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The Effect of Bottle Scratches and Lime Juice on Natural Solar Radiation Disinfection (SODIS) Techniques on Different Bacterial Colonies at ShoaRobit and Surrounding Rural *Kebeles*

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Abstract: Over one billion people on Earth do not have access to clean drinking water. Several nonprofit and government organizations are promoting low cost, household methods for water purification [1, 2]. One of best alternative approach for equator and temperate region including Ethiopia is solar water disinfection or SODIS. The previous work done at Shoarobit and Surrounding Rural *Kebeles*, shows that SODIS treatment with clear 1.5 bottles and low turbidity, disinfection is efficient at the end of six hours [3]. However frequently use of the same bottle cause for scratch on the surface of the bottle. Hence this paper addresses effect of bottle scratch and lemon juice (as a catalyst) on solar disinfection. Different level of scratches, *Less scratch bottle (LSB), Slightly Scratch Bottle (SSB) and Highly Scratch Bottle (HSB)* was analyzed. Since SODIS treatment is mainly due to UV radiation, causes for lysis the DNA of microorganisms, if it is not passes through the surface of the bottle the method became inefficient. From the result it was obtained that there is significance statistical difference between densities of scratched bottles. The disinfection efficiency different scratch bottle is decreases as the following order LSB > SSB > HSB. In addition it was tested that effect of lemon juice on solar disinfection. The pH decrease (acidity increase) and solar disinfection has a synergic effect. This was shown the disinfection efficiency increase accordingly the following pH order: pH = 3 > pH = 5 > pH = 7. In conclusion at the end of six hours almost all bacterial colonies was disinfected in all forms of scratching bottles and this disinfection efficiency of the method enhance with adding lemon juice.

Keywords: SODIS, Scratch Bottle, Lemon Juice

1. Introduction

The lack of clean drinking water for some 1.1 billion people in this world has dramatic consequences: approximately 4 billion cases of diarrhea are reported annually, of which 2.5 million ends in death. Every day around 6000 children die due to the lack of safe drinking water. Criteria for improving water supplies only consider water availability and its accessibility. However, since the drinking water quality is not taken into account, the situation is far worse as more than 1.1 billion people are exposed to unsafe drinking water. According to the World Health Organization 1.8 million of people die because of diarrhea per year and 88% of these cases are related to the ingestion of contaminated water, lack of sanitation and bad hygiene practice [4].

Among the various methods of treating drinking water at the point of use to reduce exposure to microbial pathogens, solar disinfection is a water treatment method where a drinking water sample is exposed to solar radiation to inactivate pathogenic organisms [5, 6]. It is seen as a low-cost, sustainable, and simple method of disinfecting

contaminated drinking water in developing countries where people have no access to alternative water treatment systems [7]. A combination of UV-A rays and the temperature of the water inside the bottles kills or incapacitates waterborne pathogens that can cause diseases such as diarrhea [8].

It is recommended that solar disinfected water should be consumed within 24 h to avoid the possibility of post exposure regrowth. The efficiency of the basic protocol can be enhanced by adding a number of additional steps such as:

(a) Placing filled bottles on reflective surfaces to boost the amount of sunlight absorbed by the reactor
(b) Painting the underside of the SODIS reactor black to enhance solar heating
(c) Shaking a two-thirds filled bottle vigorously for 30 s before top ping up and sealing, to increase initial levels of dissolved oxygen for solar induced oxidative inactivation processes.
(d) Filtering the water before filling the reactor[14]. Solar disinfection is not a recent technology Previous. studies have found that solar disinfection is affected by numerous variables. These variables include the wavelengths of solar radiation, water temperature, turbidity, and container selection[6]. The system uses PET (Polyethylene) transparent plastic bottles that are exposed to the sunshine for several hours. These are ordinary plastic drink bottles of the kind used for soft drinks and bottles water- they do need to be clear and transparent [9, 10].

