically inoculated into 20ml molten nutrient agar, which was swirled and aseptically plated into sterile petri dishes and were allowed to solidify. Plates were labelled in respect to the dilutions. This was done in duplicate. The plates were incubated in an inverted position at 37 °C for 24 hours. Plates were examined after the incubation period and results were recorded. This was repeated for the remaining water samples. The presence of colonies on the cultured media is indicative of viable cells, and the total viable cell count was determined using a bacterial colony counter.

Bacteriological Quality Assessment of Water Samples for The Presence of Enterobacteriaceae: Selenite-F broth is a selective growth medium for the isolation of Salmonella species obtained from fecal isolates. An inoculum from Gram negative isolate was streaked inoculated into a sterile 5 ml Selenite-F broth which was shaken to effect mixing and was incubated 37 °C for 24 hours. Bottles were examined after the incubation period, and results recorded. Positive bottles showed turbidity suspecting the presence *Salmonella typhi*.

Shigella-Salmonella Agar (SSA): A loopful of the content of the positive bottles was streaked on Shigella-Salmonella agar plates. Plates were labelled and incubated at 37 °C for 24 hours. Plates were examined after the incubation period and the colonial morphology of culture were determined and results recorded. Positive plates showed black distinct colonies.

Isolation of Fecal Coliform: Fecal coliforms were isolated using the "Most Probable Number (MPN) test.

MPN test were performed in 3 steps: (1) Presumptive test (2) Confirmatory test and (3) Completed test

Determination of Bisphenol A in exposed bottled water samples to direct sunlight using multi walled carbon nanotubes as solid phase extraction sorbent. Sample Preparation: A 50ml of sample is mixed with 100ml of Dichloromethane (DCM) and shaken for 45mins and then allow to separate, the dichloromethane portion is collected. This process is repeated for at least three times then the standard BPA (internal standard) is added to the DCM extract. The sample is mixed with anhydrous sodium sulfate, allowed to concentrate for 30 minutes minimum to 1.0 ml. The

blank was also prepared using the same method as the sample.

Procedure: GC–MS analysis was carried out using an Agilent 6890 gas chromatograph with a 5973 MS detector equipped 30-m x 0.25-mm or 0.32-mm ID fused-silica capillary column chemically bonded with SE-54 (DB-5 or equivalent), 1- μ m film thickness. (Agilent). The following temperature ramp was used: injector at 250 °C, oven initially at 200 °C, held for 1 min and heated to 230 °C (1.5 °C min⁻¹, then held for 10 min). The characterization and identification of BPA, from the sample was completed in the SCAN mode with the m/z range varied from 35 to 450. The flow rate of the nitrogen as carrier gas was 1 mL min⁻¹; manual injection; the injection volume was 1 μ L. The composition of the BPA from the sample was determined using an Agilent 6820 gas chromatograph equipped with 30-m x 0.25-mm or 0.32-mm ID fused-silica capillary column chemically bonded with SE-54 (DB-5 or equivalent), 1- μ m film thickness. Injection port. The initial oven temperature was 200 °C, which was held for 1 min, subsequently increased to 230 °C at 1.5 °C min⁻¹ and then held for 1 min. The injector was set at 250 °C, and the detector at 280 °C. Nitrogen was used as the carrier gas at a flow rate of 1 mL min⁻¹. The split ratio was 50:1, and the sample size was 1 μ L.

