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Enhancing Solar Disinfection: The Effect of the Hydroxyl Radical on Escherichia coli in a Mineral Slurry

A Thesis Presented by

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Abstract of the Thesis

Enhancing Solar Disinfection: The Effect

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Escherichia coli contamination is a continuing issue in the developing world. Lack of funding and difficulty in instituting new projects makes it necessary to discover an affordable, low-cost alternative to traditional methods of sanitation. Recent studies have shown that the hydroxyl radical, which is known to be produced from iron-bearing minerals through the Fenton reaction, can be damaging to both single-celled and multi-cellular organisms. Using hydroxyl radicals produced from low-cost, common materials would be a viable solution to the problem of contamination. It is proposed that using inexpensive, wide-spread, iron-rich materials to produce the hydroxyl radicals could lead to a new method of sanitation adaptable to various situations.

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[1] INTRODUCTION

Diarrheal illness is a continuing problem world-wide, even though new options for prevention and control have been developed in recent years. According to the World Health Organization, there are 1.5 billion cases of diarrheal illness each year in the developing world, and diarrhea accounts for the death of nearly 3 million children under the age of five annually (www.WHO.Int). Infection becomes increasingly common in areas where clean water for cooking, cleaning and drinking is not readily available, such as sub-Saharan Africa, India and South America. The prevailing cause of diarrheal illness is contamination of water with the enteropathogenic bacterium Escherichia coli (Clarke, Haigh et al. 2003).

Escherichia coli (E. coli) are gram-negative, rod-shaped bacteria which are spread through human and animal fecal matter. In developing nations, such as Tanzania, it is not uncommon for water supplies to be shared by humans and livestock, leading to contamination. E. coli is a hardy bacterium, and can survive in extreme conditions. Studies have shown that E. coli remains viable for several hours of exposure to pH as low as 2, and will continue to grow at pH 5 through 9 (Small, Blankenhorn et al. 1994; Conner and Kotrola 1995). This is due to the cell's ability to regulate its internal pH, allowing it to survive extreme conditions for extended periods of time. Additionally, E. coli continues to grow in NaCl concentrations of up to 6.5%, and is only inactivated when NaCl concentrations reach 8.5% or greater (Glass, Loeffelholz et al. 1992). Although many strains of E. coli exist, some strains are considered enteropathogenic, meaning that these strains produce a shiga-like toxin which results in disease in the intestinal tract (www.CDC.Gov). Enteropathogenic E. coli (EPEC) is highly virulent and occurs around the world (www.WHO.Int ; Clarke, Haigh et al. 2003).

Once ingested, EPEC move to the intestinal tract, where they infect a cell by forming a cup-like attaching and effacing (AE) legion at the site of contact (Clarke, Haigh et al. 2003). These AE legions cause an imbalance in the cell by disturbing the cell's ability to absorb electrolytes, which leads to diarrhea. This imbalance can lead to hemolytic uremic syndrome (HUS) in 5% to10% of cases (www.CDC.Gov). HUS is characterized by decreased urination, lethargy and loss of pink color in cheeks and eyelids, and can lead to permanent kidney damage or death. Treatment of EPEC infection ranges from hydration to hospitalization, depending on the extent of damage. Uncontaminated water is required for hydration to be effective. Antibiotics and antidiarrheal agents are not used, as they increase the likelihood of developing HUS. Although death from EPEC contamination only occurs in a small number of cases, the majority of the water supply in agricultural, non-industrialized regions is contaminated. From a recent survey of the Kondoa region of Tanzania, some villages lack access to any uncontaminated water. Thus, a higher rate of infection occurs, leading to higher rate of morbidity from EPEC in this area.

A possible solution to the problem of EPEC infection lies in preventing the infection, rather than treating the symptoms. In industrialized parts of the world, prevention is simple, and consists of treating the water with chlorine, distillation or expensive sub-micron filtration systems. Though these methods are highly effective, scarcity of water, fuel and funding makes it impossible to implement these systems in the developing world. Furthermore, some methods, such as chlorination, have been shown to have potential negative health effects (Nieuwenhuijsen, Toledano et al. 2000). Less expensive methods, such as solar-disinfection (SoDis) and adding clay minerals to the water supply, have been devised and implemented in much of the world (www.SODIS.Ch ; Haydel, Remenih et al. 2008). Addition of clay has been shown to work if special clay with bactericidal properties is used. However, the occurrence of such clay is limited and prevents large scale implementation (Haydel, Remenih et al. 2008). In this work, the focus was on developing a technique to augment the SoDis method.

The SoDis method consists of bottling water in polyethylene containers and allowing them to be exposed to direct sunlight for more than six hours. The sunlight must be above 500 W/m^2 for this method to be effective (Sommer, Marino et al. 1997). The reason for the suppression of EPEC in this system is the heat produced when water absorbs red and infrared light during exposure to sunlight. Previous studies have shown that water temperature of 45°C is needed for the denaturation of bacteria, which affects the cells' ability to process proteins and may lead to cell death (Fisher, Keenan et al. 2008). Recent work by Peter Oates, et al. (2003), has shown that in mountainous regions, direct sunlight is rarely available, and indirect sunlight does not allow water to absorb the amount of radiation needed to neutralize bacteria, although 1-day exposure to such conditions can lower the risk of infection (Oates, Shanahan et al. 2003). This makes SoDis only completely effective in subtropical, flat regions, where direct sunlight is optimal. On the basis of existing meteorological data, it is clear that solar intensity is not enough to inactivate EPEC within the water supply (Oates, Shanahan et al. 2003). The availability of meteorological data or the lack thereof, is a limitation itself. In many regions of the world, meteorological data is patchy, at best, and studies would have to be done to ensure enough exposure before the SoDis method could be implemented. Thus, it would be beneficial to augment the SoDis method so that it can lead to suppression of EPEC within a shorter timeframe for a given solar intensity, extend its use under conditions with low solar intensity, or even work in the dark. The idea pursued in this study is to add a low-cost material or materials which could augment the SoDis process and expand its utility, possibly saving millions of lives.

The notion to augment SoDis by adding a photoactive material is not new. For example, one study has shown that the addition of TiO_2 to water increases the efficiency of solar disinfection under daylight conditions (Rincon and Pulgarin 2004). In the previous study, 1 g/l of TiO_2 Degussa 25 (mainly anatase, with a specific surface area of 50 m^2/g) was exposed to artificial sunlight with an intensity of 400 W/m^2, which resulted in nearly complete denaturation of E. coli K over 60 minutes (Rincon and

Pulgarin 2004). The method of adding clay minerals and TiO_2, however, is limited by the scarcity of these minerals in large quantities, although similar results may be discovered using more common materials (Haydel, Remenih et al. 2008).

The notion of augmenting the SoDis method by adding minerals is based on earlier work that shows that minerals can produce reactive oxygen species once exposed to water (Cohn, Laffers et al. 2006; Cohn, Mueller et al. 2006; Schoonen, Cohn et al. 2006). Numerous studies have been conducted showing the toxicity of reactive oxygen species, such as hydrogen peroxide, superoxide and the hydroxyl radical (Tullius and Dombroski 1986; Imlay and Linn 1988; Halliwell and Aruoma 1991; Meneghini 1997; Fridovich 1998). When exposed to these reactive oxygen species, microorganisms undergo oxidative stress, which results in severe metabolic dysfunctions, such as peroxidation of membrane lipids, cytoskeletal disruption and DNA damage (Halliwell and Aruoma 1991). This damage is similar to the damage done in human cells from carcinogens present in cigarette smoke and asbestos (Weitzman and Graceffa 1984; Nakayama, Kaneko et al. 1985). The common cause of this damage are base modifications to the DNA, tandem legions on two separate strands, as well as single- and double-strand cleavage (Meneghini 1997; Lloyd and Phillips 1999). While such damage is known to cause cancer in complex organisms, it causes apoptosis (programmed cell death) in single-celled organisms (Halliwell and Aruoma 1991).

Various methods exist for production of the hydroxyl radical in mineral slurries. A simple and common method is creating a mineral slurry containing hydrogen peroxide and ferrous iron. Hydrogen peroxide, when exposed to ferrous iron, produces a hydroxyl radical through the Fenton Reaction:

$$Fe^{II}(aq) + H_2O_2 \rightarrow Fe^{III}(aq) + OH^- + \cdot OH \quad (1)$$

For this reaction to occur, hydrogen peroxide must be added to the system. Recent work has shown that iron-bearing minerals, such as pyrite, as well as coal, spontaneously produce hydrogen peroxide in the presence of molecular oxygen (Cohn, Laffers et al. 2006; Cohn, Mueller et al. 2006) through the following reactions:

$$Fe^{II}(aq) + O_2 \rightarrow Fe^{III}(aq) + (O_2 \cdot)^- \quad (2)$$

$$Fe^{II}(aq) + (O_2 \cdot)^- + 2H^+ \rightarrow Fe^{III}(aq) + H_2O_2 \quad (3)$$

This alleviates the need for hydrogen peroxide to be added, allowing for the hypothesis that reactive oxygen species may be formed in an oxygenated mineral slurry containing ferrous iron-bearing minerals alone. Importantly, the Fenton reaction does not require exposure to light, but the reaction is often accelerated when the system is exposed to light (Ma, Song et al. 2005). In addition to the classical Fenton Reaction, studies have shown that a hydroxyl radical may be produced through the photocatalysis of TiO_2 (Gogniat and Dukan 2007).

According to the current understanding of the Fenton Reaction and its effect on cells, it is possible that exposing EPEC to ferrous iron in a mineral slurry could cause a dramatic decrease in the viability of the bacteria. By creating conditions similar to those in Kondoa, Tanzania, it is possible to explore the effectiveness of adding inexpensive minerals to the water supply in hopes of reducing the virulence of E. coli in that region. Using meteorological and mineralogical data gained during field study, a number of experiments were run using various additives to determine the decay rate of E. coli.

