academicJournals

Vol. 14(50), pp. 3313-3324, 16 December, 2015
DOI: 10.5897/AJB2015.14971
Article Number: 18048E556616
ISSN 1684-5315

http://www.academicjournals.org/AJB

AfricanJournalofBiotechnology

Full Length Research Paper

Application of solar treatment for the disinfection of geophagic clays from markets and mining sites

Fosso-Kankeu E.[1]*, Netshitanini T. L.[1], Abia A. L. K.[2], Ubomba-Jaswa E.[3] and Waanders F. B.[1]

[1]School of Chemical and Minerals Engineering, Faculty of Engineering, North-West University, P. O. Box 558, Potchefstroom 2531, South Africa.
[2]Department of Environmental, Water and Earth Science, Tshwane University of Technology, 175 Nelson Mandela Drive, Pretoria 0001, South Africa.
[3]Natural Resources and the Environment, CSIR, P. O. Box 395, Pretoria 0001, South Africa.

Received 16 September, 2015; Accepted 12 November, 2015

Most of the microorganisms occurring in geophagic clays are undesirable and may to some extent be harmful to geophagists; it is therefore important to develop a cheap and sustainable technique for the treatment of these clays prior to consumption. In this study, a solar treatment simulated in a chamber has been investigated for its potential to inactivate the microorganisms found in the geophagic clays obtained from mining sites and from the markets. The results have shown that irrespective of the sources of the clays, they were contaminated with large amount of potentially harmful microorganisms which could have been sustained by suitable conditions such as relatively high moisture content (average of 3.6%) and the presence of organic carbon (between 1.06 and 1.5%). Treatment with simulated solar irradiation has resulted in most instances to the reduction of the number (up to 100% inactivation) of microorganisms although in few cases the same conditions have stimulated the growth of some dormant microorganisms including *Bacillus subtilis*, *Paenibacillus*, *Bacillus cereus*, *Bacillus thuringiensis*, *Bacillus humi* and *Arthrobacterarilaitensis*. This study shows that the solar treatment has the potential to significantly reduce the amount of microorganisms occurring in the geophagic clays, but require further investigation for improvement of the technique.

Key words: Geophagic clays, solar treatment, microorganisms, moisture content, organic carbon, mining sites, markets.

INTRODUCTION

Deliberate eating of soil or geophagia is a common practice reported in various countries around the world (Ekosse et al., 2015; Ngole-Jeme and Ekosse, 2015). Human and animals have been engaging in this practice for various reasons including seeking for minerals that are not provided in sufficient quantities in their normal

*Corresponding author. E-mail: kaelpfr@yahoo.fr.

diet (Songca and Oluwafemi,2015). Geophagic clays are collected from the environment and are likely to be contaminated with indigenous microorganisms which may have been involved in the genesis of the clays (Fosso-Kankeu and Bafubiandi, 2015). Using DNA-hybridization analyses to study the microbial community in the soil,Kent and Triplett (2002) found 4500 species of prokaryotic microorganisms per gram of natural soil. The presence of these microorganisms in the geophagic clays can influence the health of geophagists in different ways; beneficial bacteria such as probiotic bacteria favourably alter the intestinal microflora balance, inhibit the growth of harmful bacteria, promote good digestion, boost immune function, and increase resistance to infection (Mel'nikova et al., 1993; Smirnov et al., 1993; Fosso-Kankeu and Mulaba-Bafubiandi, 2015). However a large fraction of the microorganisms occurring in the clays especially after handling and storage, have the potential to negatively affect the health of people ingesting the clays. Most of the infections reported as the result of geophagic practices have been associated with parasitic geohelminths. Cases of people infected with geohelmints such as *Ascaris* and *Trichuris* following geophagic practices were reported in Lusaka-Zambia (Nchito et al., 2004), Jamaica (Wong et al., 1991) and Kenya (Geissler et al., 1998). Pathogenic bacteria with the potential of causing diseases such as *Clostridium tetani* and parasitic worms have also been identified in geophagic clays (Bisi-Johnson et al., 2015). A correlation has been established between the occurrence of *Yersinia enterocolitica*, *Escherichia coli*, *Streptococcus faecalis*, *Helicobacter pylori*, and *Mycobacteria* and the aetiology of Crohn's Disease which is characterized by a severe, non-specific, chronic inflammation of the intestinal wall (Liu et al., 1995; Rubery, 2002; Lamps et al., 2003). Geophagists often pretreat their clays using heat treatment (baking and burning) mostly to improve the taste of the clays (Bisi-Johnson et al., 2013). This may contribute to reduce the amount of microorganisms in the clays. However, this process uses artificial sources of energy and is therefore costly. Furthermore, the extreme and uncontrolled heat may result in the degradation of some important nutrients or compounds present in the clay. Studies have demonstrated that solar radiation can inactivate a wide range of microbes (fecal indicator, waterborne pathogenic bacteria, viruses and protozoal parasites). Millions of people in developing countries have been using solar disinfection (SODIS) for the treatment of water and mitigation of outbreaks related to waterborne diseases (Conroy et al., 1996; McGuiganet al., 1998; Conroy etal., 2001; Mani, 2006; Rainey and Harding, 2005). The principles of microorganisms inactivation using solar treatment include: (1) optical inactivation (radiation in the spectrum of UVA light); (2) thermal inactivation (increases water temperature) (Wegelinet al., 1994). Its germicidal effect is based on the combined effect of thermal heating of solar light and optical inactivation (UV radiation) (McGuigan et al., 2012). This means that the disinfection of microorganisms increases (inactivate pathogens at faster rate) when they are exposed to both thermal inactivation and UV-A light at the same time. The solar UV-A can be used in cloudy days in the presence of reflectors to enhance the optical inactivation potential of SODIS, distinct from the thermal inactivation (blackened surface) which enable to raise the temperature of water sufficiently on cloudy day (Duffie and Beckman, 2006; McGuiganet al., 2012). Solar radiation can be divided into three ranges of wavelength: UV radiation, visible light and infrared radiation. UV-B and UV-C light are absorbed by ozone layer (in the atmosphere which protects the earth from radiation coming from the space), while UV-A radiation reaches the surface of the earth (Solar water disinfection, 2002) and can therefore be used as the solar radiation. However, to the best of our knowledge no study has so far considered to investigate the impact of solar treatment on the disinfection of geophagic clays.

