



ANTIMICROBIAL EFFICACY OF SOLAR DISINFECTION OF SELECTED DRINKING WELL WATER IN BENIN CITY, NIGERIA

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ABSTRACT

Solar Disinfection (SODIS) has been identified as a relatively cheap method of purifying water against pathogens, therefore providing potable drinking water, an essential component upon which living systems and the human body depends. In this study, the antimicrobial efficacy of solar disinfection of selected drinking well water was studied. A total of thirty (30) wells was randomly sampled from six (6) local government areas in Benin City, based on accessibility and communal usage. The well water was sampled using transparent 1L polyethylene terephthalate (PET) bottles. Standard microbiological and biochemical test procedures were performed to enumerate, isolate, characterize and identify the isolates to genus level. Antibiotic susceptibility of the isolate was also ascertained. Coliform test was conducted and counts expressed in Most Probable Number (MPN). The study revealed that *Staphylococcus sp.*, *Micrococcus sp.*, and *Klebsiella sp.* and, *Penicillium sp.*, *Fusarium sp.* and *Candida sp.* were the most prevalent heterotrophic bacteria and fungi isolates in the study area in addition to fecal indicators (*Escherichia coli* and *Fecal streptococci*) with 83.33% occurrences respectively. The study also revealed that the overall percentage elimination of the identified isolates was recorded as 42.11%, while the percentage elimination of fecal indicators identified was 50%. It was observed that SODIS was very efficacious with a percentage reduction of above 95% for all identified isolates. It was also observed that SODIS is slightly more bactericidal as compared to its being fungicidal. From the study, it could not be ascertained with certainty whether or not, exposure to SODIS alters a microbial pathogens' antibiotic susceptibility. The use of SODIS did not completely eliminate all the fecal coliforms found in the studied well water, therefore making it unfit for drinking with regards to WHO recommendation. Consequently, the study recommends the use of SODIS only in conjunction with other water purification methods to ensure potability.

Keywords: Antimicrobial, Benin City, Bottles, Coliforms, Disinfection, Efficacy, Fecal, Pathogens, PET, SODIS, Solar, Water, Well

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INTRODUCTION

Water is an essential component upon which living systems and the human body depends. Moreover, the quality and potability of water is of immense importance for the optimal functioning of the human body. The WHO recommends that drinking water from any source including wells must be free from microbial pathogens for it to be considered safe for consumption. Due to pollution of drinking water with common sources of contaminants, potable water has become a rare commodity and of public health concern (Kuruf *et al.*, 2010). A quick review of literature reveals that most sources of drinking water in Nigeria are polluted with both solid and liquid contaminants. Microbial pathogens especially coliforms are frequently isolated from various water sources including well water which exceeds WHO recommendation (Obafemi *et al.*, 2018).

However, well water serve as a very cheap source of community water supply in most African and Asian countries. Well water is classified as ground water and as such, the type and level of contamination in these water sources largely depends on the edaphic, topographic and anthropological characterization of the of the location which influences and contribute to quality of the water (Palamuleni and Akoth, 2015).

Anthropogenic activities have been largely implicated with the pollution of most water bodies. Human activities such as the indiscriminate disposal of inorganic and microbial matter on land surface or through direct injection of pollutants into water bodies (Sur *et al.*, 2006). In addition, ground water sources such as well water are largely contaminated by the practice of poor hygiene in most rural areas. Practices such as the indiscriminate use of contaminated fetching barrels or buckets to draw-up water from deep wells, coughing, sneezing and chatting while fetching water have being identified as potential sources of well water contamination (Obafemi *et al.*, 2018). Such contaminated well water adversely impacts the health and wellbeing of people who ingests them without proper treatment (Obire *et al.*, 2008).

Various forms of treatment are employed all around the world to improve on the potability of drinking water. At the community and household level, common water treatment methods such as; boiling, use of alum, de-coloration, chlorination and filtration abounds. In Nigeria, boiling is the commonest method employed in household water treatment because it is relatively fast, efficient and comparatively cost-effective. However, with the current increase in cost of burning fuel, most people are now seeking more cost-effective ways of treating domestic water. One of such low-cost alternatives is the use of the solar radiation.

