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Photo-Degradation of Microbes in Hand-Dug Well-Water Using Different Radiation Sources

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***Abstract*-**Microbial bacteria in well-water have been treated using three different radiations and efficiency of treatment determined. The radiations were SUV (Sun with UV); SNoUV (Sun with NoUV, sun with UV portion filtered out) and LEDUV (radiations from LED source). Reduction in the Raman water peak at 526 nm and DOM fluorescence intensity at 550 nm indicate that the DOM experienced photo-degradation when exposed to radiation. A first-order kinetic equation was used to model this decline and degradation rate constant K calculated from the slope of the resulting natural-log graph. The half-life was determined. At 526 nm and 550 nm fluorescence peaks trend downward with time, indicating a reduction in DOM. Sample exposed to SUV showed greatest reduction and SNoUV the least. SUV had least half-life, and registered shortest time in reducing the DOM to one-half its initial value (fastest sample purification rate) while SNoUV registered the highest half-life. There was a count of coliform bacteria and Total Heterotrophic Bacteria using culture techniques before and after exposure to the radiations. Using the sum of all the microbes, the first-order kinetic equation was used to model the decrease with time and degradation rates K and half-life calculated. Exposure to SUV showed fastest decline and SNoUV slowest. This confirmed findings in LIF study, with the order of efficiency of radiation sources also in agreement with findings of the LIF. A sample was kept in the dark and analysed. For that, its fluorescence peaks fluctuated and showed just a minimal reduction in the microbes.

***Keywords*-** *Coliform Bacteria, DOM Fluorescence Intensity, Half-Life, Raman Water Peak, Total Heterotrophic Bacteria*

I. INTRODUCTION

The importance of water cannot be overemphasized as all living things need it to survive. It is one of the most abundant substances on earth and covers nearly 75% of the earth surface. Of this, about 97% is saline and resides in the ocean, with only 3% being fresh water, and even then, just

1% of fresh water is accessible and stored as aquifers, ground water or surface water [1]. In most parts of the world, majority of people living in rural areas rely on rainfall, hand-dug wells or boreholes (ground water), and other small water bodies (surface water) as their principal sources of water for their daily use. Such water, unfortunately, is often contaminated by micro-organisms such as pathogenic enteric bacteria, viruses, intestinal parasites and other unwanted chemical substances. Evidences have been documented concerning fatalities associated with polluted and bad-water consumption and therefore the quality of water has gained a critical attention as it is a powerful determinant of health [2].

The concept of water purification, which involves the removal of all contaminants to make it safe for use, has existed over the years, with new and more efficient techniques constantly being developed. Recently, ultraviolet (UV) radiation disinfection has attracted attention as it has several advantages over other purification methods. For example, distillation, which deals with evaporating water and collecting the distillate, has a low efficiency and harmful to the human body when consumed as the process demineralizes the water [3]. The chlorination technique of water treatment also leaves behind disinfection by-products (DBPs) which have adverse birth outcomes and results in bladder cancer [4]. Cryptosporidium can also not be treated using the chlorination technique, but easily inactivated in the presence of UV radiation [5].

UV radiation treatment involves exposing organism in contaminated water to radiation within the UV region (UVC 100 nm - 290 nm; UVB 290nm - 320 nm; UVA 320 nm - 400 nm) of the electromagnetic spectrum. To kill microorganisms, the UV radiation strikes and breaks through the outer cell membrane of the microorganisms and passes through the cell-body. This puts its DNA in disorder, thereby thwarting reproduction. DBPs are not formed as nothing is added except for the energy which does the killing. UV radiation has the potential of causing destruction to a lot of bacteria species, mold spores, algae, virus, and yeast based on the dose of energy delivered to the organism by the radiation [6]. Solar purification of water, which makes use of UV radiation from the sun, is currently being used as a treatment method, and the desire for constant supply of UV radiation for water treatment has led to the construction of UV-light emitting diode (LED) purification systems [7].

This study provides an in-depth knowledge on the effectiveness of treatment of pathogens or microbes in well-water using three distinct radiation sources, vis-à-vis; solar radiation with UV radiation, solar radiation without UV radiation (UV portion filtered out) and UV radiation from an LED source.