The objectives of this study were to: (1) Investigate the survival of indigenous microorganisms as well as the contribution of handling on the microbial contamination of geophagic clays; (2) To assess the potential of solar irradiation for the disinfection of geophagic clays; to the best of our knowledge, it is the first time that solar treatment is considered for the disinfection of geophagic clays.

MATERIALS AND METHODS

Study area

The study was conducted in two locations of the Gauteng province and the North-West province of South Africa. Samples of geophagic clay were obtained from different markets and mining sites. The geophagic clay samples for this study were divided into two sample-groups: Market sample group collected from vendors (purchased from markets); and field sample group collected from mining sites where the vendors obtained the commercialized clays. Geophagic clay vendors set up open stall at the local markets where the clays were displayed on the tables and stored in the plastic bags after business hours. The market samples were purchased from two different places located within Pretoria (Mabopane local market) in the Gauteng province and Potchefstroom (Ikageng local market) in the North-West province (Figure 1).

Sample collection

Geophagic clay samples were purchased from two different daily markets in Pretoria and Potchefstroom and other samples were obtained from mining sites indicated by vendors as the sources of commercialized clays. Samples collection took place within two weeks in the first semester of the year corresponding to the summer season. Clays available on the vendors tables were in the loose form or in the transparent plastic bags. The colours of the clays varied from reddish to greyish; the exact location of mining site was specified by the vendors for each clay type. The loose clays were purchased and they were randomly picked from the lot by the vendors then put in a black plastic bag as normal practice with any customer. As per arrangement the trips to the mining sites