Solar Disinfection (SODIS) has been identified as a relatively cheap method of purifying water against pathogens (Burch and Thomas, 1998). This water treatment method is a very simple process involving the use of the sun's radiation to hamper the growth and destroy pathogenic microorganisms present in water. The use of sun's radiation, which is universally available for all to use, is a free gift of nature. Being free of charge, is the basis of its being a low-cost and sustainable form of water treatment (Odeyemi *et al.*, 1988). Disinfection of water using the sun's rays has been carried out historically since the Egyptian times. The London-based scientists, Downes and Blunt in the late 1870s where the first to build an experimental setup to study the process which was reported in scientific literature thereafter for the first time (Byrne *et al.*, 2011). Solar Disinfection also known as SODIS is best used for treating small quantities of drinking water with an un-universally agreed recommendation of two (2) litres per person per day and for small family use; approximately 10-15 litres per family per day (Wegelin *et al.*, 1994). The process of solar disinfection of water, is a relatively simple, cheap, efficient, useful, renewable and environmentally friendly process.

The process employs the combination of the sun's heating potential and its ultraviolet (UV) radiation to sufficiently inactivate and destroy pathogenic microorganisms present in a water sample (Burgess, 2002). Consequently, this study was conducted to evaluate the antimicrobial efficacy of solar disinfection of selected drinking well water in Benin City, Nigeria.

MATERIALS AND METHODS

STUDY AREA

This study was conducted in Benin City located in the south-south geopolitical zone of Nigeria. Benin City is bounded by latitudes $06^{\circ} 06' N$, $06^{\circ} 30' N$ and longitudes $05^{\circ} 30' E$, $005^{\circ} 45' E$ and the capital of Edo State. Benin City falls within the tropical equatorial zone dominated by dry season (November – March) and wet seasons (April – October). The average maximum and minimum temperatures are $34^{\circ}C$ and $17^{\circ}C$ in February and December respectively, with an average annual variation of $4^{\circ}C$. The City is underlain by sedimentary formation. Its ground water aquifer for wells ranges from $28.4 m^3 hr^{-1}$ at Iyanomo (south), $125m^3 hr^{-1}$ at Uselu (central) to $208m^3 hr^{-1}$ at Ogba (northern) with a draw down ranging from 4.8m at Iyanomo, 1.8m at Uselu to 6.7m at Ogba (Short and Stauble, 1967). Samples were collected from the six (6) local government areas within the city viz; Egor, Uhumwonde, Ikpoba-Okha, Oredo, Ovia South-West and Ovia North-East. All samples were qualitative researched at the Microbiology Departmental Laboratory, University of Benin, Benin City. (Adebiyi *et al.*, 2020).