In the remainder of this thesis, field observations (Chapter 2) and a complementary laboratory study (Chapter 3) are presented. The thesis is completed with a separate appendix and a separate reference section.

[2] FIELD OBSERVATIONS

Introduction

In July of 2009, field research was conducted in the Kondoa region of Tanzania to obtain meteorological and geological data. The information gained provides guidance for the design of an applied research project seeking to augment the effectiveness of the SoDis process by adding readily available minerals to the water.

[2.1] Description of the Area

Kondoa, Tanzania, is a small town and its environs located in the north-central portion of the country. The region is part of the Dodoma Administrative District, though it is very distinct from the large urban center of Dodoma to the south. The location of Kondoa is shown in Figure 1The majority of the population in Kondoa resides in small, agricultural communities, and population density is very low (26-50 people/sq km). The central town of Kondoa is only accessible by an unpaved road from Dodoma, and the smaller villages are sometimes accessible by four-wheel-drive vehicle, when not closed off during the rainy season. The wet season lasts from October to March, when the roads to these small villages tend to be washed out. The region is located on a high plateau, between 1000 m and 1500 m in elevation, and is isolated by higher peaks to the north, east and west. The Bubu River, an ephemeral stream, drains the area in the wet season, flowing in a southerly direction. During the dry season, the Bubu becomes a stagnant remnant of itself, leaving a few isolated marshes in the lower portion of the valley.

The geology of the region varies mainly with elevation. In the low valet where many of the agricultural communities reside, the surface is dominated by Neogene and Quaternary sediments, mostly sand-sized grains of quarts. The mountains to the northwest consist primarily of neo-Achaean undifferentiated granitoids, migmatites and meta-ultrabasites. To the northeast and east, the valley is surrounded by paleo-Proterozoic plutons and metasedimentary rocks. A thrust fault runs through the

valley, with a north-by-northwest strike, separating the two geologic formations. A few small outcrops of Achaean gabbros and anorthosite can be found in the valley, though their occurrence is rare. Further to the south, the Dodoman formation dominates, consisting of paleo-Archean to mid-Archean metamorphic complexes. Further to the south, Paleo-Proterozoic meta-mafic and ultramafic rocks can be found.

Figure 1: Kondoa is in the yellow box on the map of Tanzania

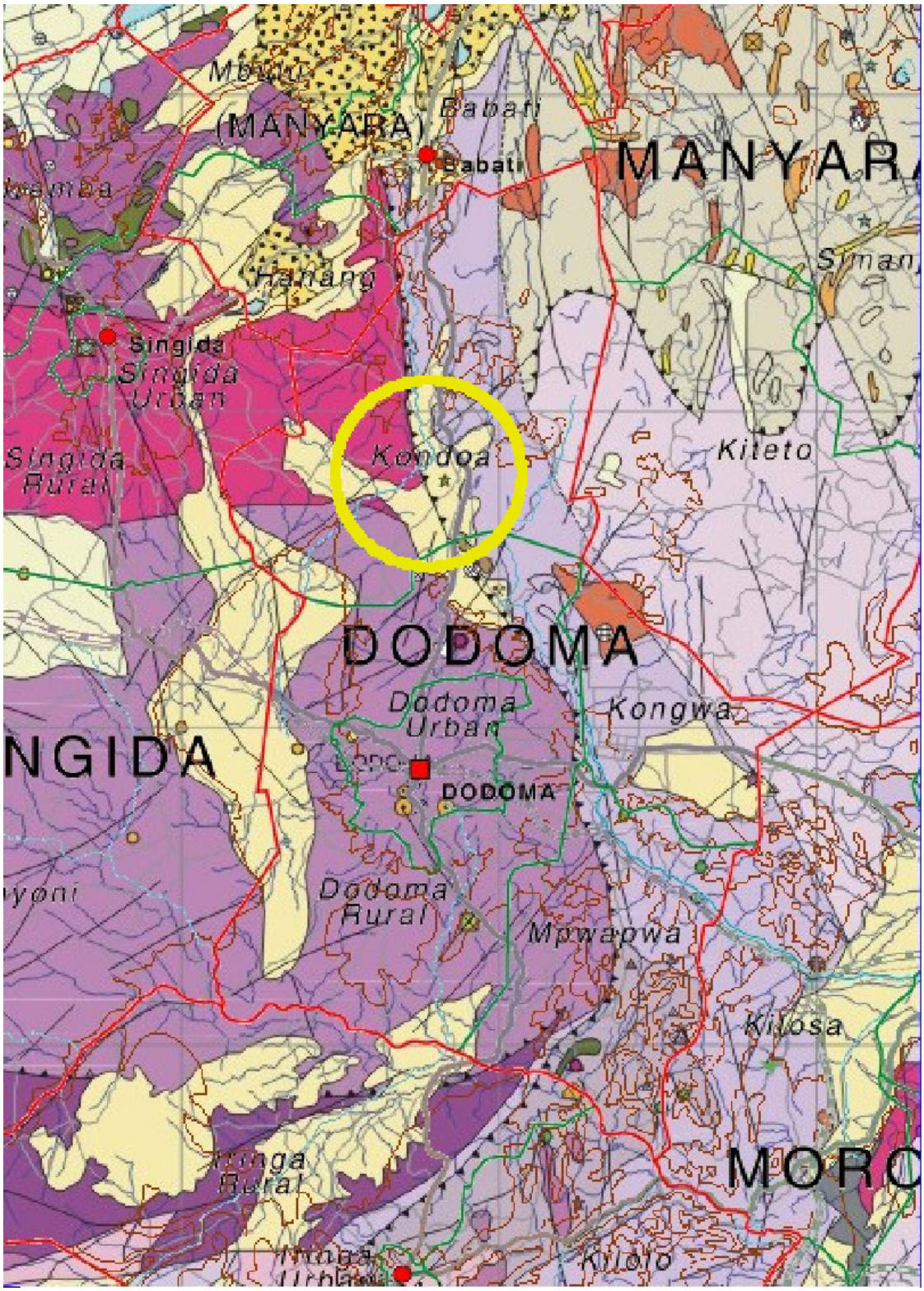
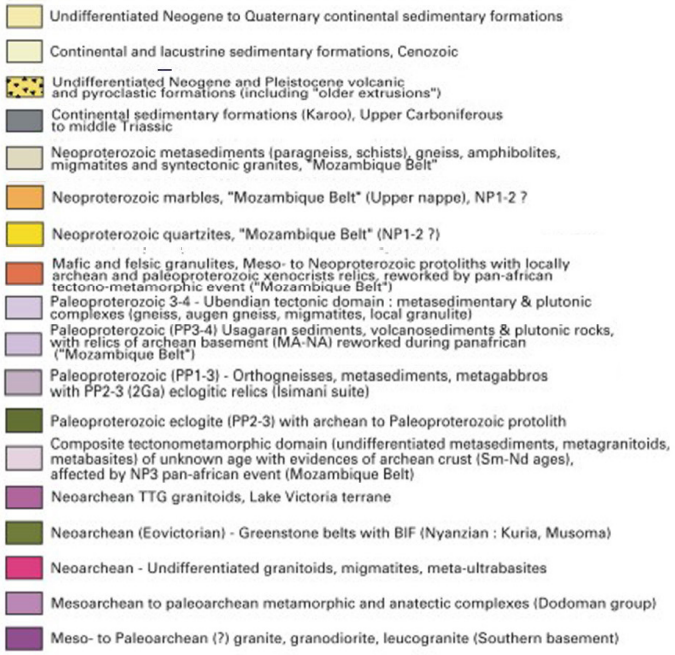
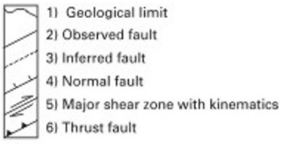


Figure 2: Geologic Map of Kondoa and surrounding regions, adapted from www.sigafrique.net

GEOLOGY





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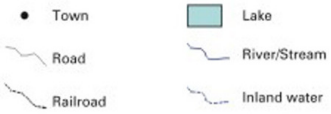

The Geology and Mineral Map of Tanzania at 1: 2.000.000 is published in the framework of the 20th Colloquium of African Geology (2-7 June 2004, BRGM Orléans, France). It synthesizes published national and international maps as well as scientific works of scientific teams including unpublished data of University of Dar Es Salaam, Tanzania Geological Survey and BRGM.

Topography simplified from DCW (Digital Chart of the World)

This map is not an autority for international boundaries

[2.2] Field Methods

During the field study, numerous villages were visited and their water supplies were tested for salinity, nitrate concentration, dissolved ferrous iron concentration and contamination by coliform bacteria. The water supplies examined were a mixture of hand-dug wells, pumped wells and direct collection from ephemeral streams. Point of use collection by the local people consisted of gathering water in a five gallon jug, usually made of polyethylene, and carrying the jug to the residence. At each site, water was collected in a 200 ml polyethylene container for later analysis. The water temperature was allowed to cool to 25°C to ensure that pH measurements were accurate. Measurements for pH and salinity were taken with a Sension electrode (Hach Company). Ferrous iron and nitrate data was collected using an RQFlex2 and test strips, provided by EM Science. The ferrous iron test was capable of measuring as low as .05 ppm and as high as 20 ppm. The nitrate test, similarly, could detect between 3 ppm and 100 ppm. Coliform contamination was measured using Oxoid Dipslides, supplied by Oxoid Inc. A 50 ml portion of the sample was transferred to a separate container for coliform analysis. The smaller sample was then tested for the presence of E. coli bacteria using Colitag Water Test System, which detects the presence or absence of coliform and E. coli bacteria. In some samples, a dipslide was inserted into the sample to determine the total colony forming units per ml. The tests used were then incubated at 37 °C overnight, with E. coli being represented as red dots on the surface of the slide. The samples selected for this special test came from various sources to give a general idea of the overall cell counts of the water supply. The Sension electrode was then inserted directly into the sample to test salinity and pH. Once these data were recorded, ferrous iron and nitrate content were determined by inserting a test strip into the sample.