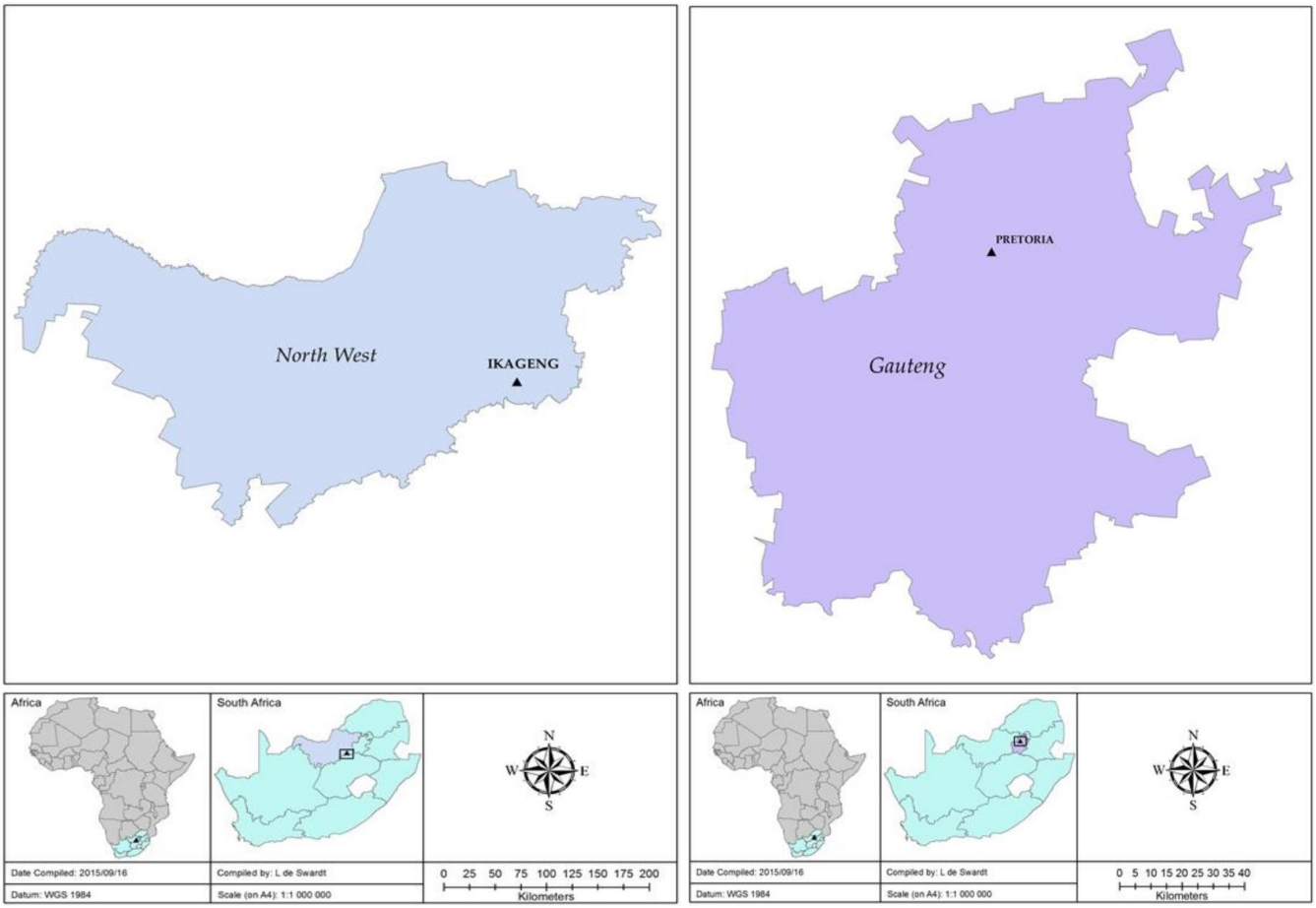

Figure 1. Sampling location in the Gauteng and North West provinces of South Africa.

were in company of the vendors who assisted to locate the exact point of mining and used the same equipment (knives, forks, and spades) to collect the samples which were packed in clean polyethylene plastic bags and transported to the laboratory for analysis.

DNA extraction

Samples from the market and mining sites were ground and homogenized into fine powders using mortar and pestle. Genomic DNA was extracted from ±250 mg of each of the six (6) clay samples using the ZR Soil Microbe DNA Mini Prep kit™ according to the manufacturer's instruction. DNA concentrations were determined spectrophotometrically with a Nano-Drop spectrophotometer (Thermo Scientific). Then extracted DNA samples were sent to Inqaba Biotechnical Industries (Pty) Ltd for sequencing using Next Generation Sequencing (NGS).

X-ray diffractometry (XRD) and X-ray fluorometry (XRF) analyses

The mineralogical composition of the clay was determined through X-ray diffractometer (XRD); The diffractometer used was the Philips model X'Pert pro MPD, at a power of 1.6 kW used at 40 kV; Programmable divergence and anti-scatter slits; primary Soller slits: 0.04 Rad; 2θ range: 4-79.98; step size: 0.017°. The elemental composition of the clay was determined using the X-ray fluorometer (XRF) which was performed on the MagiX PRO and SuperQ Version 4 (Panalytical, Netherland); a rhodium (Rh) anode was used in the X-ray tube and operated at 50 kV and current 125 mA; at power level of 4 kW.

Soil moisture

The samples were weighed ("initial weight") and then placed in a thermostatically controlled oven. Samples were dried at a temperature of 100 ± 5°C for 24 h. The samples were then weighed again ("dry weight") then their soil moisture percentage could be calculated according to the following equation:

$$\%W = \frac{W_1 - W_2}{W_1} \quad (1)$$

Where, %W = Percentage of the moisture in the sample; W_1 = Initial weight of the sample in grams
andW_2 = weight of dried sample in grams.

