



## EFFECTS OF SOLAR DISINFECTION ON *ESCHERICHIA COLI* AND *KLEBSIELLA PNEUMONIAE* ISOLATED FROM WATER SAMPLES

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### ABSTRACT

Solar disinfection of water is the use of solar radiation to eliminate or reduce the microbial load in water sample, producing disease-free water. Samples of water were collected from Kogi State University, Anyigba, Nigeria and analysed for the presence of coliform bacteria. Coliform bacteria like *Escherichia coli* and *Klebsiella pneumoniae* were isolated from the water. Disinfection of the water sample was done by subjecting the water samples contained in plastic polyethylene tetrachloride (PET) bottles to ultraviolet (UV) radiation from the sun at temperatures of about 45°C and for six hours. The two coliform organisms isolated from the water samples; *Escherichia coli* and *Klebsiella pneumoniae*, show significant sensitivity for the solar disinfection (SODIS) method. However terminal disinfection was achieved for *K. pneumoniae* and about 91 % reduction was achieved for *E. coli*. Temperature readings determined before and after disinfection are 25°C and 45°C respectively while pH readings was 7.8 before and after disinfection. This work shows that population of bacteria in water samples especially coliforms which are the index of population could be drastically reduced when subjected to solar disinfection method.

**Key words:** Disinfection, Solar radiation, Coliforms, Water sample.

### INTRODUCTION

Good quality water is odourless, tasteless and free from faecal pollution (Shiklomanov, 2000). Thus, the accessibility and availability of fresh clean water is a key to sustainable development and an essential element in health, food production and poverty reduction (Third World Water Forum on Water, 2003). A reliable supply of clean wholesome water is highly essential in a bid to promoting healthy living amongst the inhabitant of any defined geographical region (Mustapha and Adam, 1991).

Solar disinfection (SODIS) uses the sun's energy to provide an economically feasible means of providing safe drinking water. SODIS is a free and effective method for decentralised water treatment usually applied at the household level and is recommended by World Health Organization as a viable method for household water treatment and safe storage. This technology was pioneered in the late 1970s at the American University of Beirut, Lebanon, to find an inexpensive disinfection method for oral rehydration solutions (Acra *et al.*,

1984). Their exciting results gave birth to a new disinfection technique. The SODIS system consists of these basic steps: Removing solids from highly turbid water by settling or filtration, if necessary, placing low turbidity water, less than 30 nephelometric turbidity unit (NTU) in clear plastic bottles and aerating (oxygenating) the water by vigorous shaking in contact with air furthermore, exposing filled aerated bottles to full sunlight for about 6 hours (depending on the intensity of the sun). To ensure UV-A radiation overpowers pathogenic cellular defence mechanisms; a sunlight intensity of 500 W/m<sup>2</sup> should be applied for 3 to 5 hours to induce lethal effects (SODIS News No. 1, 1998). UV-A also creates highly reactive oxygen species as a secondary disinfection product in a process called photo-oxidative disinfection. UV-induced reactive oxygen species can be lethal if they are present in numbers higher than the organism is capable of attenuating. Natural dissolved organic matter can absorb ultraviolet radiation to induce photochemical reactions (Miller, 1998). The

energy transfer of a high-energy photon to absorbing molecule produces highly reactive species such as superoxide ( $O_2^-$ ), hydrogen peroxides ( $H_2O_2$ ), and hydroxyl radicals ( $OH^\cdot$ ) (Miller, 1998; Reed, 1997a), these reactives in turn oxidize microbial cellular components such as nucleic acids, enzymes, and membrane lipids, which kill the microorganisms (Reed, 1996; Reed, 1997b; McGuigan *et al.*, 1999). However, in their defence, microorganisms have evolved powerful scavenging activity toward various reactive oxygen species (Yun and Lee, 2000). Microbes cope with hydrogen peroxides using two groups of enzymes called catalases and peroxidases. Catalases eliminate hydrogen peroxide while peroxidase uses the reducing power of nicotinamide adenine dinucleotide.

It has been observed that water temperatures between 20°C and 40°C do not affect the inactivation of *Escherichia coli* by sunlight (Wegelin *et al.*, 1994). However, synergistic effects are observed at a water temperature of 45°C (McGuigan *et al.*, 1998). To increase thermal effects, bottles are painted black at the bottom. The half-blackened SODIS bottles increase the temperature by approximately 5°C. Additionally, placing the bottles on dark surfaces will also help heat the water and produce thermal effects (EAWAG/SANDEC, 1998). Indicator organisms are the most commonly used gauges of SODIS success. To assure a safe water supply, it is critical to monitor for the presence of these pathogens i.e. the coliforms. However, it would be expensive and time consuming to check the water supply for all of them; instead, an indicator organism is used to assay for faecal contamination. *Enterobacter* and *Klebsiella* are able to survive and multiply in the environment and are therefore not the best indicators of fecal pollution. The sole habitat of *E. coli* and *K. pneumoniae*, termed fecal coliforms, is the intestines of warm blooded animals. Thus, fecal coliforms are good indicators of fecal pollution and can be differentiated from other coliforms by incubating on selective media at 44.5°C. To help eliminate possible false positives, coliform of only faecal origin can be used. These organisms consist of the *Escherichia* and *Klebsiella* genera.