In shoarobit and surrounding kebeles despite being a water rich tropical place, a large percentage of the population does not enjoy access to clean drinking water. This leaves nearly half the population having to acquire their water from 'unsafe' means such as streams, rivers, unprotected wells and open water bodies. Thus diarrhea infections, and serious epidemics of cholera, dysentery and typhoid are common. Treating water to improve its quality should be combined with these health promoting practices to make a lasting change in the public health of people in shoarobit and surrounding kebeles. Having thisas [3] research reports that on his doing in 2013, The results show that on a conclusion the disinfection process was succeeded at the end of six hours using either activators or not at ShoaRobit and neighboring rural *kebeles* [3, 11].

But, The plastic bottles made from PET have proven to be an adequate and safe container for the treatment of drinking water using SODIS method. However many of the plastic bottles being used for SODIS were noticeably scratched after six months of use and study Concerns have recently been raised about the possible degradation in SODIS disinfection effectiveness due to surface scratches that accumulate on the bottles during routine, daily, handling [12].

So can the scratches would cause for inhibit the transmission UV and thus diminish the effectiveness of purification? Although SODIS has 99.99 % disinfection efficiency, but it is time consuming. It requires at least 6 hours of sun exposure and up to 48 hours if the day is cloudy. To overcome these problems, it was designed a techniques to addresses the hypothesis that, if SODIS water disinfection was related to bottle scratches, then what increased bottle scratched density would result in efficiency of solar disinfection quality. Including addition of commonly available and cheap food products/preservatives which is lemon juice [4, 13].

This research aims to provide insight on how a safe drinking water is available, in shoe robit and surrounding rural kebeles by testing whether scratch bottles and lime juice have an influence on natural solar radiation disinfection (SODIS) technique, because It has been recommended by several researchers for use in countries that receive abundant sunshine, specifically those areas between latitudes 35° N and 35° S. Different approaches, such as different backing surfaces and solar concentrators, have been used to enhance the efficiency of SODIS to achieve good water disinfection efficiency [7, 14].

2. Materials and Methods

2.1. Source of Samples and Analysis

Three tests were conducted on separate days in shoarobit, medina and Abayather. Each test followed the same procedure for contaminated water collection, sampling technique (except sample quantity), bacterial incubation, and counting.

2.2. Plastic Bottles

The same four plastic bottles were used for each of three SODIS tests. Test Bottle CB: A clear 1.5-liter plastic water bottle was used as a control. This bottle was new and not had any visible scratches. Test Bottle LSB: A 1.5-liter plastic water bottle was lightly scratched with sandpaper. Test Bottle CSB: A 1.5-liter plastic bottle was heavily scratched by scraping the bottle on concrete. Test Bottle SSB: A 1.5-liter plastic water bottle was thoroughly scratched with sandpaper.

2.3. Sampling Procedure

The technique used for sampling from wells was according to the procedure described by WHO. Then water sample (6 L) was divided into four groups (each 1.5 L) and then exposed to solar radiation on the roof water bottle with control, with less scratched, with slightly scratched and highly scratched at the research area for the period of one three and six hours, but during this period and every each hour, water samples were taken from each group for culturing to estimate the reduction in the bacterial concentration due to exposure to sunlight. After arriving in the laboratory water samples were cultured on different media at a starting time (zero time), to estimate the bacterial counts of water samples.

2.4. Microbiological Examination of Water Samples

Microbiological examinations were performed to determine the Total Coli form (TC) as described below. For

the determination of the total Coli form the original sample and each diluted sample, 0.1 ml water samples were directly pipetted into the surface of the prepared agar plates, and distributed all over using a sterile L-shaped glass loop. The plates were incubated at 35°C for 24 to 48 h. The colonies were form on the surface of the agar plate from both original and diluted samples were counted using Quebec Colony Counter and recorded as CFU/ml at DBU department of biology.

2.5. Lemon as Food Products

The test followed the same procedure for the scratched bottles. The PET bottles in the SODIS system, each with 1.5 L of contaminated water but in the other bottle there was a lemon juice and added for each 2 ml and both were exposed to sunlight with in one hours, three hours and six hourslemon was used as commonly available food products/preservatives to enhance the disinfection efficiency by decreasing the pH to around 3. the bacteria colonies were counted and recorded as CFU/ml at DBU department of biology.