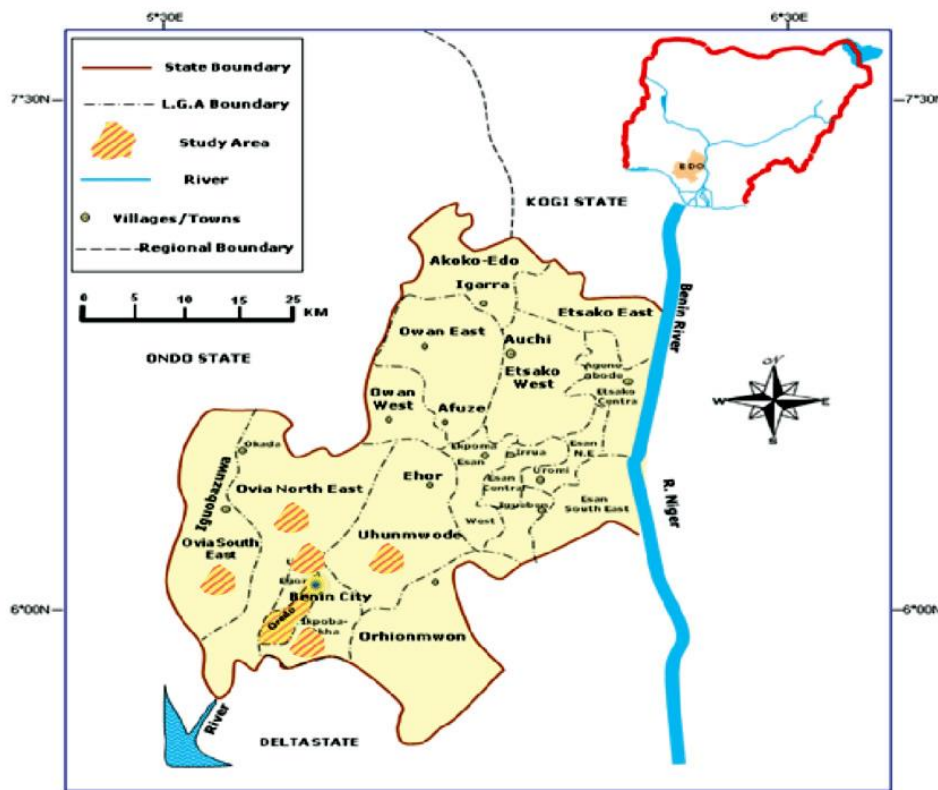


FIG.1 MAP OF EDO STATE SHOWING THE STUDY AREA

Figure 1: Map of Edo State showing the case study area. Source: Ministry of Lands and Survey, Benin City 2009

SAMPLE COLLECTION

A total of thirty (30) wells was randomly sampled from the six (6) local government areas (LGAs), based on accessibility and communal usage, with five (5) samples from each. Thirty (30) transparent 1L polyethylene terephthalate (PET) bottles were purchased from a local dealer, washed with hypo bleach, soaked overnight in Brine, rinsed with distilled water and stored in sterile polyethylene bags for later use. Well water samples were collected using the prepared PET bottles tied with a strong string to a piece of pre-sterilized metal weighing 500g. The bottle cap was aseptically removed and the weighted bottle lowered into the well to a depth of about 1-2 meters. The bottle was brought up to the surface and covered with a screw cap when no air bubbles were seen inside (Aboh *et al.*, 2015). The collected samples were immediately labelled, wrapped with black polyethylene bag to prevent photocatalytic reactions and placed in ice-packed containers and sent to the laboratory for analysis.

LABORATORY ANALYSIS

The collected samples were taken to the laboratory for analysis with emphasis on the bacteriological quality of the water samples. Standard microbiological and biochemical test procedures were performed to enumerate, isolate, characterize and identify the isolates to genus level (Cheesbrough, 2000; 2002). Antibiotic susceptibility of the isolate to common antibiotics using Kirby-Bauer disc diffusion method was also carried-out. (Bauer *et al.*, 1996) Coliform test was conducted using MacConkey broth in Durhams tubes while employing Multiple Tube Fermentation technique/ (MPN - Most Probable Number). The technique used for water analysis was the MPN (American Public Health Association 1998) (Feng *et al.*, 2014). Using the McCradys probability tables, the MPN of coliforms in the 100 ml well water sample was estimated (Mercy *et al.*, 1998). These procedures were performed before (pre) and after (post) exposure to SODIS.

PROCEDURE FOR SOLAR DISINFECTION (SODIS)

Using the Google Weather Forecast App, a very sunny day for the study was picked with a forecasted clear sky, high temperature, low humidity and very low chance of precipitation. A total of thirty (30) transparent PET bottles was employed, of which, five (5) contained well water samples from each LGA. Of these; the content of two (2) bottles were used as “control” following the processes described above. The remaining three (3) bottles were used for SODIS procedure. Of these three (3) bottles, two (2) bottles were designated “Test-bottles” and the last one designated “Temp-bottle”. The contents of the Test-bottles were used for SODIS laboratory analysis, while the Temp-bottles were used to determine the temperature of the exposed water during SODIS with the help of a thermometer. Test samples were drawn from the test-bottles before and after exposure to SODIS. During the period of exposure, the solar radiance intensity (Average Peak Radiation Intensity (W/m²)) was measured using radiation sensors. During SODIS, the bottles were placed on the roof of the laboratory (made of old, rusted, brown-colored aluminum roofing sheets) from 8am-4pm (8hrs) on the day of the study. Evaluation of the antimicrobial efficacy of solar disinfection (SODIS) of the sampled drinking well water was ascertained by assessing variation in the MPN value, re-occurrence of identified isolates and the isolates antibiotic susceptibility before (pre) and after (post) exposure to solar disinfection (SODIS).