RESULTS AND DISCUSSION

The physiochemical test of various water sample revealed that Uniport Bottle water (BW) has the highest conductivity of light (16), followed by Choba (15), then Uniport Sachet water (14) and Aluu has the lowest conductivity (12). The pH analysis of the samples showed bottle water and sachet water to be within WHO standard (6.8 – 7.2) while borehole water was slightly below with the highest acidity of 5.6. Turbidity value have bottle water recording the lowest turbidity (0.08 NTU) while borehole water Choba has the highest turbidity rate of 0.84 NTU. All water samples have a turbidity level lower than the

maximum require turbidity level for solar disinfection (30 NTU) (Udounwa and Osuji., 2005) thus having less particulate matter requiring higher temperature (Kehoe *et al.*, 2001; McGuigan *et al.*, 1999). The heterotrophic bacteria count as shown in Fig. 1 the microbial load of each sample decreases in number as hours of exposure increases. At six (6) hours of exposure, all samples showed no visible growth of microorganism. The coliform count (Fig. 2) also decreases in number as hours of exposure increases. At six (6) hours of exposure all samples showed no growth. The most prevalent isolated bacteria gotten from these samples were: *Staphylococcus*, *Pseudomonas* sp, *Echerichia coli*, *Klebsiella* sp, *Salmonella* sp, *Bacillus* sp. *Micrococcus* sp. *Proteus* sp. *Corynebacterium* sp. While the most prevalent fungi are *Penicillium* sp, *Aspergillus niger*, *Mucor* sp. *Cadida* sp. *Fusarium* sp. These organisms have been implicated in cases of water related diseases like diarrhoea (E coli), Typhoid (*Salmonella*) (Ciochetti and Metcalf, 1984) *Escherichia coli* is an indicator organism suggesting faecal contamination (Udounwa and Osuji 2005, Oyedeji *et al.*, 2010). The mean percentage reduction of total heterotrophic bacteria over the duration of solar exposure is 86.7 %, the remaining 13.3 % present after exposure is below 10³ colony forming units per millilitre while Coliform has a mean percentage reduction of 100 %. The fact that the study reveals 100 % reduction in Coliform is significant because zero percentage of *E. coli* is permissible; this is in agreement with work of other researchers (Conroy *et al.*, 1999). Statistically there were no significant different at $p \geq 0.05$ between means of total heterotrophic bacteria count of borehole (BH/c) samples, bottle water (BW) samples and sachet water samples (SW). The volume of *Staphylococcus* sp in the sample during solar exposure is less than *Salmonella* sp while *Pseudomonas* sp, *Echerichia coli*, *Klebsiella* sp, are the same (Table 1). For the fungi *Aspergillus niger* is less than *Penicillium* sp (Table 2) suggesting that solar radiation affected various organisms differently.

Table 1: Physiochemical Analysis of Water Sample

Sample Identity	Conductivity	Total hardness (mg/l)	TDS (mg/l)	DO (mg/l)	Turbidity (NTU)	pH
BHa	10	0.40	60	6.4	0.52	6.2
BHa	12	0.40	60	8.0	0.32	5.6
BW 1	10	0.43	60	8.0	0.08	6.8
BW 2	16	0.43	60	6.8	0.68	6.6
SW 1	13	0.41	60	6.8	0.72	6.9
SW 2	14	0.41	60	6.8	0.41	6.7
BHc	15	0.40	60	8.0	0.84	6.5
BHc	11	0.40	60	8.0	0.78	6.4

Key: BHa =Borehole water Aluu, BW = Bottle water, SW = Sachet water, BHc = Borehole water Choba

The Bisphenol analysis (Table 3) revealed that the Bisphenol-A concentration in the sachet and bottle

water sample gotten from Uniport Bottling Company located in Unipark, Abuja, University of Port Harcourt

before exposure of the water sample to sunlight was 0.167mg/L and 0.196 mg/L respectively. After exposure of the water samples to sunlight, the quantitative concentration of Bisphenol-A in the bottled water sample increased to 5.461mg/L while that of the sachet water sample increased to 7.129mg/L (Table 3) having a percentage increase of 96.4 % and 97.7 % respectively. The study revealed that the longer the time of exposure of the water samples to sunlight, the higher the concentration of Bisphenol-A in the sample this correspond with studies of other researchers (Omer et al., 2016).