2.6. Statistical Analysis of the Efficient of Various Reflectors and Sunlight Only

The relative disinfection efficiency of SODIS method different degree of scratched and lemon juice as activator were analyzed based on descriptive statistics and tested their significances by AnalysisOf Variance *(ANOV*A).

3. Results and Discussion

3.1. Sample Water Characteristics

Drinking water sample from ShoaRobit, Medina kebele pond water has a natural pH value range about 7 - 8, with low initial turbidity (especiallyShoaRobit) and normal room temperature of about 24°C. These high pH values in the sample water could be due to the presence of lime in the ground around the interested area. The microbial contamination of the drinking water at *ShoaRobit* is most probably due to the surface municipal wastes from the town, and agricultural wastes. In the same way the microbial contamination of Medina *kebele* pond water mostly due to the wastes from cattle and agricultural effluents from the surrounding farmlands. These microbial concentrations could decrease considerably once free from the drinking water sources from these wastes. However, it is difficult completely free from source, from microbes due to various reasons. Instead, small-scale disinfection efficiency with low cost recommended with all Shoa robit and surounding rural kebeles.

3.2. Effect of Bottle Scratch on Solar Disinfection

The extent of disinfection is mainly due to UV radiation sourced from sunlight, So in this section it was tested that the UV radiation transmission efficiency on different scratch bottles; Control bottle (CB), less scratched bottle (LSB), Slightly scratched bottle (SSB), and Highly scratched bottle (HSB). The disinfection at the end of one, three and six hours were tested.

3.3. The Disinfection of Different Scratch Bottles at the end of One and Three Hour (Shoa Robit)

The table below (Table 1) shows the analysis of variance (ANOVA) for time one hour. From theANOVA table it is clear that the null hypothesis can be rejected for the statement "all Treatments are the same"; the Fobs value is 72.5159, which is much greater than the F_{table} Value, 3.49.

Table 1. *The ANOVA analysis at the end of one hour.*

source	df	SS	mean squaree	Fobs	$F_{table}(3; 0.05)$
-Treatment Agents	3	9.69345E+11	3.23115E+11	72.5159	3.49
-With in treatment.	12	53469398894	4455783241		
-Total	15				

The table below (Table 2) shows the analysis of variance (ANOVA) for time Three hour. From the ANOVA table it is clear that the null hypothesis can be rejected for the statement "allTreatments are the same"; the Fobs value is 16.75957, which is much greater than the F_{table}. Value, 3.49.

Table 2. *The ANOVA analysis at the end of three ours.*

Source	df	SS	mean square	F_{obs}	F_{table} (3; 0.05)
-Treatment agents	3	797808	265935.972		
-With in treatment.	12	191990	15999.1736	16.62185677	3.49
-Total	15				

As figure (1) shows that Disinfection efficiency increases by increasing time and therewasalmost total coli form removal in the SODIS system from the time three hour to six hour. The total coli form inactivation increased by by high percent in SODIS at the time six hour when compared tothe sample at the time one and three hour. In addition to this from the table that obtain for the sample water in Medina kebelesthe analysis of variance (ANOVA) for time six hour shows that a difference is occur between the type of scratched bottle that we use for treatment. So that as the scratched bottle density increases the disinfection quality decrease. And also the disinfection ability of pathogenic bacteria colonies is decrease as the scratched bottle density increase.