II. MATERIALS AND METHODS

A. Study Area

The well-water used for this study was fetched from a well drilled at Amamoma, a community located in the Central Region of Ghana (05° 06′ 00″ N, 01° 15′ 00″ W). This community has a large number of students of the University of Cape Coast, and as a result of lack of water, most inhabitants within this community depend on hand-dug wells for water. Figure 1 is a composite figure in which (a) is a map of Ghana showing the Central Region, (b) depicts the study area Amamoma and (c) illustrates the area details within Amamoma indicating the investigated well.

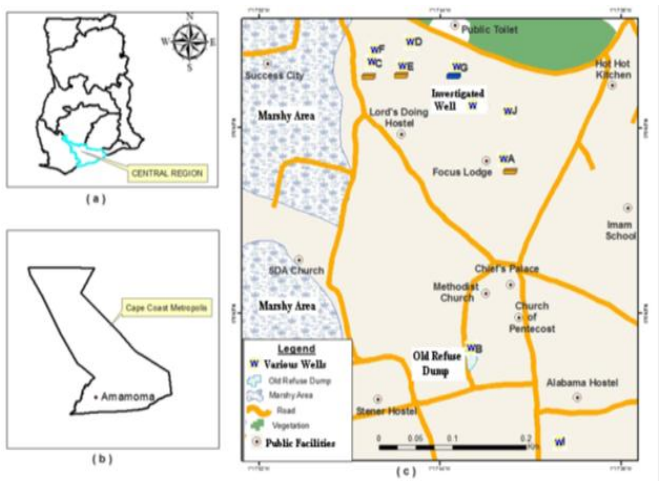

Figure 1. (a) Map of Ghana showing the Central Region, (b) Study area Amamoma, (c) Details within Amamoma showing the well from which the water sample was fetched.

B. Overview of Materials and radiation sources

Three different radiation types were used; (a) radiation labelled SUV (Sun with UV), which are radiations from the sun. For this, the sample was stored in a material (Material A) which is completely transparent to all radiations from the sun and therefore allowed the transmission of all incident wavelengths. The optical properties of this material were determined. (b) radiation labelled SNoUV (Sun with NoUV) which involved the exposure of the sample to radiation from the sun, but with the UV portion filtered out. The filtering was achieved by housing the sample in a material (Material B) that selectively absorbs all UV (UVB and UVA) radiation but transmits all other wavelengths. This optical property was also confirmed in this study. (c) radiation labelled LEDUV which involved the exposure of the sample to UV radiation from an LED source in a specially constructed purification chamber, which is similar to that used in a previous study [7]. The optical properties of materials 'A' and 'B' were determined using a technique similar to that used in a previous study [8]. Table 1 summarizes the optical properties of Materials A and B and the wavelengths of radiation they transmit when sunlight is incident on them. It also gives the characteristics of the LED source.

TABLE I. CHARACTERISTICS OF WAVELENGTHS EMITTED BY THE RADIATION SOURCES USED

Item	Properties	Assigned Radiation Name	Characteristics of Radiation
Material A	Completely transparent to all radiations	SUV Sun with UV	Completely transparent to all radiations and therefore UVB, UVA, visible and infrared radiations presentin the sun are transmitted and incident on the sample.
Material B	Completely opaque to UV radiations	SNoUV Sun with NoUV	Completely opaque to UV radiation and therefore UVB and UVA from the sun are terminated. Visible and infrared are transmitted and incident on sample.
LED	Emits only UV radiation	LEDUV	Peaks at 396 nm but broadly emits within the interval 375 nm-432 nm

C. Microbiological Experimental Procedure

The method used to analyse the sample for coliform bacteria (Total coliform TC, Fecal coliform FC and Escherichia coli EC) and Total Heterotrophic Bacteria (THB) is the same as that used in a previous study [7] and presented.

Using the Ghana Standards Authority guidelines [9], the pour-plate technique was used to analyse the sample for coliform bacteria and THB, all in Colony Forming Unit per millilitre CFU/mL. The samples were collected into sterilized plastic containers before noon, kept under ice and transported to the laboratory for processing within a few hours of collection. Culture media (Plate Count Agar [Oxoid Ltd., Hampshire, England] and Eosin Methylene Blue Agar [Oxoid Ltd., Hampshire, England]) were prepared according to the manufacturer's instructions and sterilized at 121°C, 15 psifor 15 minutes. Each sample was shaken vigorously and the area around the lid of the bottle wiped with clean tissue soaked with 70% ethanol (Aseptic technique).

