Inactivation of *Escherichia Coli*, *Salmonella Enterica* and Naturally Occurring Microorganisms in Edible Geophagic Clay under Real Sunlight

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***Abstract*—**Geophagic practice is associated with dirt because of the natural source of clay. The consumption of clay is therefore always considered as unhealthy. It is of interest to develop a low cost, simple and environmentally sustainable method to disinfect the edible geophagic clay. Experiments were carried out in winter and summer season by inoculating 10^9 CFU/ml^{-1} concentration of *Escherichia coli* and *Salmonella enterica* in geophagic clays. The aim of the study was to investigate the effect of real sunlight on the inactivation of *E. coli*, *S. enterica* and naturally occurring microorganism in geophagic clays. Geophagic clays were exposed to real sunlight for periods up to a cumulative time of 5 hours. Inactivation was determined by a log reduction in the growth of the organisms. The clay temperature (°C) was taken at every time point and the climatic conditions were monitored. During periods of strong sunlight, complete inactivation of bacteria occurred under 4 h, even at a clay temperature of <35°C. Under dark exposure and low clay temperature, non-complete inactivation was observed. No regrowth of bacteria occurred within 24 h and 48 h after the complete disinfection of exposed clays. However, regrowth in clay exposed to the dark occurred within 24 h and 48 h and in low level and no reactivation of bacteria. The naturally occurring microorganisms showed a far higher sensitivity than *E. coli* and *S. enterica*. The study confirms that significant clay disinfection can be achieved using real sunlight exposure which is available to the community at no cost, to reduce negative impact to human health.

***Index Terms*—** Real sunlight; Regrowth*; E. coli; S. enterica;* Naturally occurring microorganisms; Geophagic clay; Health risk.

I. Introduction

Geophagia is widespread in sub-Sahara Africa and also prevalent in the following countries: South Africa, Swaziland, Nigeria, Uganda, Cameroon, Malawi, Tanzania, Zambia, Zimbabwe and Kenya [1; 2; 3; 4; 5]. However, geophagia is considered as a medical condition by the World Health Organization [6]. The main reason for geophagia practice is the supplementation of minerals from the consumption of clays, moreover, there are some other reasons including hunger and protection from toxins and pathogens [4; 7]. Geophagia is a risk factor in the transmission of geohelminth parasitic infection, microbiological infection and development of iron depletion and anemia, furthermore, anemia or iron deficiency can cause a craving for soil [7; 8]. A study conducted in the Eastern Cape Province, South Africa reported that geophagic materials are contaminated by either one of the following: *geohelminths, Ascaris lumbricoides, Trichuris trichuriasis, Nectar Americans, Ancylostoma duodenal* and *Strongyloides stercoralis* [9; 10]. Geophagic materials can alleviate the detrimental effects of plants chemicals and microbes by either adsorbing pathogens or toxins within the gut lumen or covering the surface of intestinal endothelium thereby causing it less permeable to toxins and microbes [11]. Nchito *et al* [2] reported that 53%-80% of the girls in Zambia and South Africa consume soil and 37% of the pregnant women ingest soil [12]. Solar disinfection (SODIS) is a low cost and simple water treatment method used to eliminate pathogens to improve the microbiological quality of water and it uses both the optical effects (UV solar radiation) and thermal effect (water temperature greater than 45°C) [13; 14]. SODIS involves exposing contaminated water in transparent bottles (polyethylene terephthalate bottles) for at least 6 hours to direct sunlight [14; 15]. The inactivation of microbes is through the synergistic effect of both U-VA radiation (wavelength: 320-4000nm) and temperature increase which must reach at least 45°C [13; 16; 17]. The types of earth materials consumed vary from one locality to the other. Nyanza *et al.* [18] reported that pregnant women in Tanzania preferred soil from the wall of houses, termite mounds and ground soil but commonly (69%) eat soil sold in the markets. And other studies reported that most of the geophagists (95%) purchase it from the markets [19; 20]. The geophagic clays are mined in large quantities and distributed for sale in markets in countries such as Nigeria, Ghana and West African sub-region [20; 21]. Henry and Cring [22] reported that the marketed soil is cleaned, baked, shaped and cooked. Relatively high amount (71-90 g/day) of earth materials are consumed by women in Vhembe District [10]. A Recent study demonstrated the inactivation of microorganisms found in the geophagic clays obtained from mining sites and from markets exposed to UV- A lights (solar treatment in simulated chamber) [5]. This study uses the real sunlight to inactivate the microorganism in geophagic clays. The potential regrowth of organisms was studied.