In addition to field testing of water quality, sediment samples were retrieved from the sites using both 8 µm and 0.45 µm filters. Each sample was drawn into a 50 mL syringe and forced through the filter, leaving a fine film of sediment. The solid residues trapped on the filter were then stored for future analysis using a scanning electron microscope.

Data were gathered daily regarding the solar radiance in this mountainous region, allowing for calibration of exposure in the laboratory. These data were taken using a Heavy Duty Light Meter, made by Extech Instruments, on an hourly basis. The light meter was exposed, facing the sun, and held in place for five minutes. This resulted in an average solar radiance for the time exposed.

[2.3] Preparation of materials for Scanning Electron Microscopy

Ten of the twenty samples were selected for this analysis based on the amount of residue trapped on the filter, site location, and type of source. These filters were then carefully cut and affixed to a copper grid. A thin film of gold was sprayed onto the sample, resulting in a small Au peak in the SEM analysis. Three to four spectra were then taken on each of the samples, to ensure complete analysis. The scanning electron microscopy was conducted under the guidance of Dr. Jim Quinn, Material Science Research Laboratory, at Stony Brook University. The analyses were conducted on a LEO1550 SFEG-SEM equipped with a Phoenix Sapphire energy dispersive detector.

Table 1: Field observations of water supply in Kondoa, Tanzania

	Supply type	pH	Salinity	Fe^{2+}(ppm)	NO_3^+(ppm)	Presence of Coliform (cfu/mL)
Mondo 1	Hand-dug	7.1	0.1 ‰	1.1	50	Y
Mondo 2	Hand-dug	NA	NA	NA	NA	Y
Mondo 3	Hand-dug, shared with livestock	5.4	0.0 ‰	1.3	4	Y
Mondo 4	Manual Pump	7.0	0.5 ‰	<.5	10	Y (10^4)
Mondo 5	Household tap (unique)	7.8	0.2 ‰	0.7	40	Y
Simba 1	Gas-powered pump from aquifer	7.3	0.4 ‰	<.5	31	N
Losangi 1	Hand-dug	7.0	0.1 ‰	<.5	12	Y (10^6)
Losangi 2	Manual pump	8.0	1.9 ‰	NA	NA	Y
Losangi 3	Hand-dug	7.0	0.2 ‰	NA	NA	Y
Losangi 4	Manual pump	7.8	0.0 ‰	NA	NA	Y
Lyongi 1	Manual pump	7.7	0.3 ‰	1.1	20	Y (10^5)
Lyongi 2	Hand-dug	7.8	0.1 ‰	<.5	15	Y
Kingale 1	Hand-dug	7.6	0.3 ‰	<.5	20	Y
Kingale 2	Gas-powered pump from aquifer	7.7	1.4‰	<.5	>100	N
Kilema 1	Hand-dug	7.3	0.1 ‰	<.5	5	Y
Kilema 2	Manual pump	7.7	0.3 ‰	<.5	25	Y
Mongoroma 1	Gas-powered pump from aquifer	7.5	0.3 ‰	<.5	7	Y
Mongoroma 2	Spring, shared with livestock	7.3	0.1 ‰	<.5	11	Y (10^6)

Serya	River	7.5	0.3 ‰	<.5	2	Y (10^6)
Murungi	Manual Pump	7.0	0.2 ‰	<.5	3	Y

[2.4] Results

The water quality tests conducted in the field show that drinking water within the region falls well within the range for potability in terms of pH. All but one observation fall between pH 7 and pH8 (Table 1). One sample, collected at a manual pump in the town of Mondo, had a pH of 5.8. The pumps tended to contain a higher salinity than the wells, as high as 190 ppm, with a mean of 70 ppm. It was unclear where the salinity was coming from, but it is possible that salt was added to the water in an attempt to disinfect it. In both the hand-dug wells and the pumps, the ferrous iron content was below detection levels (0.5 ppm) for the majority of samples, and never reached levels which may be considered dangerous. Nitrate, conversely, was consistently above safe levels, possibly due to extensive agriculture and use of sub-standard cesspool systems. The mean nitrate level was 22.19 ppm, more than double the standard set by the EPA for US drinking water (www.EPA.Gov). In one sample (Kingale-2), the nitrate content was too high to be measured, though the equipment used could measure up to 100 ppm. Of the twenty samples we had taken, eighteen contained coliform bacteria, including E. coli. In the United States, the EPA regulation on safe drinking water does not allow the presence of any coliform bacteria. Since coliform bacteria is only spread though fecal contamination, a positive test is enough to denote contamination. Cell count information was recorded at various sites to obtain a general understanding for the extent of contamination. The cell counts ranged from 10^4 cfu/mL to 10^6 cfu/mL. The mere presence of E. coli is enough to consider the water contaminated. The cell count data would be used to enhance our experimental study, to replicate the population density. The two which

did not contain coliform were both pumped (Kinglae-2 and Simba-1), and the water was stored in above ground tanks for later use. These two systems were distinct, as many pumps drew from an aquifer directly to the faucet. The faucets were located outdoors in the towns, sometimes with a stopper locked to prevent unauthorized use, and were shared by the entire village.

The results of the SEM analysis indicate that the sediment load in the region consists primarily of aluminosilicate minerals, with minor amounts of iron and potassium-bearing minerals, as shown in Figure 3. Concentration of both calcium and iron ions were normalized to aluminum content to show the overall concentrations, as shown in table 2. The one sample which stands out is sample 7 (Mondo-5, Figure 3, panel g). It was the only sample available from a household tap. This sample contained a high amount of calcium in addition to the standard ions. The reason for this difference is unknown, though it is common for water stored in carbonate rock, such as limestone or chalk, to contain raised levels of calcium. For the purposes of this study, the iron levels of primary importance. The concentration of iron is at most 17% of the total ion content. The addition of iron to the water supply would have little effect on the color or odor of the water, as normal levels are well below the accepted maximum.

Table 2: Al:Ca and Al:Fe ratios of sampled water supplies in Kondoa, Tanzania

	Al:Ca	Al:Fe
Sample 1.1	0.0181752	0.1404762
Sample 1.2	0.0182149	0.1406844
Sample 1.3	0.0235415	0.1263158
Sample 2.1	0.030066815	0.104827586
Sample 2.2	0.020912548	0.082294264
Sample 2.3	0.01553063	0.118811881
Sample 3.1	0.01793722	0.104829211
Sample 3.2	0.0220126	0.096
Sample 3.3	0.017917133	0.133086876
Sample 4.1	0.014395393	0.070543375
Sample 4.2	0.015701668	0.096194503
Sample 4.3	0.339697169	0.088871097
Sample 5.1	0.015250545	0.093984962
Sample 5.2	0.025954198	0.082066869
Sample 5.3	0.020703934	0.119804401

Sample 6.1	0.030955585	0.137323944
Sample 6.2	0.038057743	0.154228856
Sample 6.3	0.033161385	0.129844961
Sample 7.1	0.04519774	0.169847328
Sample 7.2	0.056720099	0.16146789
Sample 7.3	0.042372881	0.126153846
Sample 8.1	0.051282051	0.100840336
Sample 8.2	0.059278351	0.144366197
Sample 8.3	0.042553191	0.078947368
Sample 8.4	0.020408163	0.032258065
Sample 9.1	0.03875969	0.135693215
Sample 9.2	0.054968288	0.155223881
Sample 9.3	0.051565378	0.148558758
Sample 9.4	0.046052632	0.108910891
Sample 10.1	0.024939662	0.049856184
Sample 10.2	0.029339853	0.059467919
Sample 10.3	0.022222222	0.060689655