STATISTICAL ANALYSIS

Various statistical analysis was carried out to analyses the resulting data using Statistical package SPSS Version 22.0 and Microsoft Excel version 2015. Results are expressed in percentages, frequencies and graphical representation. (Ogbeibu, *et al.*, 2015).

RESULTS AND DISCUSSION

TEMPERATURE AND AVERAGE PEAK RADIATION INTENSITY

Table 1 shows the mean temperature and average peak radiation intensity on SODIS bottles on the study day. The highest temperature reading was observed at midday with mean value of 56°C. The average peak radiation intensity recorded was 653.0W/m².

Table 1: Mean Temperature and Average Peak Radiation Intensity on SODIS bottles

Mean Temperature (°C)	Average Peak Radiation Intensity (W/m ²)
56.0	653.0

MICROBIAL ISOLATES DISTRIBUTION

Table 2 shows the distribution of microbial isolates from the sampled well water in the six LGAs of Edo State. It was observed that the among the isolated heterotrophic bacteria, *Staphylococcus sp.*, *Micrococcus sp.*, and *Klebsiella sp.* were the most prevalent in addition to the global fecal indicators (*Escherichia coli* and *Fecal streptococci*) with 83.33% prevalence. Similar results were also observed by Ogueri *et al.*, 2020, who conducted a similar research on the “Bacteriological Quality of Borehole and Sachet Water from a Community in Southeastern Nigeria”. Among the fungi isolates, *Penicillium sp.*, *Fusarium sp.* and *Candida sp.* were most prevalent with 83.33% frequency of occurrence. It was also observed that Egor LGA had the highest prevalence of the isolates with frequency of 78.95%. A similar result was reported by Erah *et al.*, (2002) who studied the “The Quality of Groundwater in Benin City: A baseline study on inorganic chemicals and microbial contaminants of health importance in boreholes and open wells”. In this study, the researchers observed that Okhoro District in Benin City had very high levels of microbial contamination in their open wells. Coincidentally, this district is within the Egor LGA. One possible reason for this high microbial prevalence may be due to relatively shallower nature of the wells in this LGA. It was observed that only Ikpoba-Okha (*Fecal streptococci*) and Ovia South-West (*Escherichia coli*) LGAs had only one fecal indicator each as opposed to all other LGAs that had both. The high number of heterotrophic bacterial count observed in the study area indicates that the groundwater in that area may be densely contaminated (Nwankwo *et al.*, 2019).

Table 2: Distribution of microbial isolates from sampled well water in six LGAs of Benin City, Nigeria

Isolates	Local Government Area						Frequency of isolates (%)
	Egor	Uhunmwonde	Ikpoba-Okha	Oredo	Ovia South-West	Ovia North-East	
Bacteria							
<i>Staphylococcus sp.</i>	+	-	+	+	+	+	83.33
<i>Clostridium sp.</i>	-	+	+	+	-	+	66.67
<i>Salmonella sp.</i>	+	+	-	+	-	-	50.00
<i>Micrococcus sp.</i>	+	+	+	-	+	+	83.33
<i>Serratia sp.</i>	+	+	+	-	+	-	66.67
<i>Bacillus sp.</i>	+	-	+	-	+	+	66.67
<i>Shigella sp.</i>	-	+	-	+	-	-	33.33
<i>Klebsiella sp.</i>	+	+	+	+	-	+	83.33
<i>Proteus sp.</i>	-	+	-	+	-	-	33.33
<i>Citrobacter sp.</i>	+	-	+	-	+	-	50.00
Fecal Indicators							
<i>Escherichia coli</i>	+	+	-	+	+	+	83.33
<i>Fecal streptococci</i>	+	+	+	+	-	+	83.33
Fungi							
<i>Aspergillus sp.</i>	+	+	-	+	+	-	66.67
<i>Penicillium sp.</i>	+	+	+	+	+	-	83.33
<i>Rhizopus sp.</i>	+	-	+	+	-	+	66.67
<i>Fusarium sp.</i>	+	+	+	-	+	+	83.33
<i>Trichoderma sp.</i>	-	+	+	-	+	-	50.00
<i>Cladosporium sp.</i>	+	-	-	+	-	+	50.00
<i>Candida sp.</i>	+	+	-	+	+	+	83.33
Frequency per LGA (%)	78.95	73.68	63.16	68.42	57.90	57.90	X ² =5.0000 p=0.9989*