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II. Methodology

2.1. Sample collection

Samples were purchased from open markets in Pretoria and mined from the respective sources. The collected clay

samples were packed into sterile plastic bags and the loose clays were purchased and picked from the lot and stored in plastic bags as a normal practice with any customer. And other samples were already packed in the plastics by vendors for commercialization. Sample collection took place within different seasons; in summer (2015) and winter season (2016). The samples were kept in the refrigerator at 4°C until analyzed.

2.2. Bacterial growth

Laboratory strain of *E. coli* and *S. enterica* were obtained from frozen stocks (Liquid Stock Culture) and 100 µl inoculated on agar plates and incubated at 37°C for 15-18 h. A single colony was then inoculated into 250 mL of sterile Luria broth and was then incubated at 37°C for 18 h on a rotary shaker to obtain a stationary phase culture. The volume of 500 µL of stock was inoculated into clays, respectively, in order to obtain a 109 CFU mL^{-1} starting concentration of bacteria for each experiment.

2.3. Real sunlight inactivation of *E. coli, S. enterica* and naturally occurring microorganisms

Inactivation of *E. coli, S. enterica* and naturally occurring microorganisms using real sunlight was carried out in the metal zinc aluminum painted roof of CSIR Environmental Building No 33, in the full sunshine. The clay samples were autoclaved to inoculate the bacteria *E. coli* and *S. enterica* (the starting concentration was indeed 109 CFU mL^{-1}). Samples were collected in the experimental day and exposed same day, to remove any naturally occurring microorganisms in the market and mining clay samples. The experimental configuration was composed of clay samples exposed to sunlight covered by Polyethylene terephthalate (PET) bottles and other clay samples exposed to dark covered by PET bottles, wrapped with paper towels. The clay temperature (°C) was taken at every time point and the climatic conditions were monitored. The clay samples were irradiated to real sunlight for periods of 5 hours. Samples were taken every 1 hour of real sunlight exposure until the end of the experiment (5h). The last clay samples were stored in dark at room temperature and sampled at 24 h and 48 h to monitor regrowth. All the experiments were conducted in triplicate and each sample was plated in triplicate. Samples were serially diluted in sterile phosphate-buffered saline (PBS) solution and plated on their respective media using the drop count technique. The volume of 40 µL of the approximately diluted sample was spread on Membrane Lactose Glucuronide agar plates and Nutrient agar plates in triplicate and incubated at 37ºC for 18 h and counted the following day.

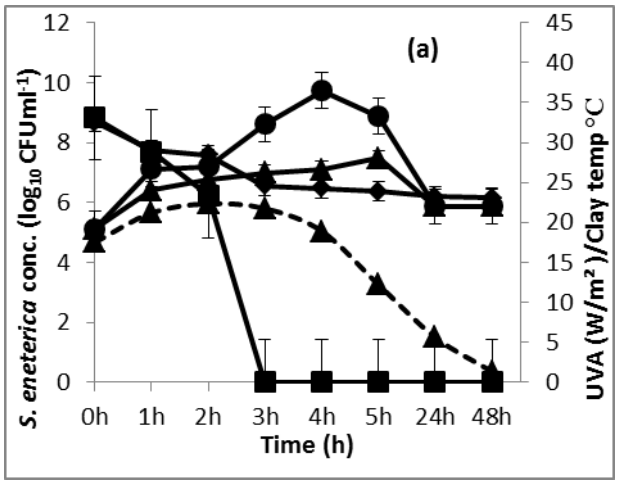
SODIS of E. coli kept under real sunlight was carried out as the control assays. Polyethylene terephthalate (PET) bottles of 2 L in volume were filled with distilled water. Subsequently, each bottle was inoculated with 1 mL of stock concentration, giving an initial population of 109 CFU/mL. These bottles were placed on the roof of the building and exposed to full sunshine and were prepared in triplicate. During the experiment, water temperature was monitored. The solution was irradiated for 6 h. Samples were taken after 15 min, 30 min, 45 min, 60 min, 90 min 120 min, 180 min, 240 min, 300 min, 360 min of sunlight exposure. Serial dilutions were performed in PBS and incubated for overnight and counted using the plating techniques described previously.