Duplicate dilutions of 0.1 mL and 1 mL of each sample were inoculated on plate count agar using the spread and pour plate technique respectively and incubated at 37°C for 48 hours. All colonies were counted, and an average of duplicate samples recorded as THB counts/mL (CFU/millilitre) for the sample.

Similarly, 2 duplicate dilutions of 0.1 mL and 1 mL of each sample were plated on Eosin Methylene Blue agar and one incubated at 37°C for 48 hours to observe for Total coliform and the other duplicate incubated at 44°C for 48 hours to observe for Fecal coliform. All purple colonies were counted,

and an average of duplicate samples recorded as TC and FC counts/mL (CFU/mL) respectively for the sample.

For Escherichia coli each of the presumptive colonies (metallic green sheen colonies on the FC) was sub-cultured in 10 mL of Peptone Water (Oxoid) for biochemical testing. Each colony was grown in peptone water and incubated at 44°C for 24 hours. A drop of Kovac's reagent was then added to the tube of peptone water. All the tubes showing a red ring colour development after gentle agitation indicated the presence of indole and recorded as a confirmation of Escherichia coli. All colonies of that morphological type were then enumerated and recorded.

D. Construction of Purification Chamber and Optical System for LEDUV

The method used in the construction of the purification chamber and the optical arrangement for the LEDUV is the same as that used in a previous study [7] and is presented.

A vacuum thermos flask (1 litre volume) was used as the purification chamber as it could provide and ensure an efficient and easy-to-maintain system. The reflecting inner surface of the flask ensured an even distribution of light within the purification environment. A glass tube was driven through a rubber cork and the LEDUV arranged on strips wound round the tube. The terminals of the LED were passed through the rubber cork to allow connection to a 12 V DC supply. A specially fabricated white glass tube made of quartz was then lowered to the base of the cork to serve as an outer shield to protect the LED, as shown in Figure 2a. The quartz glass is transparent to UV radiations, as has been established in a previous study [8]. Figure 2b shows the powered LEDUV and Figure 2c shows the purification chamber with the shielded LEDUV lowered into it.

Figure 2. (a) LEDUV arranged on strips and wound round fabricated tube. (b) Powered LEDUV. (c) Purification chamber (flask) with the shielded LEDUV lowered into it

E. Laser Induced Fluorescence (LIF) Set-up

LIF was used to monitor and analyse the degradation of the dissolved organic matter (DOM) in the water samples (the well-water and Voltic purified drinking water used as reference) before and after exposure to the radiation sources. A sample was also kept in the dark (the same environment except for light conditions) throughout the study period without exposure to any radiation. The study was carried out continuously for seven (7) hours, with measurements being carried out after every hour. The experimental set-up is as shown in Figure 3 and consists of a diode laser source emitting at 445 nm, a detector mounted perpendicular to the laser and an optical fibre cable that couples light from the detector into a spectrometer. A computer was used for data collection and analysis