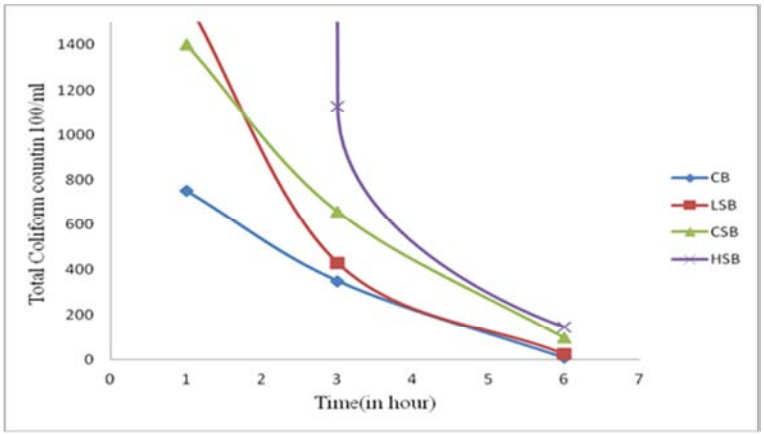

Figure 1. *The disinfection quality of different scratch bottles varrries with time in Shoarobit. The graph addapted from Appendix I, II ana III.(The traces from top to bottom represents Controled Bottle (first trace), Less Scratched Bottle (second trace), Slightly Scratched Bottle (third trace) and Highly Scratched Bottle (fourth trace).*

3.4. The Disinfection of Different Scratch Bottles at the End of One and Six Hour in Medina Kebele

The table below (Table 3) shows the analysis of variance (ANOVA) for time one hour. From theANOVA table it is clear that the null hypothesis can be rejected for the statement "alltreatments are the same"; the Fobs value is 455.0245247, which is much greater than the F_{table}. Value, 3.49.

Table 3. *ANOVA analysis at the end of one hour (Medina Kebele).*

Resource	df	SS	mean square	Fobs	F_{table} (3; 0.05)
-Treatment agents	3	1.29E+08	42943129.11	455.0245247	3.49
-within treatment	12	1132505	94375.41667		
-Total	15				

The table below shows the analysis of variance (ANOVA) for time six hour. From theANOVA table it is clear that the null hypothesis cannot be rejected for the statement "alltreatments are the same"; the Fobs value is 0.56912596, which is much less than the F_{table}. Value, 3.49.

Table 4. *The ANOVA analysis at the end of six hours.*

Resource	df	SS	mean squar	Fobs	Ftable (3; 0.05)
-Ttreatment Agents	3	285.4167	95.13888889	0.56912596	3.49
-Within treatment	12	2006	167.1666667		
-Total	15				

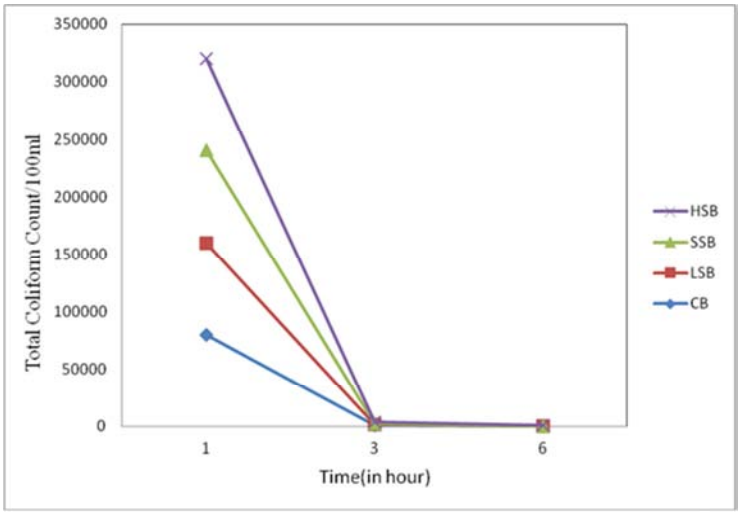

Figure 2. *The disinfection quality of different scratch bottles varrries with time in Medina kebel. The graph addapted from Appendix IV, V and VI.(The traces from Bottom to Top represents Controled Bottle (first trace), Less Scratched Bottle (second trace), Slightly Scratched Bottle (third trace) and Highly Scratched Bottle (fourth trace).*

As figure (2) shows that Disinfection efficiency increases by increasing time and therewasalmost total coli form removal in the SODIS system from the time three hour to six hour. The total coli form inactivation increased by by high percent in SODIS at the time six hour when compared tothe sample at the time one and three hour. In addition to this from the table that obtain for the sample water in Medina kebelesthe analysis of variance (ANOVA) for time six hour shows that nothing difference isprovide between the type of scratched bottle that we use for treatment.