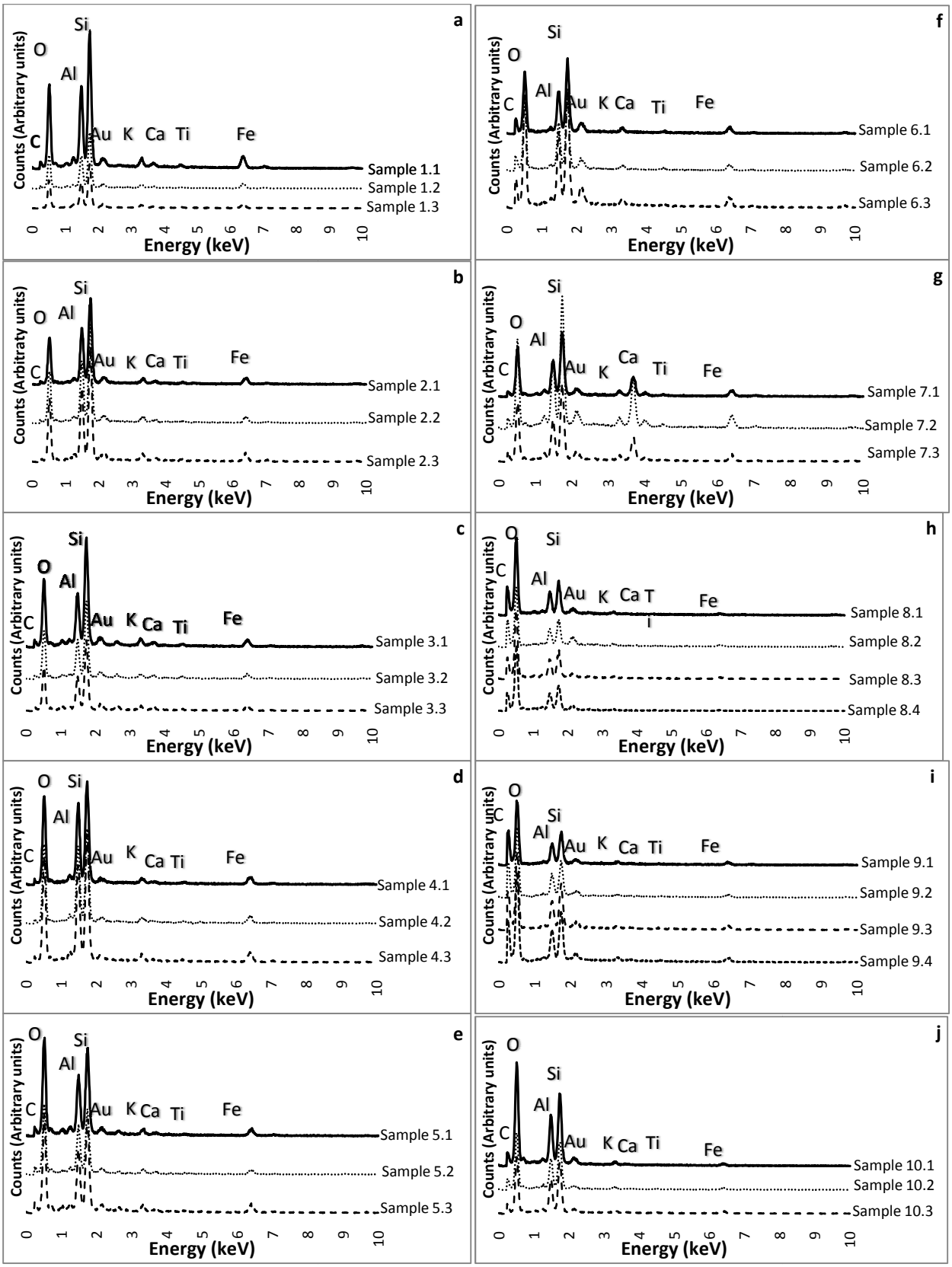

Figure 3: SEM Analysis of Sediment Samples from Kondoa, Tanzania

The average solar radiance was 1030 W/m^2, with a minimum of 272 W/m^2 and a maximum of 1410 W/m^2. The minimum represents full cloud cover at 2 p.m., and the maximum is full sun at 11:00 a.m. There appears to be a large variance in solar radiance over the course of one day, and it is difficult to tell what a long-term average might be. For the purposes of our experiments, the average was used to simulate the solar radiance for batches run under simulated daylight.

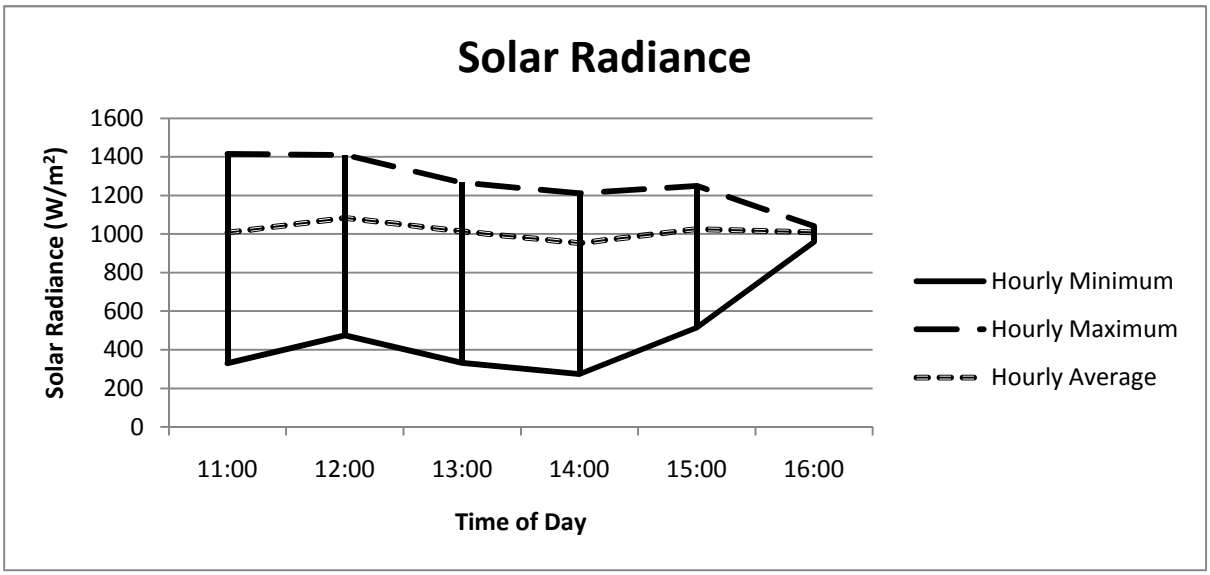

Figure 4: Measured solar radiance in Kondoa, Tanzania between 7/14/2009 through 7/22/2009

Discussion

Although the study represents only a small number of observations in the Kondoa area of Tanzania, it is clear that the water quality is severely impacted by the presence of E. coli. The field sampling kits returned 18 out of a total of 20 determinations a positive indication of E. coli. Not all E. coli are a health threat, but exposure to EPEC is a valid concern. The solar intensity measurements indicate that there is enough sun for SoDis to be functional on a daily basis, although at the minimum intensity recorded the method would not be an option. At the time of writing, SoDis was not being practiced in the region. The waters potentially used for SoDis contain aluminosilicates and some samples (1-7, 9) contain a few percent iron, although mostly in the trivalent state based on the fact that the soils are red/brown. The study area is in a sedimentary geologic terrain, transported from felsic

plutons, which means that minerals such as quartz, orthoclase and plagioclase are prevalent. To the north of the study area there is widespread alkaline volcanism. Hence obsidian, olivine, ilmenite, pyroxene and other minerals common to alkali basalts are possibly available. To the south, Paleoproterozoic ultra-mafic rocks outcrop, giving access to weathering products such as hematite and goethite.

Conclusions

The implementation of the SoDis method in this area is on the basis of the solar irradiation data presented in the study show that the method would indeed be possible. The average solar radiance falls well above the 500 W/m^2 minimum for effective use of the SoDis system. Even though the solar irradiation data presented in this study suggest that there is enough light, periods of cloudiness over a normal day will impair the SoDis method. Under normal conditions, solar intensity falls short of the 500 W/m^2 minimum at numerous points during the day. It is for those conditions that an augmentation of the process is needed. Based on the geology of the local area, hematite and goethite are available at low cost, while obsidian, olivine, ilmenite and pyrite may be acquired within the country at low cost. These minerals could be added as point of use capsules or powders. In a five gallon container (roughly 18 L), a ten gram supplement could possibly serve as a disinfectant. These could easily be distributed from the local health center on a daily basis, with simple instructions for use.

[3] EXPERIMENTAL STUDY

[3.1] INTRODUCTION

Solar disinfection, a method used to provide drinking water in developing nations, has been shown to lack effectiveness in many regions of the world (Sommer, Marino et al. 1997; Oates, Shanahan et al. 2003; Fisher, Keenan et al. 2008). This simple method involves using clear polyethylene bottles and exposing them to direct sunlight in an effort to heat the water to a point where bacteria are nullified (Sommer, Marino et al. 1997; Fisher, Keenan et al. 2008). The relative absence of technology for this method to be effective, as well as few harmful side effects (Nieuwenhuijsen, Toledano et al. 2000), make it attractive as a solution to increased demand for water throughout the world. In areas where the method is less effective, due to little direct sunlight, it is not possible to implement solar disinfection in its current form (Oates, Shanahan et al. 2003).

A possible improvement to the method is the addition of iron-rich minerals to the water. Hydroxyl radicals can be generated with very little effort by adding iron-rich minerals to water (Cohn, Mueller et al. 2004; Cohn, Laffers et al. 2006; Schoonen, Cohn et al. 2006). These hydroxyl radicals have been shown to cause cell death in microbes, and would be a cost-effective addition to improve the solar disinfection method (Tullius and Dombroski 1986; Imlay and Linn 1987; Halliwell and Aruoma 1991; Meneghini 1997; Fridovich 1998). This improvement would allow solar disinfection to be a viable disinfection method regardless of exposure to direct sunlight. By testing the ability of various minerals to disinfect water with and without sunlight, it is possible that an inexpensive additive could be discovered and implemented as part of the solar disinfection method.

[3.2] METHODS

Bacteria used. The bacterium utilized in this study was E. coli K12, purchased from Carolina Biological Supply. The liquid cultures were prepared in the lab of Dr. Gordon Taylor, SUNY Stony Brook, School of Marine and Atmospheric Sciences, inoculated in Tryptone broth and stored in liquid nitrogen until needed. The resulting cultures were then diluted with a saline base and adjusted to 10^7 cfu/ml and kept in a sample freezer between experiments. The cell counts were conducted using an Accuscope 3032 inverted fluorescent microscope, provided by Accu-scope, Inc.

Materials tested. The minerals used in these experiments were research-quality samples. Each mineral was analyzed for specific surface area using the BET method, and analyzed for bulk chemical components using XRF. Table 3 shows the results of this analysis. The results of the XRF analysis are truncated to show only major constituents, excluding any trace elements, explaining the resulting total of <100%. The XRF analysis was conducted using a Brucker-AXS S4 Pioneer X-ray spectrometer. A Nova 2200e Surface Area and Pore Size Analyzer (Quantachrome Instruments) was used for surface area analysis. In some tests, hydrogen peroxide was added to create a 1 mM solution before the addition of minerals.