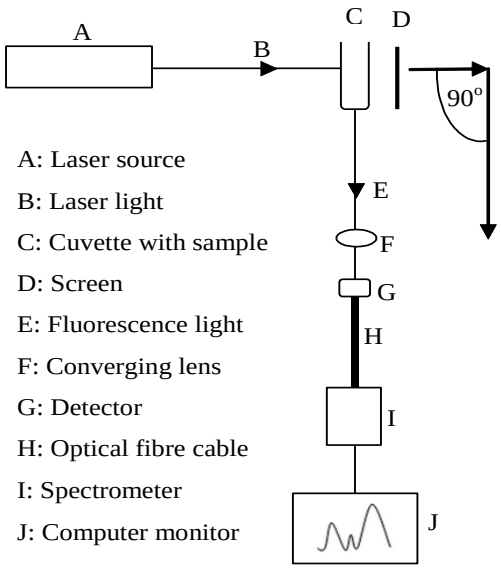

Figure 3. LIF setup for DOM measurements

III. RESULTS AND DISCUSSIONS

A. Spectra of radiation sources used

The spectra of the radiation sources used were taken to determine the wavelengths and their individual variations in intensity responsible for the photo-degradation of the DOM in the water sample. The spectrometer used was a USB 4000. Figure 4 is a composite graph for the normalized spectra in which (a) shows the plots for SUV, SNoUV and LEDUV compared with Sun (radiation directly from the sun). (b) shows a plot for SUV and SNoUV compared with 'Sun', with the wavelength range restricted within the UV spectral region. Sun was used as a reference to confirm that radiation SUV really had all wavelengths present as can be found in the sun, while radiation SNoUV had the UV portion of the sun filtered out. It can be seen from this graph (Figure 4 (b)) that the spectra for SUV is similar to that of 'Sun', while that of SNoUV lies on the '0' mark of the intensity and runs parallel to the wavelength axis. As has been established in a previous study [8], this means that within this spectral region Material A is completely transparent to radiations from the sun and therefore all wavelengths present in the sun are also present in SUV, while Material B, which is completely opaque to UV radiations, blocks the UV portion of radiations from the sun, and therefore the absence of any UV wavelengths for SNoUV.

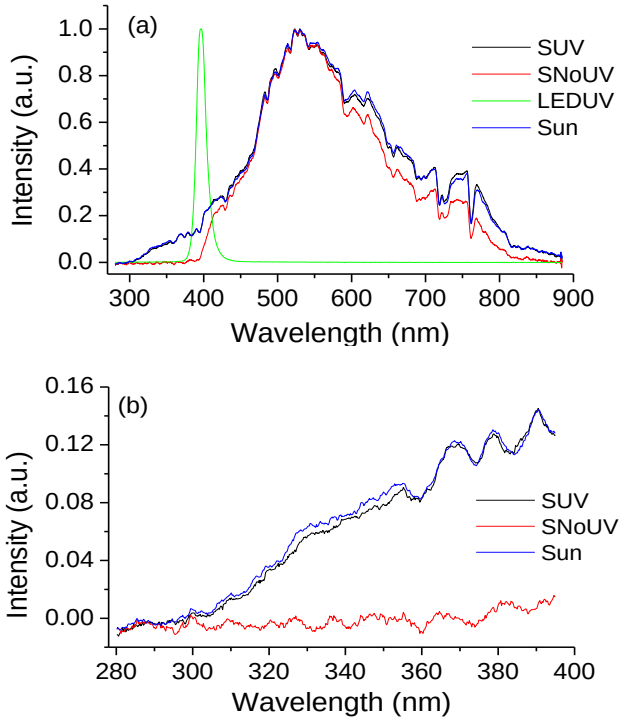

Figure 4. (a) Radiation sources SUV, SNoUV and LEDUV compared to sun's spectrum. (b) Comparison between SUV and SNoUV and sun's spectrum specifically within UV spectral regions. This shows transmitted and terminated wavelengths.

B. Temperature measurements

Temperatures of the samples were determined within the measurement period before any measurement was carried out and the findings presented in Figure 5. The temperature of the sample kept in the dark throughout was also determined. The initial temperature at the start of the entire procedure was 29 0C.

From Figure 5, the temperature of the sample stored in the dark remained constant, the sample exposed to LEDUV had the lowest temperature while the sample exposed to radiation SNoUV had the highest throughout the measurement period. SNoUV recorded the highest temperature because Material B, which is opaque to UV radiations, dissipates absorbed UV light as heat by reversible intra-molecular proton transfer by dispersing UV light into a lower energy state, thereby acting as an optical filter [10, 11] (UV absorbers 1; 2, 2018). The sample exposed to SUV had an intermediate temperature.

C. LIF measurements and First Order Kinetic Equation

The LIF peak intensities at 526 nm (Raman water peak) and 550 nm (DOM fluorescence intensity) were determined after exposure of the well-water sample to the various radiation sources. For the Voltic purified drinking water (used as reference), only the peak at 526 nm was observed. DOM is only detected for contaminated water samples and was therefore observed for the water samples collected from the well and not for the Voltic purified drinking water. This is in agreement with the findings of a precious study [7]. For the water samples from the well, the intensities of the two peaks reduced with irradiation time for all the various radiation sources. The LIF spectra of the sample kept in the dark was also determined. Figure 6is a plot of the fluorescence spectra after exposure of the water samples to the LEDUV. This is presented (only for the LEDUV radiation source) to show the reduction in peak intensity with time of exposure. Insert are the peak intensities at 526 nm showing the reduction in intensity with time of exposure.