III. Results And Discussion

During winter season naturally occurring microorganisms were not observed in marketed clays and mining site clays; naturally occurring microorganisms were observed in clays only in summer season. Both mining site and marketed samples were contaminated with microorganisms (Fig 3). Mining site clays were observed with a high concentration of naturally occurring organisms compared to marketed clay. Geophagic clays in the open markets are exposed to the sunlight, each day prior to the collection of mined clays [5]. This could explain the inactivation of naturally occurring microorganism in marketed clays. [22] reported that marketed soil is prepared in a cleaned way, however, microorganisms were observed in such clays. Geophagic materials from the environment are expected to be contaminated with indigenous microorganisms which may be involved within the beginning of the soil [23]. The contamination of marketed clays probably results from the spreading of dust and handling [5]. After 2 h of sunlight exposure, the naturally occurring microorganisms from mining clay were completely inactivated, whereas naturally occurring microorganisms in the market clays were totally inactivated after 1 h (Fig 3). High UV irradiance values were observed within 2 h of experiment ranging from 39.5- 30.4 W/m2 and with the maximum ambient temperature of 40°C. Climatic conditions varied from winter to summer, with ambient temperatures in winter ranging between 8°C and 23°C during the experiments, and between 12°C and 40°C in summer, and UV irradiance levels were of 15 W/m2 average in winter and 36 W/m2 in summer. UV radiation values were doubled in summer corresponding with the study of [24] who observed doubled irradiation values in summer. Inactivation of *E. coli* during the winter season resulted from synergistic effect of clay temperature and UV radiation. The complete inactivation of *E.coli* was achieved after 5 h of sunlight exposure (Fig 2a) during winter and in summer the complete inactivation of *E. coli* was achieved after 4 h of sunlight exposure (Fig 2b). Difference of 1hour for complete inactivation of E. coli between summer and winter experiments is due to variations of climatic conditions. After sunlight exposure of *S. enterica* during summer, complete inactivation was achieved after 4 h (Fig1a), however, after 2 h exposure there was 4-log reduction of the concentration of *S. enterica* and in 3 hours there was 3-log reduction. The averages of UV irradiance levels were 46.01 W/m2 in summer and 15.1 W/m2 in winter, and ambient temperature max of 38°C in summer and 23°C in winter. These results indicate that useful inactivation can still occurs also when there is low UV irradiance [25]. The complete inactivation of *S. enterica* was achieved after 3 h sunlight exposure in winter. Non- complete disinfection was observed when *E. coli* and *S. enterica* contaminated clays were kept in the dark; this was likely due to insufficient UV irradiance and thermal heat of solar light received by the samples.

No regrowth was observed on the fully inactivated sample, however, in samples exposed to the dark, regrowth of *E. coli* and *S. enterica* were observed after 24 h and 48 h and no reactivation of bacteria were observed. The results are in

agreement with [26] study that observed no E. coli regrowth in the water disinfected using SODIS; other literatures also reported no regrowth of E. coli in water disinfected for 2-3 days [13; 16; 25; 27; 28; 29; 30; 31]. When the water reached complete disinfection according to the WHO drinking Water Guidelines, there should be 0 CFU/100ml [32]. [33; 34] observed a regrowth in incompletely disinfected water incubated at 37°C for 24 h. Regrowth of *E. coli* and *S. enterica* occurred within 24 h and 48 h in clays exposed to the dark because of the level of disinfection achieved (Fig 1a and Fig 2a). Not all microorganisms in the clays are killed, and because they were only injured, they recovered and multiplied [35]. From the results the *S. enterica* bacterial showed to be more resistant to real sunlight than *E. coli* bacterial. In SODIS treatment, the inhibition of *E. coli* was mostly due to the effect of solar radiation and temperature, however, some species may be more resistant as [36] reported that *Salmonella typhimurrium* were more resistant to SODIS treatment.



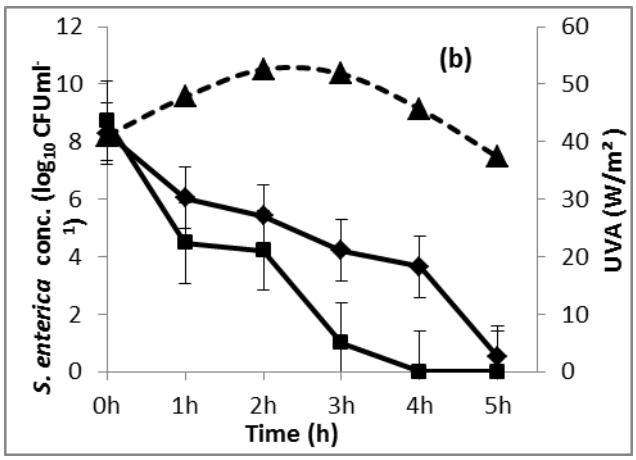
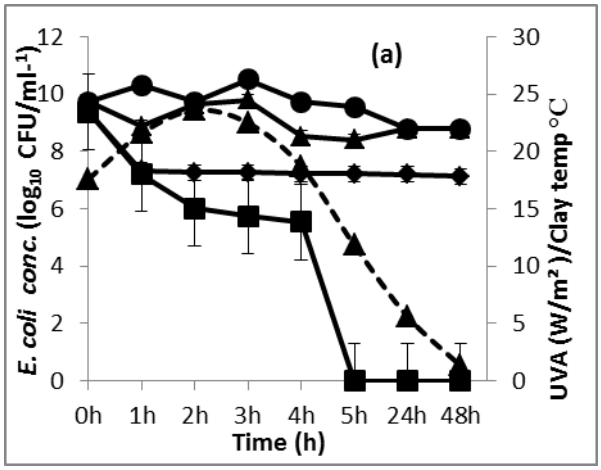