X²= Chi square; *=not significant; LGA=Local Government Area

OCCURRENCE OF ISOLATE BEFORE AND AFTER SODIS

Table 3 shows occurrence of identified microbial isolates before and after SODIS. It was observed that the among the identified heterotrophic bacteria, *Clostridium sp.*, *Salmonella sp.*, *Shigella sp.*, *Proteus sp.* and *Citrobacter sp.* were un-detectable after exposure to SODIS. The fecal indicator (*Fecal streptococci*) was also un-detectable. Among the identified fungi isolates, only *Rhizopus sp.* and *Candida sp.* became un-detectable. This goes to show that SODIS was more efficacious against bacteria as compared to fungi isolates. This might be attributed to the presence of spore and other vegetative forms of fungi which helps build resistance to radiations and adverse environmental conditions (Shuryak, 2019). The overall percentage elimination of the isolates identified in this study was recorded as 42.11%. While the percentage elimination of fecal indicators identified was 50%.

Table 3: Occurrence of microbial isolates before and after SODIS

Isolates	Before	After
Bacteria		
<i>Staphylococcus sp.</i>	+	+
<i>Clostridium sp.</i>	+	-
<i>Salmonella sp.</i>	+	-
<i>Micrococcus sp.</i>	+	+
<i>Serratia sp.</i>	+	+
<i>Bacillus sp.</i>	+	+
<i>Shigella sp.</i>	+	-
<i>Klebsiella sp.</i>	+	+
<i>Proteus sp.</i>	+	-
<i>Citrobacter sp.</i>	+	-
Fecal Indicators		
<i>Escherichia coli</i>	+	+
<i>Fecal streptococci</i>	+	-
Fungi		
<i>Aspergillus sp.</i>	+	+
<i>Penicillium sp.</i>	+	+
<i>Rhizopus sp.</i>	+	-
<i>Fusarium sp.</i>	+	+
<i>Trichoderma sp.</i>	+	+
<i>Cladosporium sp.</i>	+	+
<i>Candida sp.</i>	+	-
% Elimination of Isolates		42.11

MICROBIAL POPULATION OF THE WATER SAMPLES

Table 4 shows the Most Probable Number (MPN) count of the isolates per 100ml of the sampled water. It was similarly observed that Egor LGA had the highest count for all isolates. Again, this might be attributed to the relatively shallower nature of the wells in this LGA. This assertion is corroborated by Rajasekaran and Abinaya (2014) who posited that anthropogenic sources, such as seepages from domestic sewages and livestock wastes can also affect the quality of shallow groundwater sources in many parts of the world.

Table 4: Microbial population of the water samples across six LGAs of Benin City, Nigeria

LGAs	Isolates		
	MPN count for bacteria (per 100 ml)	MPN count for fungi (per 100 ml)	MPN count for coliforms (per 100 ml)
Egor	402	220	18
Uhunmwonde	346	184	7
Ikpoba-Okha	303	202	13
Oredo	268	106	15
Ovia South-West	188	87	9
Ovia North-East	132	103	12

MPN = Most Probable Number

MICROBIAL POPULATION BEFORE AND AFTER EXPOSURE TO SODIS

Table 5 shows the Most Probable Number (MPN) count of the isolates per 100ml before and after exposure to SODIS. It was observed that SODIS was very efficacious with a percentage reduction of above 95% for all identified isolate. It was also observed that SODIS is slightly more bactericidal as compared to its being fungicidal. However, it was observed in this study that SODIS did not completely eliminate coliforms as it recorded 97.30% reduction. As a result, the disinfection process did not meet the WHO standard of zero (0) coliforms per drinking water sample. A similar result was observed by Ojo *et al.* (2011) who reported that coliforms in his study area (Ibadan, a relatively close by state to Edo State) were high in all the examined wells with values above the WHO standard.