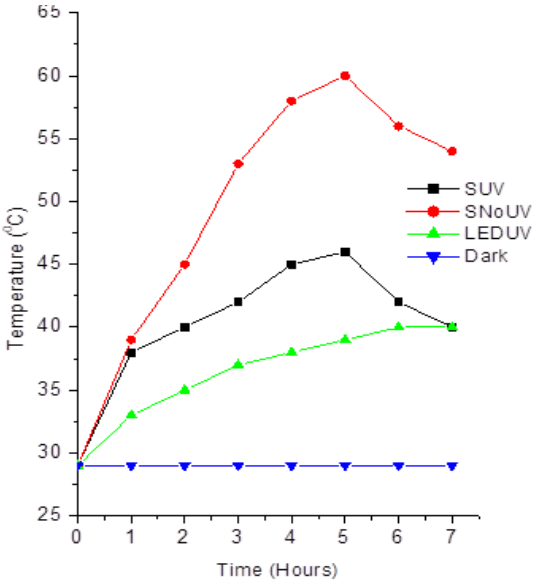

Figure 5. Temperature of the samples before measurements within the study period, and for the sample kept in the dark.

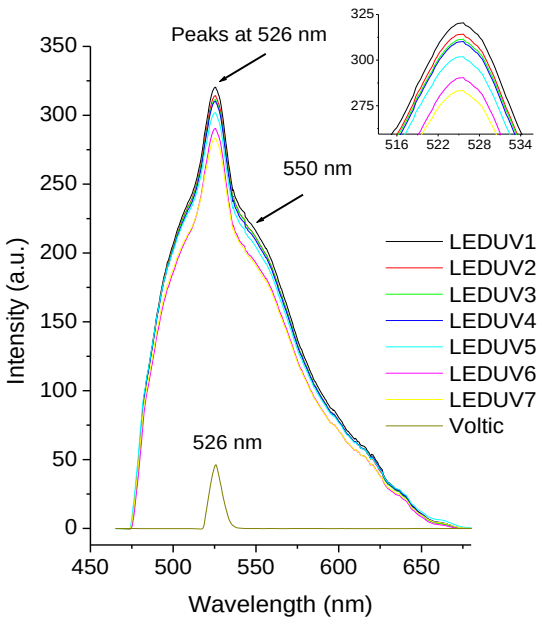

Figure 6. Variation in peak intensity with time of exposure for the water samples when exposed to the LEDUV radiation source.

The reduction in fluorescence peaks indicates that the substances characterizing the peaks (DOM) experienced photo-degradation with time when exposed to the radiation sources. The fluorescence intensity of the sample kept in the dark environment fluctuated (increased and decreased) during the measurement period and may be due to the sequential series of growth phases (lag, exponential, stationary and logarithmic decline phases) followed by bacteria [12, 13].