Fig. 1. Inactivation of *S. enterica* in the geophagic clay exposed to real sunlight and in dark exposed to sunlight wrapped in paper towels. Experiment during the winter season (Fig a) and experiments during summer season (Fig b). Solar irradiance on the day of the experiment (--►--), temperature of dark exposed clay (--▲--), the temperature of exposed clay (--●--), *S. enterica* inactivation in exposed clay (--■--), in dark exposed clay (--♦--). Errors bars indicate standard error of triplicate measurements.



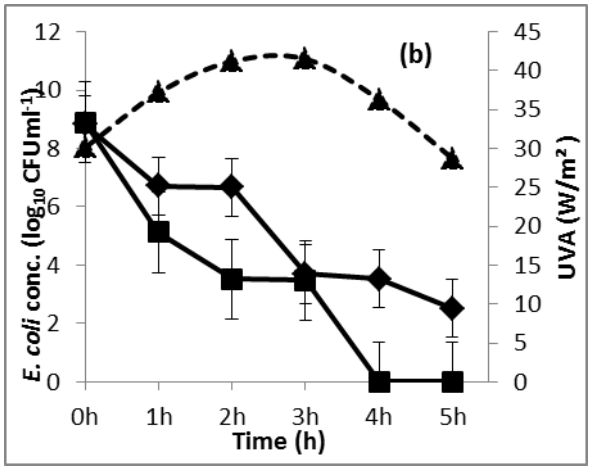


Fig. 2. Inactivation of *E. coli* in the geophagic clay exposed to real sunlight and in dark exposed to sunlight wrapped in paper towels. Experiment during the winter season (Fig a) and experiments during summer season (Fig b). Solar irradiance on the day of the experiment (--►--), temperature of dark exposed clay (--▲--), the temperature of exposed clay (--●--), *E. coli* inactivation in exposed clay (--■--), in dark exposed clay (--♦--). Errors bars indicate standard error of triplicate measurements.

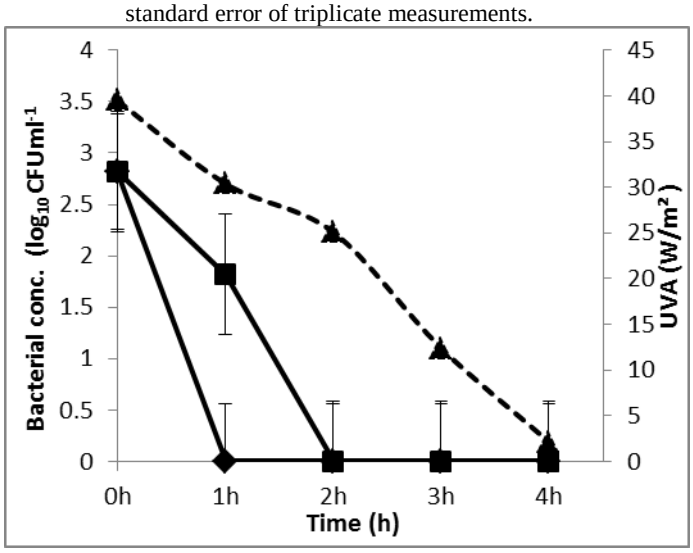


Fig. 3. Inactivation of naturally occurring organisms in markets clays (--♦--) and mining clays (--■--), solar irradiance (--►--). Errors bars indicate standard error of triplicate measurements.

III. Conclusion

This study confirms that significant inactivation of microorganisms in geophagic clays can be achieved using a real sunlight exposure, environmental sustainable and free method. SODIS is approved for water treatment. The treated clays that has been completely disinfected (values of 0 CFU/mL), are therefore considered according to WHO drinking water guideline, safe for consumption with regard to the microbial quality. It poses no health risk associated to potential regrowth of *E. coli* and *S. enterica* in completely disinfected clays. The risks are minimum, when clays are exposed to sunlight in dark because of the level of inactivation achieved. It is recommended to expose clays from the mining sites to direct sunlight for few days to ensure inactivation of the naturally occurring microorganisms prior to commercialization Inactivation studies of other microbial organisms are still to be conducted.

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