	Surface Area	Major constituents
Fayalite	2.294 m^2/g	FeO ≈ 69%, SiO_2 ≈ 25.3 %, MnO ≈ 4.3%, MgO ≈ 0.4%
Goethite	11.617 m^2/g	Fe_2O_3 ≈ 68%, SiO_2 ≈ 30.3%
Hematite	3.263 m^2/g	Fe_2O_3 ≈ 88.4%, SiO_2 ≈ 8.2%, Al_2O_3 ≈ 1.4%
Ilmenite	1.271 m^2/g	FeO ≈ 48.2%, TiO_2 ≈ 35.3%, SiO_2 ≈ 5.9%, Al_2O_3 ≈ 4.2%, MgO ≈ 3.2%, CaO ≈ 1.2%
Obsidian	0.349 m^2/g	SiO_2 ≈ 74.3%, Al_2O_3 ≈ 13%, K_2O ≈ 5.6%, Na_2O ≈ 4.2%,

		$Fe_2O_3 \approx 1.4\%$
Olivine	2.755 m^2/g	$SiO_2 \approx 44.4\%$, $MgO \approx 43.3\%$, $FeO \approx 9.9\%$

Table 3: Surface area and XRF analysis of minerals tested

Inoculation and containment of sample. Each mineral was ground with a Retch PM 100 ball mill, using and agate cup set aside for iron-bearing minerals, and filtered between 400 µm and 125 µm. Once ground, two separate doses of 0.01 g of each was placed into a 50 ml Pyrex bottle, then heated at 375°C for six hours to remove any possible contaminant bacteria. The samples were then removed from heat, covered and cooled to room temperature, and 20 mL of de-ionized water was added, to make a slurry with .5 g/L sediment. The slurry was then well-shaken and 20 µl of E. coli broth was added via pipette, for a resulting 200 colony- forming units (cfu) per 5 µl of sample.

Exposure technique. Each mineral sample was then exposed to two separate conditions to determine effects of light exposure on the experiment. Each mineral was exposed to simulated sunlight, while a control sample of the same mineral was kept in a dark container. The light was filtered through clean water to further reduce radiance to 1000 W/m^2 and filter out IR radiation. The temperature of the samples exposed to simulated solar light remained below 37°C for the entire experiment, assuring that heat did not have any effect of the decay of the bacteria.

Cell counts. Every hour during each batch, a 1 ml sample was taken from the batch and 3 µl of Invitrogen Live/Dead bacterial viability assay was added. This assay consists of two separate dyes, SYTO-9 and propidium iodide. The dyes are mixed and added to the sample. The samples were then allowed to sit in a second dark container for 15 minutes while the dye interacted with the cells. After the dye had sufficiently set, 5 µl of sample was placed on a microscope slide and analyzed optically using a fluoroscope. The SYTO-9 dye is membrane-permeant, meaning it is able to pass through the cell

membrane and stain both living and dead cells. Conversely, the propidium iodide dye is membrane-impermeant, and is thus only able to stain cells with a compromised cell membrane. Thus, dead cells appear red under fluorescent light, and living cells appear green. A single bandpass filter was used for viewing each dye, to optimize contrast of cells. For viewing SYTO-9, an Omega Optical Aloha Vivid XF 100-2 was used, and for viewing propidium iodide, an Omega Optical Alpha Vivid XF 103-2 filter cube was used. The resulting view allowed for optical counting of bacteria on the slide, as well as pictures to be taken of the cells.

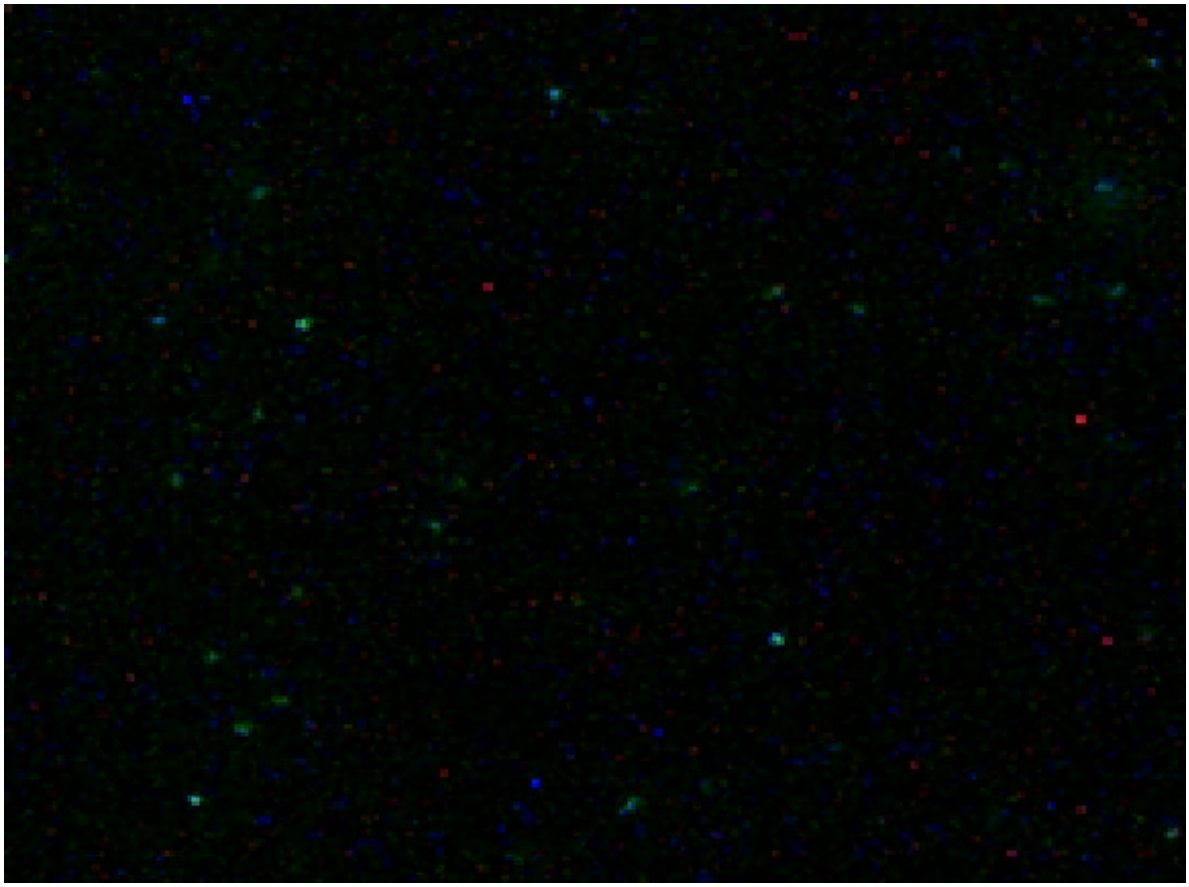

Figure 5: View of E. coli through fluoroscope. Red pixels represent dead cells, green pixels represent living cells, blue pixels represent noise.

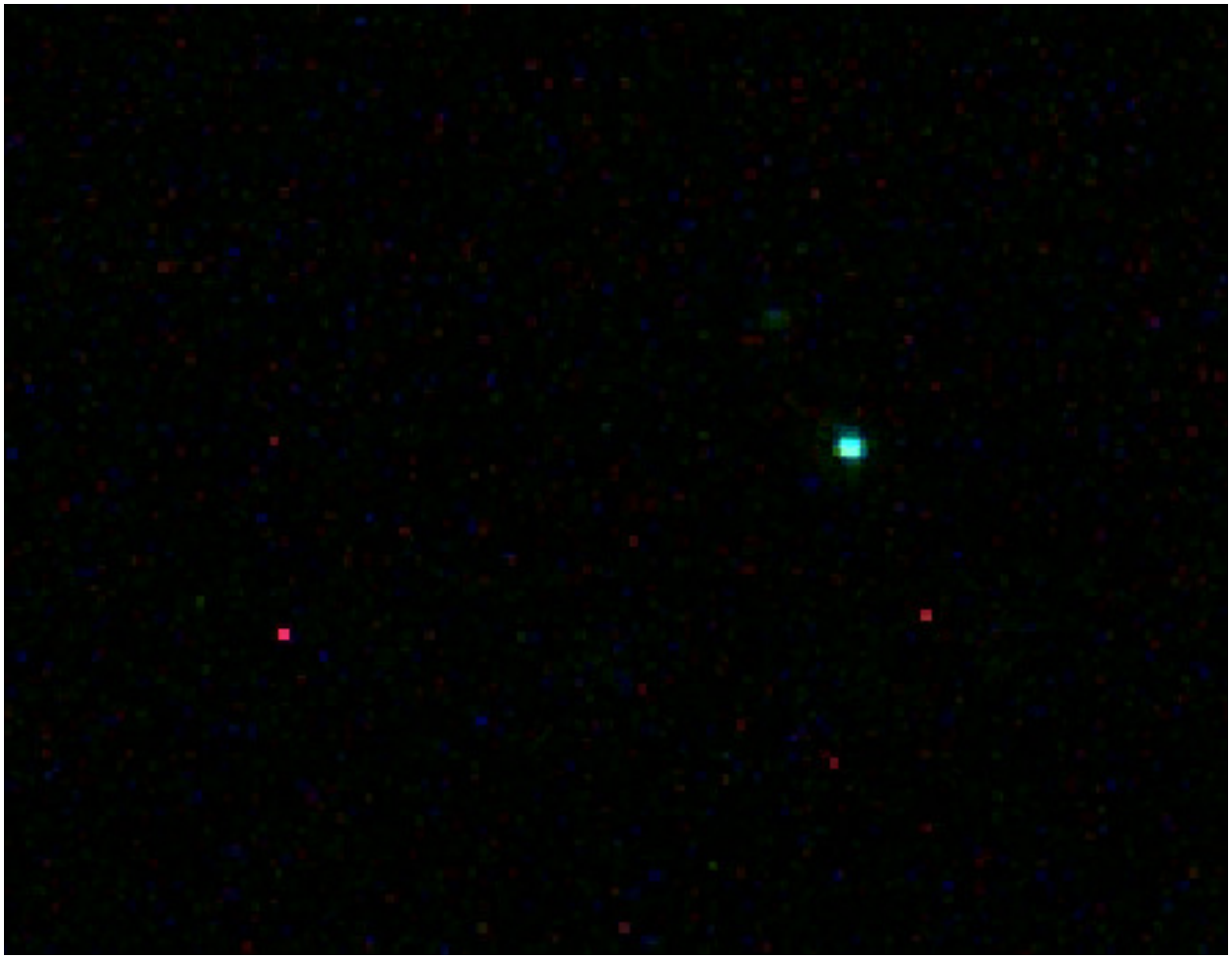

Figure 6: Close up of fluoroscope view shows difference in magnitude between green and red pixels.