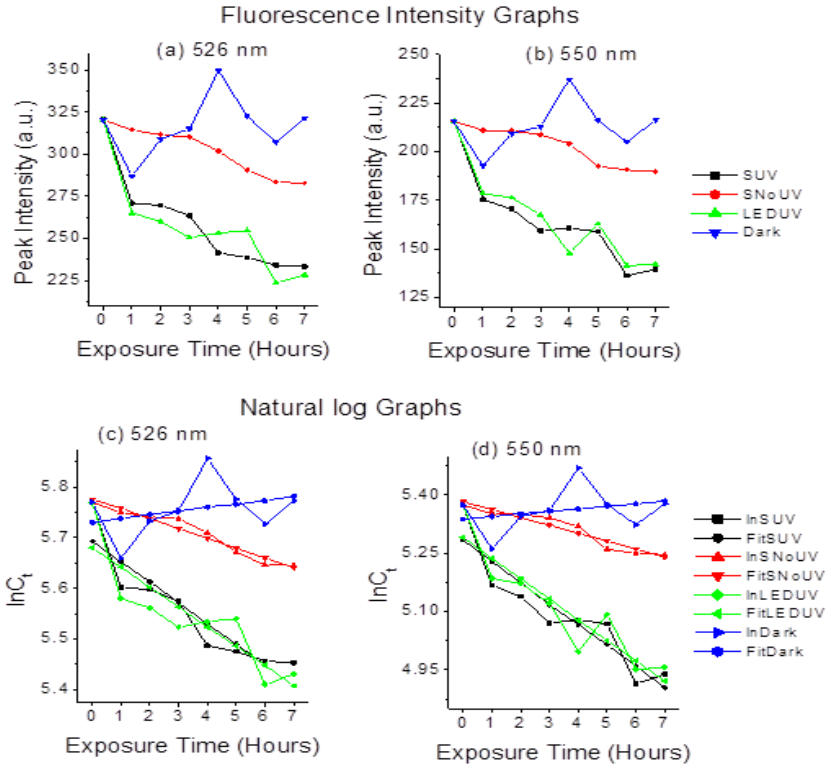

Figure 7. Variation of peak intensity with exposure time at 526 nm (a) and 550 nm (b) Natural log of fluorescence peak intensities at 526 nm (c) and 550 nm (d).

TABLE II. DEGRADATION RATE CONSTANTS K, HALF-LIFE VALUES $T_{1/2}$ AND RANKINGS FOR THE VARIOUS RADIATION SOURCES AND SAMPLE STORED IN THE DARK ENVIRONMENT.

	K Value	526 nm		550 nm		Ranking
		Half-life T1/2(h)	Ranking	K Value	Half-life T1/2(h)	
SUV	-0.0405	17.1111	1st	-0.0537	12.9050	1st
SNoUV	-0.0194	35.7214	3rd	-0.0202	34.3069	3rd
LEDUV	-0.0390	17.7692	2nd	-0.0529	13.1002	2nd
Dark	0.0071	97.6056	4th	0.0064	108.2813	4th

A first-order kinetic equation can be used to model this decline in fluorescent peaks with time [14]. If C_0 is the initial peak intensity; K the degradation rate; and t the degradation time, the peak intensity at any time t C_t can be expressed as:

$$C_t = C_0 e^{-Kt} \quad (1)$$

The first-order degradation rate constant K can be determined for each radiation source by taking the natural log and fitting the graph between $\ln C_t$ and t as:

$$\ln C_t = -Kt + \ln C_0 \quad (2)$$

The slope of the resulting L-graph gives the value of K. The higher the value of K, the better is the efficiency of the radiation source to degrade the DOM in the sample, which will mean a faster rate of purification of the water sample.

The half-life$T_{1/2}$, which indicates the time when C_t equals half of C_0, can be expressed as:

$$T_{1/2} = 0.693/K \quad (3)$$

A smaller $T_{1/2}$ value will indicate a shorter time required for the DOM present in the water samples to reduce to one-half its

initial value, which will mean a faster rate of purification of the water sample. Figure 7 is a plot showing the variation in peak intensity with time for the various radiation sources at 526 nm (a) and 550 nm (b). Figures 7 (c) and (d) are the plots of the natural log of the fluorescence peak intensity values with time at 526 nm and 550 nm respectively. Values of the degradation rate K calculated from the fitted L-graphs in Figures 7 (c) and (d), the calculated half-life $T_{1/2}$ and a comparison with respect to efficiency of the various radiation sources are presented in Table 2. The sample stored in the dark environment was also taken through the same analysis.

At 526 nm, the peak intensities for SUV, SNoUV and LEDUV reduced respectively by 28.85% (320.4540-228.0069), 11.73% (320.4540-282.8806) and 28.75% (320.4540-228.3308), while that for the sample stored in the dark increased by 0.28% (320.4540-321.3617). At 550 nm, the peak intensities for SUV, SNoUV and LEDUV reduced respectively by 35.26% (215.6257-139.5956), 11.95% (215.6257-189.8639) and 34.08% (215.6257-142.1479), while that for the sample stored in the dark increased by 0.30% (215.6257-216.2711).

The K-values registered by the samples exposed to the radiation sources were negative, as shown in Table 2, meaning that their fluorescence peaks trend downward with exposure time. This indicates a reduction in the DOM with time. The fluorescence intensity for the sample kept in the dark environment fluctuated (increased and decreased) during the measurement period, and on the whole had a positive K-value being calculated from its fitted L-graph. This means that its fluorescence peaks trend upward with time.