3.5. The Disinfection of Different Scratch Bottles at the End of One, Three and Six Hour for (Abayatir Kebele)

The table below (Table 5) shows the analysis of variance (ANOVA) for time three hour. From the ANOVA table it is clear that the null hypothesis can be rejected for the statement "alltreatments are the same"; the Fobs value is 188.4462, which is much greater than the F_{table}. Value, 3.49

Table 1. *The ANOVA analysis at the end of three hours (AbayAtirKebele).*

Resource	df	SS	mean square	Fobs	Ftable (3; 0.05)
-Treatment agent	3	50215258	16738419	188.4462	3.49
-Within treatment.	12	1065880	88823.333		
-Total	15				

The table below (Table 6) shows the analysis of variance (ANOVA) for time one hour. From theANOVA table it is clear that the null hypothesis cannot be rejected for the statement "alltreatments are the same"; the Fobs value is 0.55426, which is much greater n than the F_{table}. Value, 3.49

Table 2 *The ANOVA analysis at the end of one hours.*

Resource	df	SS	mean square	Fobs	Ftable (3; 0.05)
-Treatment agent	3	1661.67	553.8888889	0.55426	3.49
-With in treatment	12	11992	999.3333333		
-Total	15				

The table below (Table 7) shows the analysis of variance (ANOVA) for time six hour. From theANOVA table it is clear that the null hypothesis can not be rejected for the statement "alltreatments are the same"; the Fobs value is 1.5297, which is less than the F_{table}. Value, 3.49.

Table 7. *The ANOVA analysis at the end of six hours.*

Resource	df	SS	Mean square	Fobs	Ftable (3; 0.05)
-Treatment agent	3	751.083	250.361111	1.5297	3.49
-Within treatment	12	1964	163.666667		
-Total	15				

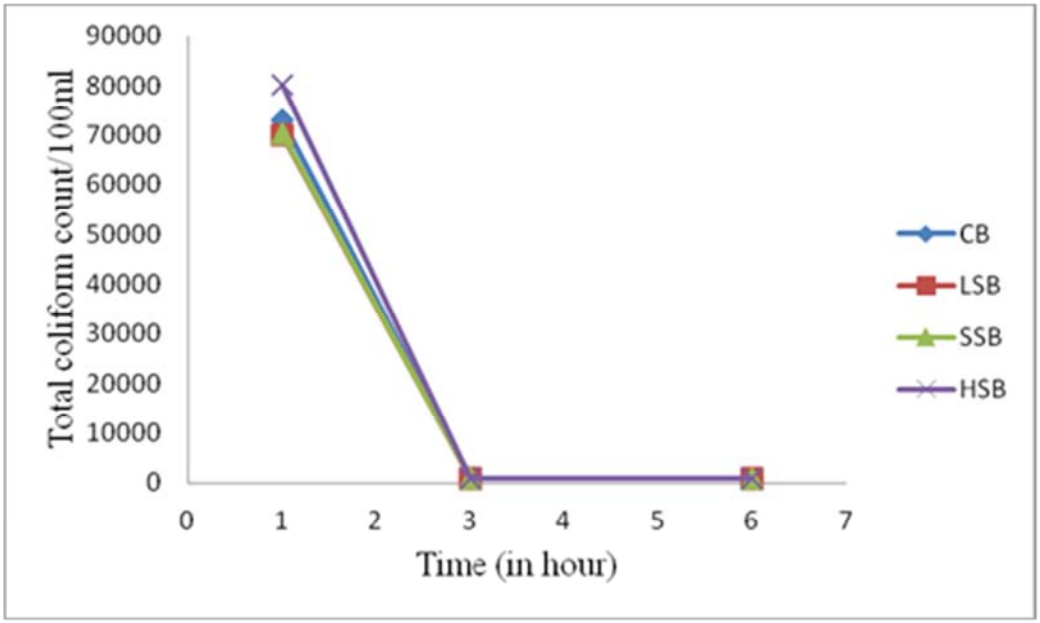

Figure 3. *The disinfection quality of different scratch bottles varrries with time in Abayatir kebel. The graph addapted from Appendix VII, VIII anaIX.(The traces from top to bottom represents Controled Bottle (first trace), Less Scratched Bottle (second trace), Slightly Scratched Bottle (third trace) and Highly Scratched Bottle (fourth trace).*