[3.3] RESULTS

The results of the experiments conducted in this study are presented in Figures 5 through 12. The resulting cell counts were plotted against the time exposed, and a decay rate was found for each dose. The decay rates were calculated using equation 1, where N(t) is the number of colony forming units at time t, given as a ratio of live cells/total cells, N_0 is the original number of colony forming units, also given as a ratio. The variable –kt is the decay rate at time t. Table 4 illustrates the effective decay rates for each experiment, where y represents the number of colony forming units at time x. Decay rates are given in hr^{-1}. Figure 7 is a graphical representation of these decay rates, illustrating the discrepancy between light and dark results.

$$N(t) = N_0\, e^{-kt} \quad (1)$$

	Decay rate under daylight conditions	Decay rate under dark conditions
Control	$Y=0.9351e^{-0.025x}$	$Y=-0.8898e^{-0.008x}$
Ilmenite	$Y=0.932e^{-0.03x}$	$Y=0.9953e^{-0.025x}$
Hematite	$Y=0.8495e^{-0.038x}$	$Y=0.9335e^{-0.036x}$
Goethite	$Y=0.918e^{-0.024x}$	$Y=0.9346e^{-0.037x}$
Obsidian	$Y=0.8676e^{-0.033x}$	$Y=0.9234e^{-0.036x}$
Fayalite	$Y=0.9067e^{-0.029x}$	$Y=0.9201e^{-0.031x}$
Olivine	$Y=0.9064e^{-0.028x}$	$Y=0.9341e^{-0.03x}$
Pyrite		$Y=0.9335e^{-0.027x}$
Hydrogen Peroxide		$Y=0.9034e^{-0.063x}$
Pyrite + Hydrogen Peroxide		$Y=1.0111e^{-0.075x}$
Goethite + Hydrogen Peroxide		$Y=0.9916e^{-0.073x}$

Table 4: Exponential decay rates of experimental slurries

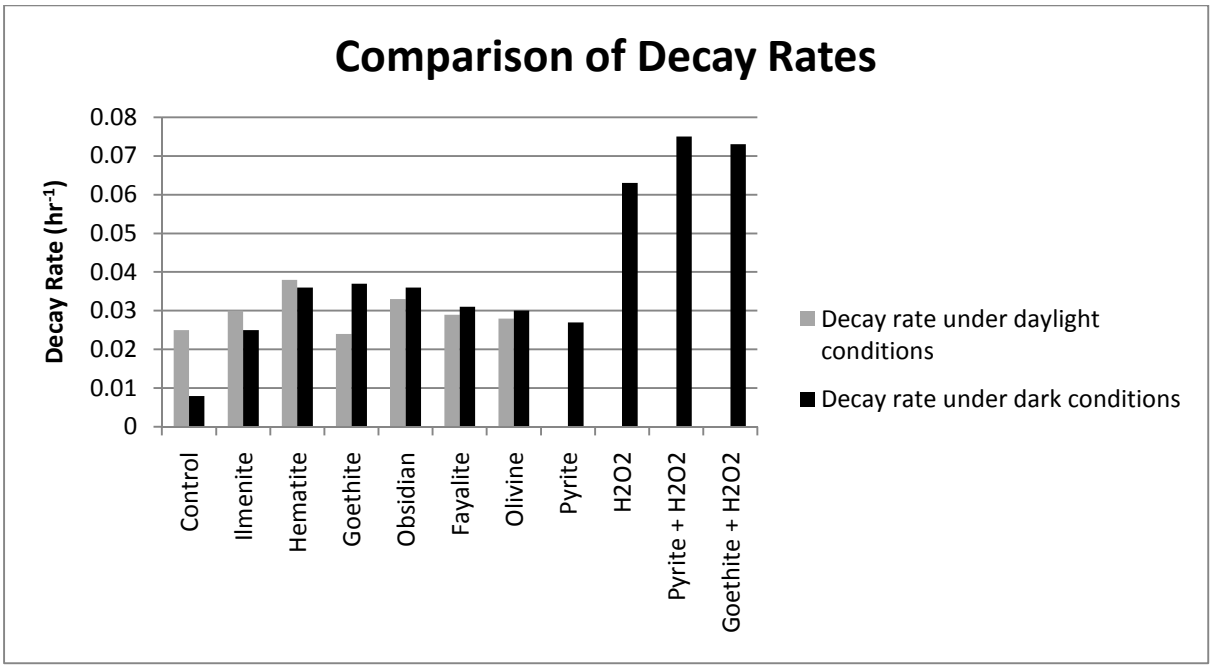

Figure 7: Graphical comparison of decay rates.

Exposure to solar illumination. The results of the control batches, shown in figure 8, show a large difference in the decay rates of E. coli in regards to light exposure. In the control experiments with exposure to light, about 80% of the cells remain viable. Additions of olivine, obsidian or fayalite (figures 9-11) do not lead to lower cell viabilities. Also, there is little or no difference in the dark or solar illumination experiments with these minerals. By contrast, exposure to hematite and ilmenite in combination with daylight reduces viability of E. coli within six hours to 70% (hematite) and 80% (ilmenite), as seen in Figures 12 and 13. This difference, however small, is an indication that these minerals in combination with solar light decrease the viability of the cells present in the slurry.

Exposure to hydrogen peroxide. The second part of the experiment, exposing E. coli to a mineral slurry which also contained hydrogen peroxide, showed varied results. The resulting viability when hydrogen peroxide is added decreases to 55% (figure 14), regardless which mineral is added. In separate batches, both pyrite and goethite were inoculated with hydrogen peroxide. The decrease in viability, when compared to the same minerals under different conditions, shows that the addition of

hydrogen peroxide increases the rate of reaction within the slurry. Additionally, the rate of decay is greater with the addition of minerals to a batch containing only hydrogen peroxide and water.

[3.4] DISCUSSION

The experiments revealed that while there is some effect of direct light on the viability of Escherichia coli K, it is not the driving force in the destruction of bacteria in solar disinfection. As seen in the blank experiments, where no minerals or hydrogen peroxide were added, there is only a small difference between the trials that were run under dark conditions and those run under artificial daylight at 1000 W/m^2. The change in decay constants, from $e^{-0.008x}$ to $e^{-0.025x}$, can be attributed to ultraviolet radiation. This satisfies the assumption that, under less than ideal conditions, solar disinfection is not a useful means of water treatment by itself. As the heat was controlled in these experiments to maintain a constant temperature of 35°C for six hours, it is possible that raising the temperature of the slurry would yield greater results. This also suggests that using black bottles might further increase the efficiency of the process.

Results from the addition of minerals varied greatly, and certain minerals seem more effective under different conditions. In particular, the ilmenite used contained 35.3% TiO_2, and showed an increased effect under ultraviolet radiation. This reflects experiments run by Rincón and Pulgarin, in which a mixture of rutile and anatase was used to increase efficiency under daylight conditions (Rincon and Pulgarin 2004). Ilmenite produces more photoelectrons than either rutile or anatase, but the energy of the photoelectrons is lowered (Rincon and Pulgarin 2004). Thus, while rutile and anatase may produce greater results, ilmenite is an inexpensive alternative for exposure in regions with ample daylight.

Experiments conducted using olivine and fayalite, both of which contained ferrous iron, as well as other constituents, showed no difference between dark conditions and simulated daylight. It can be assumed that there is no photocatalysis of Fe^{2+} occurring, and these minerals would be worthwhile

choices for regions where direct sunlight is less than 500 W/m^2 daily. The reduction in E. coli viability in these batches was from $e^{-0.008x}$, in the dark control experiment, to $e^{-0.03x}$ with olivine and to $e^{-0.031}$ with ilmenite under similar conditions. This is evidence of the Fenton reaction occurring in the slurry and neutralizing the contamination, without the assistance of ultraviolet radiation.

The minerals which contained ferric iron, hematite and goethite, also showed signs of increased efficiency. Previous work has shown that ferric iron is also a powerful oxidizer, and it can be assumed that the Fenton reaction is occurring in these batches as well (Guerinot and Yi 1994). Hematite also showed a higher bacterial effectiveness with exposure to simulated daylight, which may imply that photocatalysis occurs when a large portion of the mineral is purely ferric iron. Further experiments must be conducted to verify this assumption. Conversely, goethite seems to have seen a reduction in efficiency under daylight conditions. The chemistry cannot explain this disparity, and further study is required.

Additional experiments were conducted to understand the role hydrogen peroxide plays in the Fenton reaction with the minerals concerned, and the results showed that there is indeed an increase in efficiency with the addition of hydrogen peroxide to select minerals. Merely adding hydrogen peroxide increased the efficiency of the experiments further than use of minerals alone. The addition of pyrite and goethite, however, further increased the ability of the hydrogen peroxide to disinfect the slurries. This is likely due to increased activity in the slurries through the inclusion of a reactive oxygen species. While oxygenation of the slurry introduces a small amount of dissolved oxygen to drive the Fenton reaction, hydrogen peroxide is a much more efficient reagent.

The research conducted implies that addition of minerals to drinking water in regions similar to Kondoa, Tanzania, could increase availability of clean water to some extent. The inclusion of minerals containing ferrous iron, such as fayalite, or ferric iron, in the case of hematite, shows promise as a

disinfectant. These minerals are fairly common, and in some cases considered a waste mineral by the mining industry. In addition to adding minerals, it is possible that dosing drinking water with a small amount of hydrogen peroxide will further increase disinfection. A combination of the two methods would allow for increased efficiency regardless of the amount of sunlight available. These minerals are added in small enough amounts that there is no threat to the health of the people in areas where there is little iron already in the water, such as Kondoa.