Using the fluorescence intensities at 526 nm and 550 nm, the efficiency of the radiation sources in degrading the DOM in the water samples was deduced using the calculated K-values. SUV had the highest value while SNoUV had the least. Consequently, SUV had the least half-life while SNoUV had the highest. This translates to the fact that SUV had the shortest time required for the DOM present in the water samples to reduce to one-half its initial value, which will mean the fastest rate of sample purification. SNoUV had the slowest rate of sample purification. The sample stored in the dark was the worst performer with respect to the reduction in DOM concentration.

D. Coliform and THB counts

Samples were taken before and after exposure to the radiation sources and analyzed with culture techniques. There was a count of coliform bacteria and THB in CFU/mL every hour for the duration of the study. Figure 8 is a composite graph showing the counts for TC, FC, EC, THB and the sum of all these microbes after exposure to the radiation sources. 'Dark" represents counts obtained for the sample stored in the dark environment.

Using the data for 'sum of all microbes', the first-order kinetic equation (equation 1) was used to model the decrease in microbes after exposure of the samples to the radiation sources. The first-order degradation rates K and half-life for the microbes were also calculated. The sample stored in the dark was also taken through the same process. Figure 9 is a plot of the natural log of the count with time of exposure. Values of the degradation rate K calculated from the fitted L-graphs, the half-life $T_{1/2}$ and a comparison made between the various radiation sources and the sample stored in the dark environment are presented in Table 3.

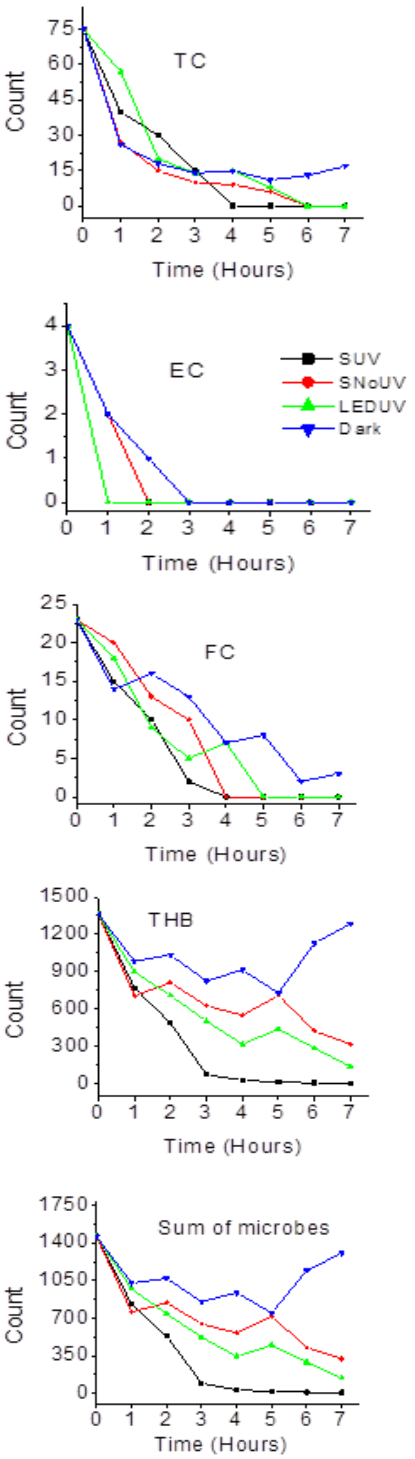

Figure 8. Composite graph showing the counts for TC, FC, EC, THB and sum of all these microbes after exposure to the different radiation sources. 'Dark" represents counts obtained for the sample stored in the dark environment