As figure (3) shows that Disinfection efficiency increases by increasing time and therewasalmost total coli form removal in the SODIS system from the time three hour to six hour. The total coli form inactivation increased by by high percent in SODIS at the time six hour when compared tothe sample at the time one and three hour. In addition to this from the table that obtain for the sample water in abayatirthe analysis of variance (ANOVA) for time six hour shows that nothing difference isprovide on the type of scratched bottle that we use for treatment.

3.6. Effect of Lemon Juice (P^H Value) on Solar Disinfection

The effects of safe, readily available, and inexpensive food preservative, such as lemon and vinegar, with a target of decreasing the pHvalue, were evaluated in relation to disinfection efficiency especially under weakweather conditions. The objective was to achieve complete disinfection at low pH using lemon in a quantity that does not cause smell or taste problems. The main reason for using weak weather was the inefficiencyof the SODIS system in this weather for all of the microbial parameters.

The ground water of ShoaRobit, Medina and Abayatir pond water sample has a pH value of usually around 7-8. The corresponding lemon juice concentrations for that were used for pH adjustment. However there is no health-based guideline for pH in drinking water quality guideline (WHO, USEPA, etc.)although the pH range that is recommended is to avoid the corrosion inthe distribution system. The annual report of the National Health andMedical Research Council in 1996 (NHMRC, 1996) indicates that the consumption of food or beverages with low (2.5) or high pH (11) doesnot result in adverse health effects.

3.7. The Disinfection of Different Bottles with Lime Juice at the End of One, Three and Six Hours for (Shoarobit)

The table below (Table 8) shows the analysis of variance (ANOVA) for time one hour. From theANOVA table it is clear that the null hypothesis canbe rejected for the statement "alltreatments are the same"; the Fobs value is 7.91161, which is Much greater than the F_{table}. Value, 3.49.

Table 8. *The ANOVA analysis at the end of one hour (Shoa Robit).*

Source	df	SS	mean square	Fobs	Ftable (3; 0.05)
-Treatment agent	3	175608	58536.0437	7.91161	3.49
-With in treatment	12	88785.1	7398.75694		
-Total	15				

The table below (Table 9) shows the analysis of variance (ANOVA) for time three hour. From theANOVA table it is clear that the null hypothesis canbe rejected for the statement "alltreatments are the same"; the Fobs value is 144.558, which is much greater than the F_{table}. Value, 3.49.

Table 9. *The ANOVA analysis at the end of three hours (ShoaRobit).*

source	df	SS	mean square	Fobs	Ftable (3; 0.05)
-Treatment agent	3	161125	53708.26587	144.558	3.49
-With in treatment.	12	4458.42	371.5347222		
-Total	15				

The table below (Table 10) shows the analysis of variance (ANOVA) for time six hour. From theANOVA table it is clear that the null hypothesis canbe rejected for the statement "alltreatments are the same"; the Fobs value is 28.3265306, which is much greater than the F_{table}. Value, 3.49.

Table 10. *The ANOVA analysis at the end of six hours (ShoaRobit).*

Source	df	SS	mean square	Fobs	Ftable (3; 0.05)
-Treatment agent	3	90.88095	30.29365079	28.3265306	3.49
-With in treatment	12	12.83333	1.069444444		
-Total	15				

From the above table obtain the point that as the disinfection time increase with lime juice, the disinfection efficiency also increases.

As figure (4) indicates that Disinfection efficiency increased by decreasing initial pHvalues and there is almost total coli form removal in the SODIS system at lowest pH of around 3. The total coli form inactivation increased by by high percent in SODIS at the lowest adjusted pHof 3 when compared tothe sample without any lemon concentration.