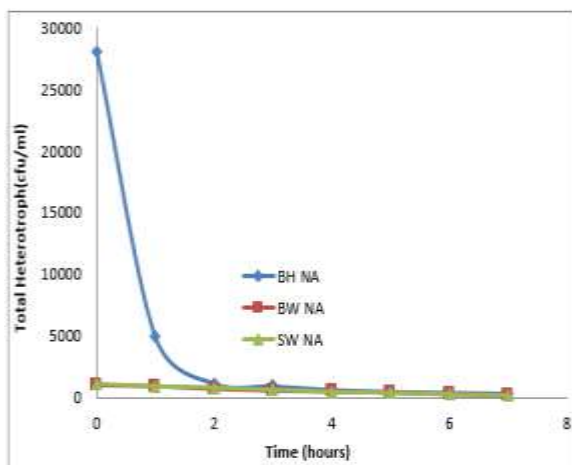


Fig. 1: Total Heterotrophic bacteria count of various sample over time. Key: BH= Borehole water, BW = Bottle Water, SW = Sachet water

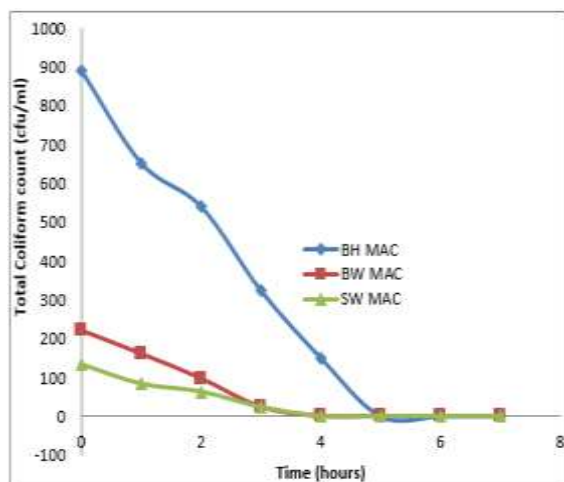


Fig. 2: Coliforms count of various water samples over time. Key: BH= Borehole water, BW = Bottle Water, SW = Sachet water

The following Bisphenol-A and it's derivatives were detected; Methylene Chloride (0.150 mg/L), Vinyl Chloride (0.036 mg/L) Benzene (BDL) Toluene (0.010), Bis (4 – hydroxyphenl) methane (BDL), Bis (4 – hydroxyphenl) sulfone (BDL), 1,1-Bis (4 – hydroxyphenl) 1-phenyl ethane (BDL), 1,1-Bis (4 –

hydroxyphenl) ethane (BDL), 2,2-Bis (4 – hydroxyphenl) butane (BDL), 2,2- Bis (4 – hydroxyphenl) propane (BDL) for water samples in bottles before solar exposure, After solar exposure samples recorded Methylene Chloride (0.174 mg/L), Vinyl Chloride (0.439 mg/L) Benzene (0.105) Toluene (1.826), Bis (4 – hydroxyphenl) methane (0.625), Bis (4 – hydroxyphenl) sulfone (0.159), 1,1-Bis (4 – hydroxyphenl) 1-phenyl ethane (1.186), 1,1-Bis (4 – hydroxyphenl) ethane (0.224), 2,2-Bis (4 – hydroxyphenl) butane (0.597), 2,2- Bis (4 – hydroxyphenl) propane (0.126).

Table 2: Frequency of different bacteria in the samples during the time of exposure

Hour	<i>Penicillium</i> sp	<i>Aspergillus</i> sp
0	+	+
1	+	-
2	+	-
3	+	-
4	+	-
5	-	-
6	-	-
7	-	-
8	-	-

Table 3: Frequency of the fungi present in the samples during the time of exposure

Hour	<i>Staphylococcus</i> sp	<i>Pseudomonas</i> sp	<i>E. coli</i>	<i>Klebsiella</i> sp	<i>Salmonella</i> sp
0	+	+	+	+	+
1	+	+	+	+	+
2	+	+	+	+	+
3	+	+	+	+	+
4	-	+	+	+	+
5	-	+	+	+	-
6	-	-	-	-	-
7	-	-	-	-	-
8	-	-	-	-	-