A conventional technology used for disinfection of unpotable water includes ozonation, chlorination, filtration and boiling (Pelizzetti, 1999). None of these methods however, is entirely free from practical problems like taste from using boiling

method and chlorination, and cancer from chlorination. These calls for the development of an alternative disinfection technology that is effective, practical, and simple enough to be applied by individuals at the household level. Under the right conditions, solar water disinfection, or SODIS, could solve the problem. The objective of this study is to expose water samples collected in PET bottles to solar radiation in order to achieve disinfection of the water sample.

## MATERIALS AND METHODS

### Collection of samples

The site of sample collection is Kogi State University, Anyigba, Nigeria. Four samples of water were collected aseptically from borehole into sterile Polyethylene tetrachloride (PET) bottles. They were first filled to about  $\frac{3}{4}$  of the filling capacity, thereafter aerated by shaking vigorously, before filling to full capacity. Thereafter were transferred quickly to the laboratory for the isolation of coliforms and their population determination, before exposure to solar radiation. (Ciochetti and Metcalf, 1984).

### Microbiological analysis

The Multiple tube fermentation test method for enumeration of total coliform and *Escherichia coli* (APHA, 1998), was used to assess the bacteriological quality of water samples. MacConkey broth was used for presumptive test. The confirmatory test was done by re-inoculating the positive tube from presumptive test into a confirmatory broth and the MPN index per 100ml of sampled water was obtained with reference to MPN tables. Acid and gas formation confirmed positive test. Eosin methylene blue (EMB) agar was used for the completed test and was incubated for 24-48 hrs at 44°C

### Solar disinfection of water samples using PET bottles

The water samples contained in PET bottles of two litres capacity were exposed to ultraviolet rays from the sun at temperatures of about 45°C, and in a slant position. After about five hours of exposure, the samples were taken to the laboratory for analysis. Coliform count was determined after the exposure to sunlight.

## RESULTS.

The mean counts of the two coliforms isolated from the four water samples collected before and after disinfection are shown in the table 1 below.

**Table 1: Mean counts of coliforms isolated from the water samples**

Coliforms isolated	Mean counts (cfu/ml) (before disinfection)	Mean counts (cfu/ml) (after disinfection)
<i>Escherichia coli</i>	5.25x10 <sup>5</sup>	5.0x10 <sup>4</sup>
<i>Klebsiella pneumoniae</i>	2.0x10 <sup>5</sup>	0

The temperature and pH of the water samples collected before and after disinfection are shown below in table 2:

**Table 2: Temperature and pH determination of water samples before and during/after disinfection.**

	Temperature (°C)	pH
Before disinfection	25	7.8
After disinfection	45	7.8

## DISCUSSION

The borehole water in Kogi State University (KSU), Anyigba, Nigeria had very low turbidity therefore a pre-filtration step was not necessary and SODIS was directly applied. The presence of *E. coli* in this water samples is a strong indicator of recent sewage or animal waste contamination which could be through improper handling of the water, and animals which graze around the area. *Escherichia coli* were less sensitive to the lethal effect of UVR. This is in agreement to Acra *et al.* (1984) which suggest that *E. coli* should be used as an indicator organism due to its relative resistance to lethal effect of UVR. This could be as a result of deletion of genes for adenylate cyclase or cAMP protein in *E. coli* which confers a phenotype that includes resistance to UVR.

The mean counts of the two coliform bacteria isolated from the water sample before and after disinfection shown in the table 1 concurs with Yukselem *et al.*, (2003) reports which show that solar radiation methods when adequately applied could yield significant results in coliform bacteria mortality. Terminal disinfection in this research was achieved for *Klebsiella* while about 91% reductions in population were achieved for *E. coli*. However, increase in exposure time could further decimate this organism in the water. The temperature at which disinfection was carried out was 45°C (Table 2). Rijal and

Fujioka (2001) reported that for terminal disinfection of microorganisms in drinking water using solar heat a minimum disinfection temperature of 60°C must be reached. This temperature however was not achieved during the period of this work as the research was carried out during the raining season. The pH however at 7.8 was almost adequate for disinfection to take place.

## CONCLUSION

For the principle behind SODIS to be effective, the environment must be sunny and hot enough, (preferably in the dry season) water must be clear enough to allow the light to penetrate, and the type of bottle being used must not substantially hinder these processes. In addition, for this technology to become a reality, people must be able to afford it, and they must believe in it, or it would never be applied. It is very attractive as it could provide a safe source of water at the cost of a plastic bottle. It is hoped that this relatively new disinfection method will produce an economically and technically friendly technology to improve water quality and public health in KSU, Anyigba, and by extension, Nigeria.

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