[4] FUTURE DIRECTIONS

The experiments conducted show what there is an effect on the viability of Escherichia coli by the addition of ferrous iron into the water supply. The possibility remains that increasing the amount of minerals added to the slurry could increase the efficiency of the process, though the results of this work has shown there is an effect, albeit a small effect, even in small amounts. Increasing the dosage amount could possibly increase the effectiveness of this method. An alternative approach is to focus on minerals that can promote the Fenton reaction (a dark reaction) and augment the heating of the waters by adding black tape to the bottles. This would have the effect that the water temperature would be higher, while still having the minerals producing hydroxyl radicals. The addition of the black tape would provide little benefit as the sunlight is dim.

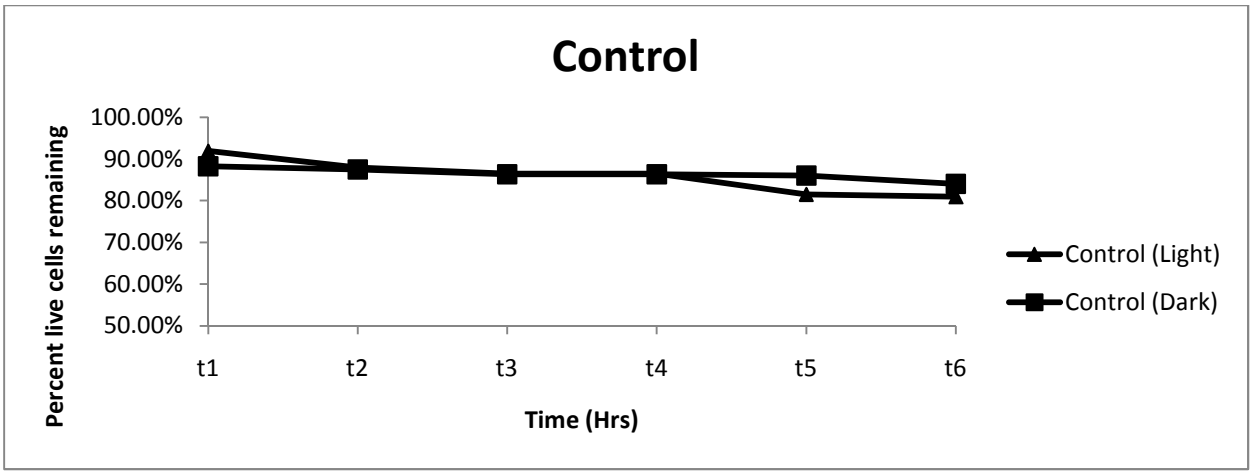

Figure 8: Control

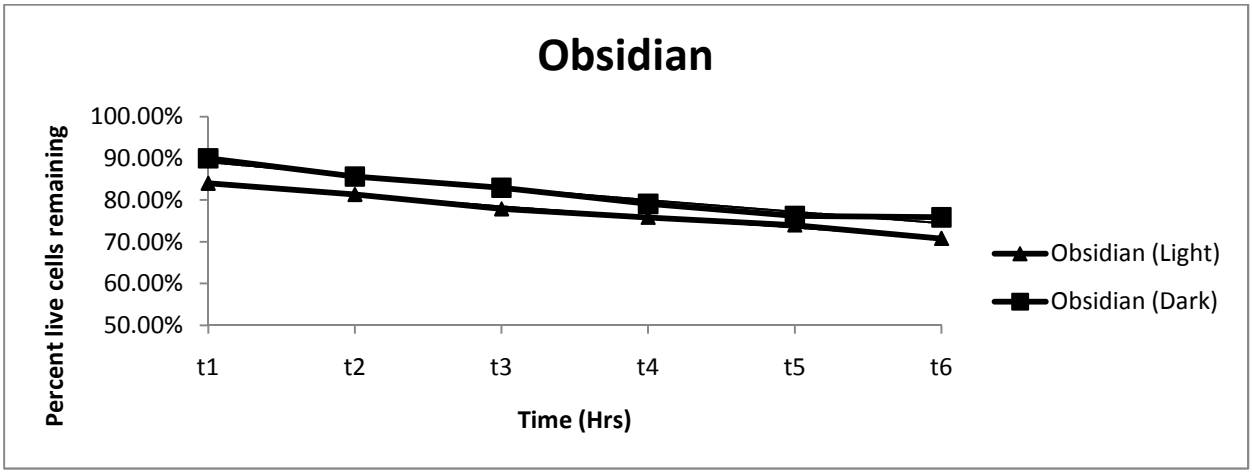

Figure 9: Obsidian

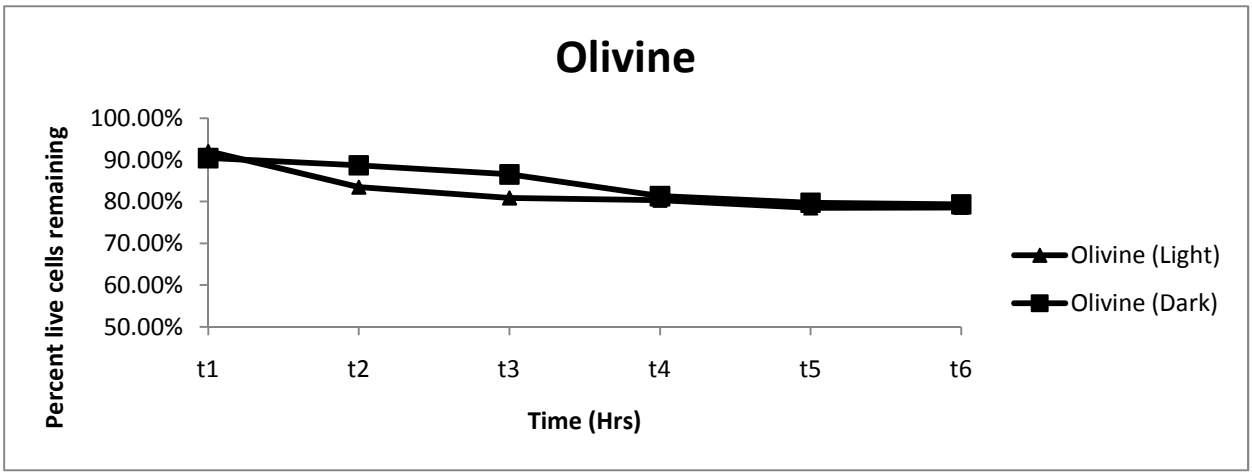

Figure 10: Olivine

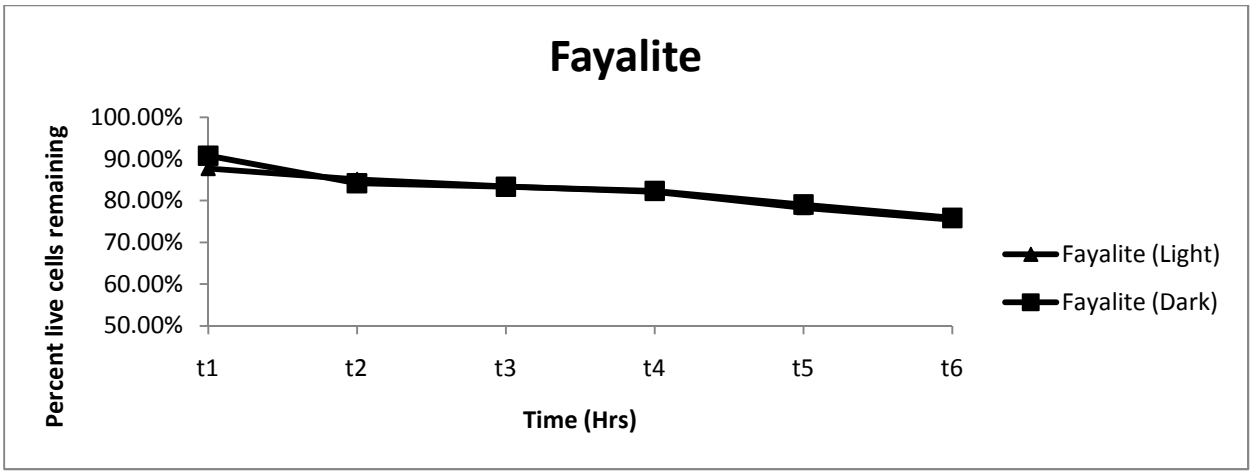

Figure 11: Fayalite

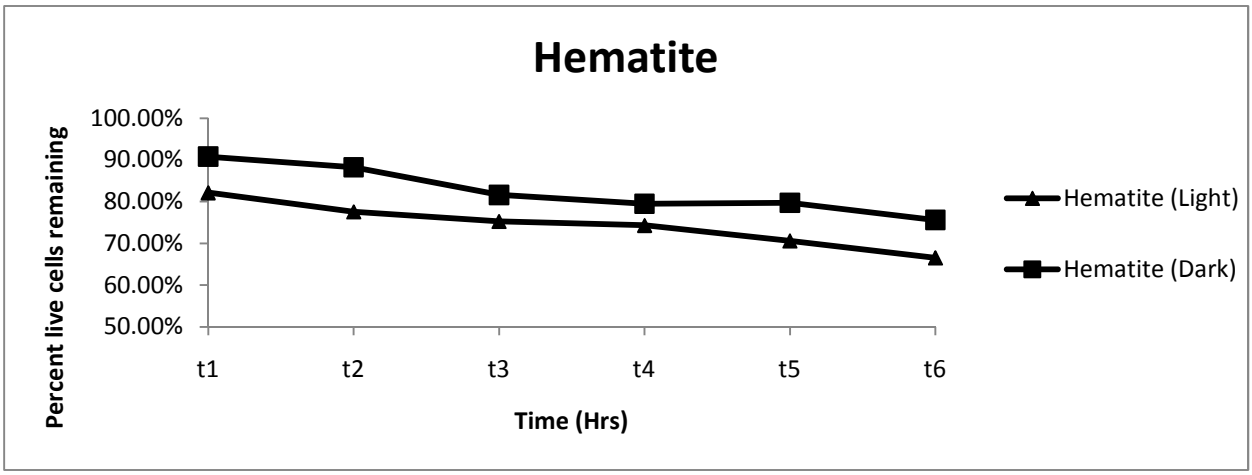

Figure 12: Hematite

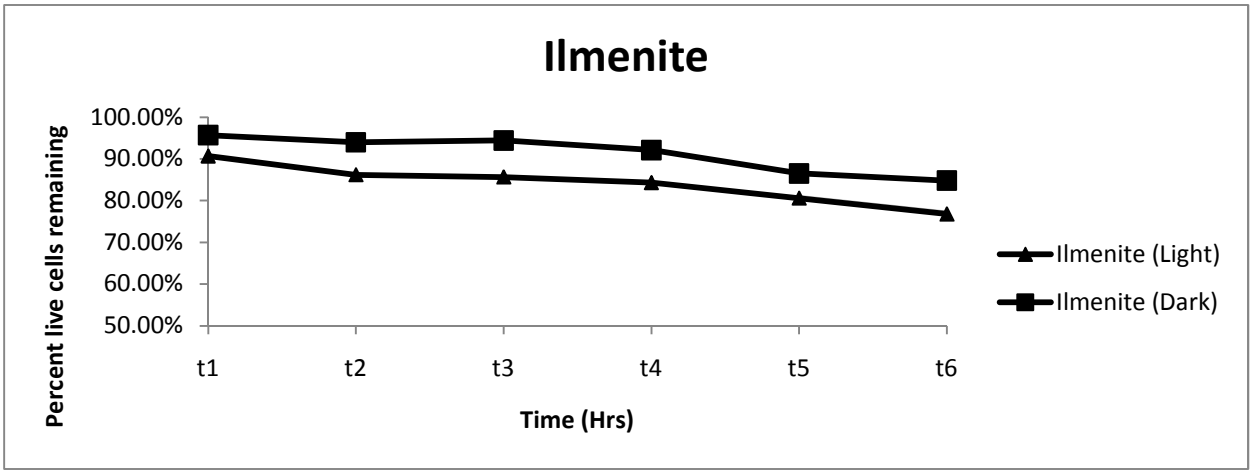

Figure 13: Ilmenite

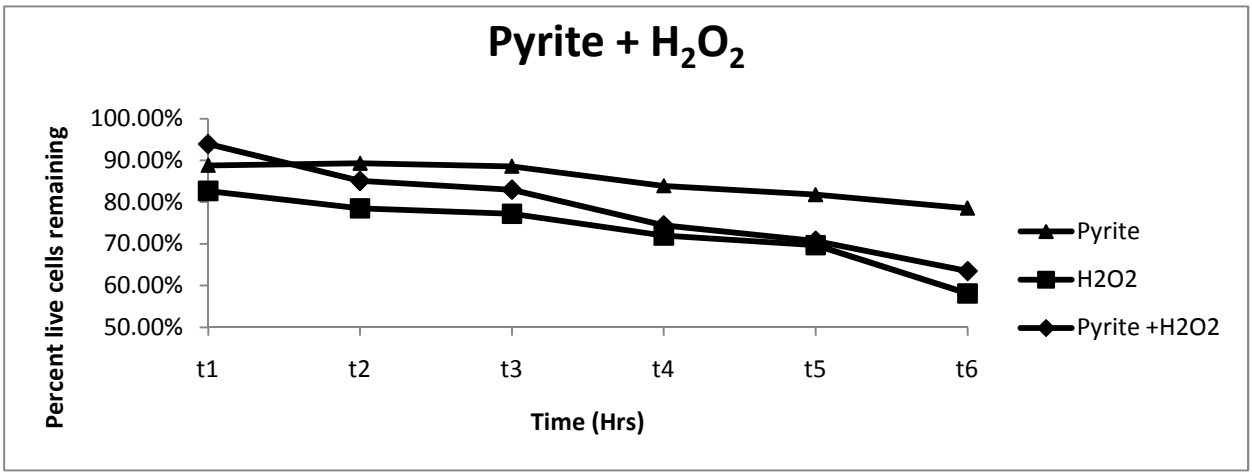

Figure 14: Pyrite + Hydrogen Peroxide

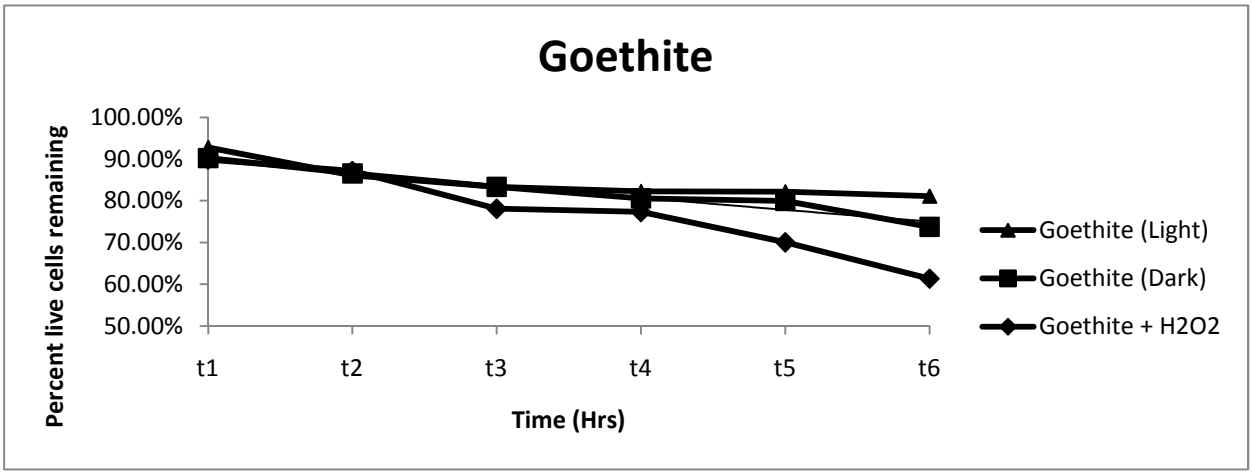

Figure 15: Goethite

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APPENDIX

EXPERIMENTAL PROTOCOL

1) Sample Preparation
   a) Early preparation of mineral samples (before experiments begin)
      i) Grind mineral for 5 minutes at 250 rpm in Retch PM 100 ball mill, using five agate balls and agate cup set aside for iron-bearing minerals.
      ii) Using brass sieve set, filter out 0.125-0.45 μm size fraction of ground mineral.
      iii) Repeat process until desired amount of mineral is achieved.
   b) Container preparation (24 hours before individual experiment begins)
      i) Preheat oven to 375° C.
      ii) In a clean 50 mL glass bottle, place 0.01 g of desired mineral.
      iii) Remove cap from bottle and replace with aluminum foil.
      iv) Heat bottle in oven for 6 hours.
      v) Remove bottle from oven carefully and set on heat-resistant surface. Let cool for at least 12 hours, with aluminum foil covering opening.
   c) Slurry preparation (1 hour before individual experiment begins)
      i) Remove aluminum foil and immediately replace with cap to avoid contamination from airborne bacteria.
      ii) In cooled bottle, place 20 mL de-ionized water.
      iii) Replace cap and shake well.