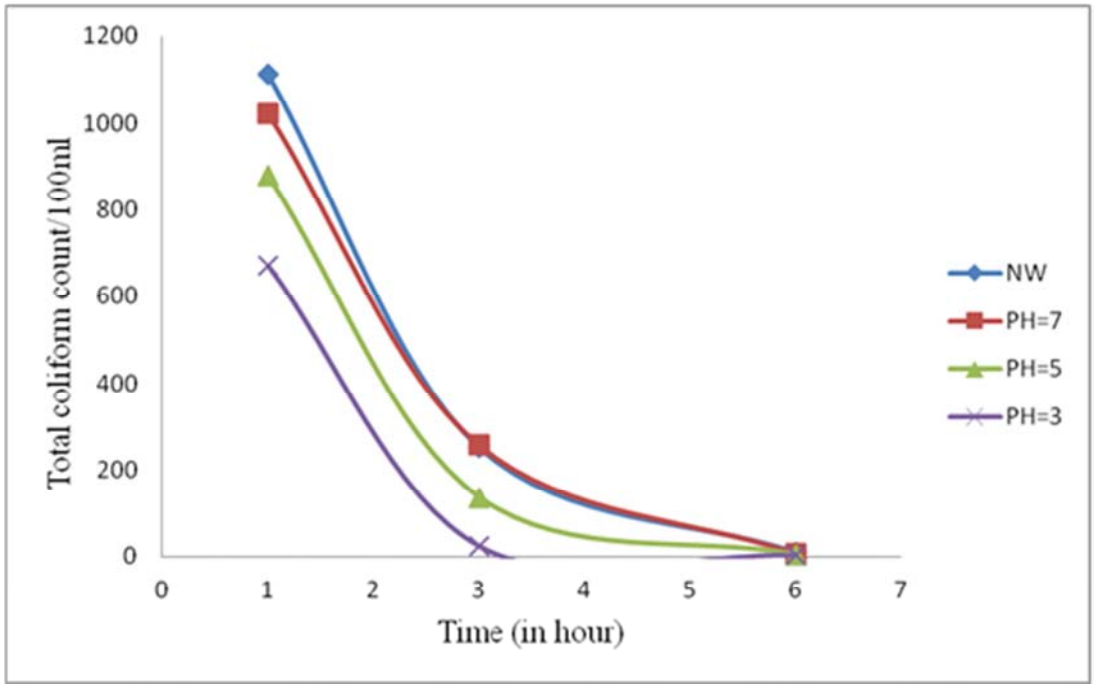

Figure 4. *The disinfection quality of different scratch bottles varrries with time and PH in Shoarobit town. The graph addapted from Appendix X, XI and XII..(The traces from top to bottom represents Normal Water (first trace), PH=7(second trace), PH=5 (third trace) and PH=3(Fourth trace).*

4. Conclusionsand Recommendations

The application of this solar based disinfection technologies to obtain safe drinking water to meet the daily demands of individuals or a family in shoarobit and surrounding rural kebeles is investigated by SODIS water disinfection quality related to bottle scratches and byadding some commonly available and inexpensive food preservative like lemonbased on the betterperformance of these systems at low pH.

Our results suggest that although SODIS disinfection effectiveness is reduced when densities of bottle scratches areincrease. In fact, the six hour treatmentalmost all bacterial colonies were disinfected in all forms of scratching bottles. But, in the 1 to 3 hour treatment the highly scratched HSB bottle performed highpercent ofcoli form than the other scratch bottles and unscratched CB bottle. And also the choice of catalyst (lemon in this study) was also an important factor in addition to low pH fordisinfection using sunlight. in such away that, bottle scratches do not influence the quality of SODIS disinfection, however the SODIS disinfection is greatly influenced by ambient temperature and Lemon increase the disinfection efficiency in SODIS. Therefore SODIS should be an effective method for household water disinfection at temperatures as low as $20^{O}C$ irrespective of bottle scratch density and with adding lemon juice.

From our hypothesis that we proven, It is recommended that, for the comunity of shoarobit and surrounding kebeles Long term education processes are required which involve participatory tools for hygiene education and careful coaching through community workers at community gatherings on how SODIS is work.

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