Sachet water sample before solar exposure has the following Biphenyl-A and it,s derivative concentration; Methylene Chloride (0.026 mg/L), Vinyl Chloride (0.057 mg/L) Benzene (BDL) Toluene (0.084), Bis (4 – hydroxyphenl) methane (BDL), Bis (4 – hydroxyphenl) sulfone (BDL), 1,1-Bis (4 – hydroxyphenl) 1-phenyl ethane (BDL), 1,1-Bis (4 – hydroxyphenl) ethane (BDL), 2,2-Bis (4 – hydroxyphenl) butane (BDL), 2,2- Bis (4 – hydroxyphenl) propane (BDL) after solar exposure Methylene Chloride (0.328 mg/L), Vinyl Chloride (1.659 mg/L) Benzene (0.182 mg/L) Toluene (0.539 mg/L), Bis (4 – hydroxyphenl) methane (1.267 mg/L), Bis (4 – hydroxyphenl) sulfone (0.155), 1,1-Bis (4 – hydroxyphenl) 1-phenyl ethane (1.180 mg/L), 1,1-Bis (4 – hydroxyphenl) ethane (0.944 mg/L), 2,2-Bis (4 – hydroxyphenl) butane (0.130 mg/L), 2,2- Bis (4 – hydroxyphenl) propane (0.745 mg/L). Statistical analysis of the results gives positive correlation (+1)

between Bisphenol-A concentration and the duration of solar radiation exposure, showing that as the time of exposure increases; the concentration of Bisphenol A in the water sample increases. This result is in agreement with studies of other researchers (Omer et al., 2016).

Table 4: Mean concentration of Bisphenol A in water samples

Sample	Before Exposure (0hr)	After Exposure (3hrs)
Sachet water	0.167	7.129
Bottle water	0.196	5.461

The microbial load gives a negative correlation (-1) to the duration of solar radiation exposure, as the time of exposure increases the bacteria count decreases. This corresponds with our results (Table 1 and 2) and also in agreement with studies of other researchers (Conroy et al., 1999, Caslake et al., 2004; Udounwa and Osuji 2005; Narain et al., 2012). There is negative correlation (-1) between Bisphenol-A concentration and the microbial load in the various samples over solar radiation exposure time. This shows that as the microbial load decreases over the solar radiation exposure time; the concentration of bisphenol-A increases suggesting leaching from the plastic container into the water sample as duration of solar radiation exposure increases.

Bisphenol-A exposure has been linked to a number of disease conditions such as cancer, diabetes, obesity and reproductive disorders (Sonavane and Gassman, 2019). Research shows that bisphenol-A leaches from the plastic bottles (Omer et al., 2016) especially when there is an increase in temperature. Bisphenol-A influences some hormones receptor (estrogen and thyroid) altering their functions (Kim and Part, 2019; Morgan et al., 2017).

The study revealed an increase in the concentration of bisphenol-A with increase in temperature while increase in temperature leads to reduction in microbial load showing effectiveness of solar radiation. But, there is the need to monitor the concentration of bisphenol-A, since 5 mg per kg of bodyweight per day is considered safe (FDA, 2014). There is need for more research to understand if there is truly a permissible safe limit for bisphenol-A with regard to human health. Individuals should limit the use of plastics, make use of bisphenol-A free plastic and glass bottles or stainless steel containers.

Conclusions: Contaminated water can be purified by using sunlight as the UV rays reduces the microbial load of the sample to a point where the water could be used without threat of epidemic outburst of disease.

While exposing water samples can reduce the microbial load, this research further revealed that the water sample should not be exposed to sunlight when contained in nylon bags or plastic bottles made from PVC— Polyvinylchloride as the Bisphenol-A chemical used to prevent this substances from rusting can easily diffuse into the water and the increased concentration of it is harmful to the human body.

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