      iv) Remove E. coli container from freezer and warm with hands. The temperature of the container should not be above body temperature at any time.
      v) Pipette in desired amount of E. coli broth. The resulting mixture should be 200 cfu/ 5 μL of slurry. The exact amount will vary based on the concentration of the broth used.
      vi) Stir vigorously to distribute bacteria and broth in sample as well as increase the amount of dissolved oxygen in the slurry.
      vii) In addition, remove Live/Dead dye from freezer and thaw between hands to room temperature. Pipette equal parts of both components into locking 2 mL vial and shake vigorously. Set aside.
2) Experimental procedure
      i) For dark experiments, cover bottle completely with aluminum foil and place in sealed container. A drawer will suffice.
      ii) For simulated daylight, ignite mercury bulb and lower amps to 20. Place bottle containing tap water 6” from aperture. Place bottles on stand 28” from aperture in center of beam. Use light meter to ensure the resulting exposure is no more than 1000 W/m^2.
      iii) Immediately pipette 1 mL of sample into locking 2 mL vial.
      iv) Pipette 3 μL dye mixture into vial. Shake well to distribute dye.
      v) At room temperature, place dye in sealed container to avoid light contamination.
      vi) Let dye react for 15 minutes to set.
      vii) Pipette 5 μL of dyed sample onto a slide. Trap with cover slip if necessary.
      viii) Repeat the procedure of capturing samples onto slides once per hour over six hours.

3) Counting procedure
    i) Turn on fan to ventilate darkroom.
    ii) Place slide on microscope stand and change to highest objective.
    iii) Slide filter cube tray into 100-2 position and turn off darkroom light.
    iv) Beginning with the apex of the sample, count all visible bacteria. Under this filter set, the bacteria will appear as slightly oblong green spots. The background may be a duller green color, as residual staining from the dye may intercept some light. In this case, the bacteria will still be much brighter than the background and should easily stand out.
    v) Slowly rotate the sample clockwise, keeping a tally of bacteria as they become visible. The object here is to complete a full circuit of the sample, counting all bacteria which appear. This visual method is tedious, but has been shown to be very reliable as a counting method.
    vi) Upon returning to the apex, slide the filter tray into the 103-2 position. The sample should now have a red tint, rather than green. As before, residual staining may give the background a deep red color. Dead bacteria will appear as brighter spots on the red background. Repeat the procedure of rotating the sample clockwise.