Table 5: Microbial population before and after exposure to SODIS

Isolates (MPN)	Exposure to SODIS		% Reduction
	Before	After	
Bacteria	1639	36	97.80
Fungi	902	29	96.78
Coliform	74	2	97.30

ANTIBIOTIC SUSCEPTIBILITY PATTERN OF ISOLATES BEFORE AND AFTER SODIS

Figure 2 shows the antibiotic susceptibility pattern of the isolates before and after SODIS. The figure shows that *Staphylococcus sp.* and *Escherichia coli* exhibited low antibiotic susceptibility before and after exposure to SODIS. Thus, SODIS did not alter the antibiotic susceptibility pattern of these isolates. However, *Micrococcus sp.*, *Serratia sp.*, *Bacillus sp.* and *Citrobacter sp.* showed a remarkable increase in antibiotic susceptibility after exposure to SODIS. However, this result could not be attributed with certainty to the effect of SODIS since this result was not observed for all the isolates tested.

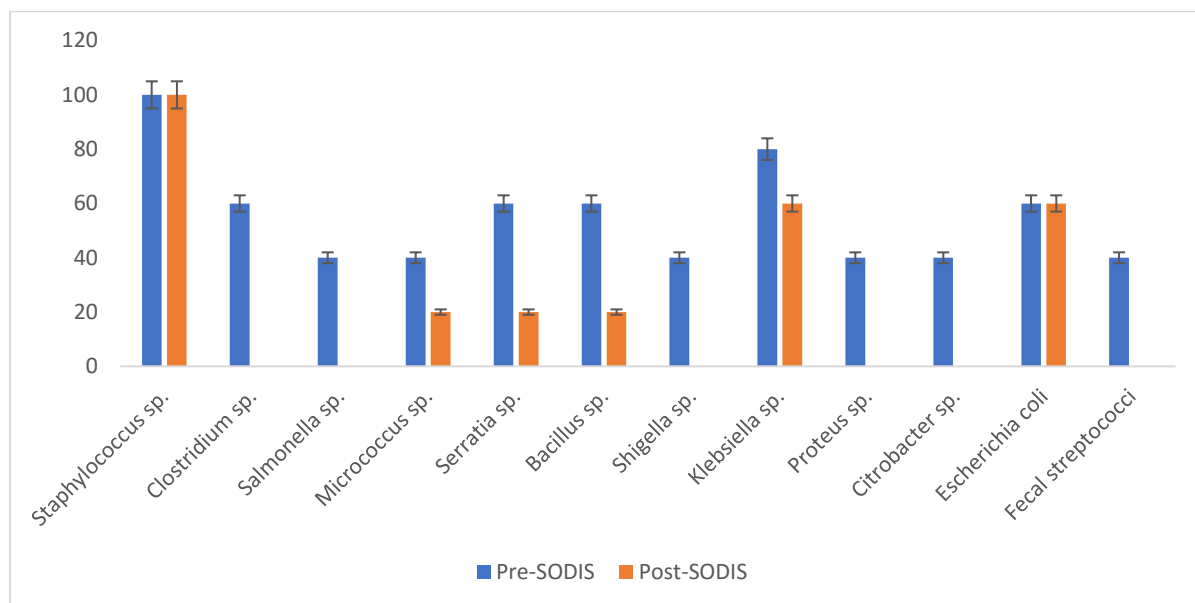


Figure 2: Percentage antibiotic susceptibility pattern of isolates before and after SODIS

CONCLUSION

Water is an essential component upon which living systems and the human body depends. Moreover, the quality and potability of water is of immense importance for the optimal functioning of the human body. Well water serve as a cheap source of community water supply. The WHO recommends that drinking water from any source including wells must be free from microbial pathogens for it to be considered safe for consumption. Solar Disinfection (SODIS) has been identified as a relatively cheap method of purifying water against pathogens. In this study, the antimicrobial efficacy of solar disinfection of selected drinking well water was studied. The study revealed a plethora of microbial contamination of the well water in the study area including fecal coliforms. The use SODIS had a moderate elimination efficacy on the identified isolates, though with a very high percentage reduction in the isolate count. From the study, it could not be ascertained with certainty whether or not, exposure to SODIS altered a microbial pathogens' antibiotic susceptibility. The use of SODIS did not completely eliminate all the fecal coliforms found in the studied well water, therefore making it unfit for drinking with regards to WHO recommendation. Consequently, the study recommends the use of SODIS only in conjunction with other water purification methods to ensure potability.

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