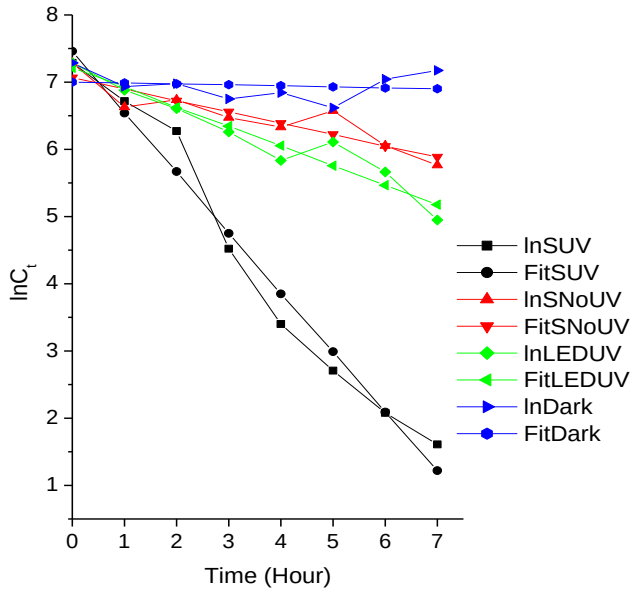

Figure 9. Natural log and fitted L-graphs of bacteria count (after exposure of the water samples to the radiation sources) with time, including counts of the sample stored in the dark.

TABLE III. DEGRADATION RATE CONSTANTS OF THE MICROBESK, HALF-LIFE VALUES T1/2 AND RANKINGS FOR THE VARIOUS RADIATION SOURCES AND SAMPLE STORED IN THE DARK ENVIRONMENT

	Sum of Microbes		
	K Value	Half-life $T_{1/2}$(h)	Ranking
SUV	-0.8899	0.7787	1st
SNoUV	-0.1686	4.1103	3rd
LEDUV	-0.2902	2.3880	2nd
Dark	-0.0147	47.1429	4th

The decrease in the total number of microbes using SUV, SNoUV and LEDUV respectively was 99.66% (1462-5), 78.18% (1462-319) and 90.36% (1462-141), while that for the sample stored in the dark decreased by 10.74% (1462-1305). From Table 3, radiation from the SUV yielded the highest value of K(and therefore shortest half-life) while SNoUV had the lowest K-value (longest half-life). This means that within the study period, the sample exposed to SUV experienced the fastest decline in microbes while the sample exposed to SNoUV showed the slowest decline. The sample stored in the dark was the worst performer as it experienced a reduction of just 10.74%.

IV. CONCLUSION

The varied results obtained after exposure of the same water sample to different radiation sources have shown that different wavelengths have different effects.

With respect to temperature, exposure of the different radiations resulted in the water samples having different temperature, with the sample exposed to SNoUV having the highest, LEDUV the least and SUV showing an intermediary temperature. The sample kept in the dark (in the same environment except for light conditions) maintained the same temperature throughout the study period.

The reduction in LIF peak intensities at 526 nm (Raman water peak) and 550 nm (DOM fluorescence intensity) indicates that the DOM experienced photo-degradation when exposed to the radiation sources. The use of a first-order kinetic equation to model this decline, and the subsequent calculation of the degradation rate constant K (from the slope of the resulting natural log graph) and the half-life helped determine the efficiency of the radiation sources in degrading the DOM in the water. At 526 nm and 550 nm, the fluorescence peaks for the samples exposed to the radiation sources trend downward with time (indicating a reduction in the DOM), with SUV showing the greatest reduction and SNoUV showing the least. SUV had the least half-life, meaning it registered the shortest time required for the DOM present in the water samples to reduce to one-half its initial value, which means the fastest rate of sample purification. SNoUV registered the highest half-life. The fluorescence peaks for the sample kept in a dark environment trend upward with time, meaning the DOM experienced growth during the study period.

There was a count of coliform bacteria (Total coliform TC, Fecal coliform FC and E.coli EC) and Total Heterotrophic Bacteria (THB) using culture techniques before and after exposure to the radiation sources. The sample kept in the dark was also analysed. The sum of all the microbes was determined every hour and the first-order kinetic equation used to model the decrease. The calculated degradation rates K and half-life for the microbes showed that SUV experienced the fastest decline while the sample exposed to SNoUV showed the slowest. The sample stored in the dark was the worst performer.

This finding is in agreement with the findings of the LIF as the reduction in fluorescence peaks has actually confirmed that the substances characterizing the peaks, which are the DOM, experienced photo-degradation with time when exposed to the radiation sources. The order of the reduction in the microbes count is also in agreement with the findings of the LIF results.

It is worth noting that although the sample exposed to SNoUV had the highest temperature, in terms of efficiency in reducing the number of microbes in the water sample, it exhibited the worse performance, while SUV with an intermediary temperature proved to be the most efficient.

This study has provided an in-depth knowledge on the effectiveness of treatment of pathogens or microbes in well-water using UV radiation sources.

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