Carbon analysis

Clay organic carbon content was determined according to the Modified Walkley-Black (Walkey and Black, 1934) chromic acid wet

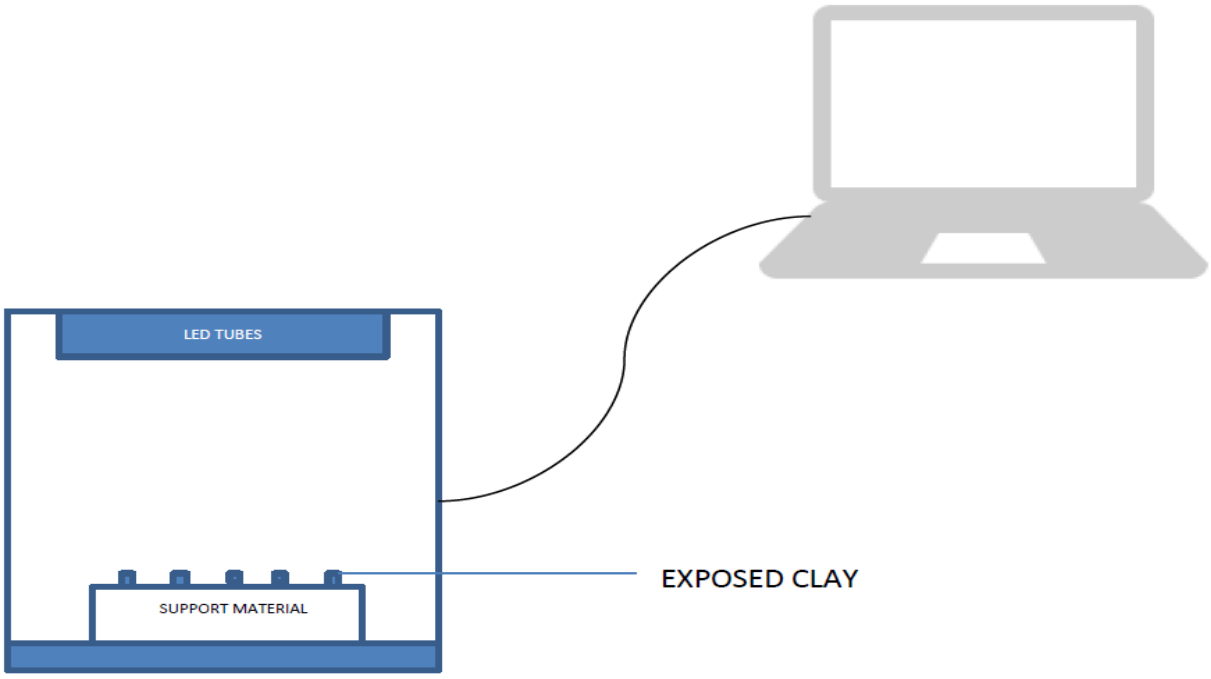

Figure 2. Schematic diagram of the solar treatment reactor system.

oxidation method: 1g of dried clay (ground to < 60 mesh) was mixed with 10 mL 1N $K_2Cr_2O_7$ in a 250 mL Erlenmeyer flask, then 20 mL of concentrated H_2SO_4 was added and the content of the flask gently swirled to mix. The mixture was then heated on a hot plate till a temperature of 135°C was reached for half a minute. The clay suspensions was adjusted to 200 mL with distilled water after 30 min and four drops of Ferroin were added then titrated with 0.4 N $FeSO_4$ till an end point at which the organic content could be calculated.

The percentage of organic carbon was determined by the following formula:

$$\%C = \frac{0.003\,g \times N \times 10\ ml \times (1-\frac{T}{S}) \times 100}{ODW} \quad (2)$$

Where, N is the normality of $K_2Cr_2O_7$ solution, T is the volume of $FeSO_4$ used in sample titration (mL), S is the volume of $FeSO_4$ used in blank titration (mL) and ODW is the oven-dry sample weight (g).

Solar treatment

The reactor chamber (Figure 2) was mounted with wooden materials and was equipped with three 8 W LED tubes (Philips) attached in parallel to the roof emitting light at a wavelength in the range 315 - 400 nm; to deliver the power within the visible spectrum, LED was chosen due to the good correlation between the spectral irradiation of the LED and the spectral radiation of power delivered by the sun. Small holes were perforated at the backwall of the chamber to ensure proper circulation of air. A temperature monitoring device made with a thermocoupler was fitted at the back of the chamber and linked to the laptop for instant recording of the temperature in the photocatalytic chamber.

Clay samples were prepared by carefully cutting into square pieces of 2 cm × 2 cm with a thickness of 0.3 cm under aseptic conditions to minimize cross contamination. Solar treatment was applied by exposing the cut clay samples to UV-A lights for 8 h a day in the photocalytic chamber linked to the laptop; every day the clay was turned over to expose the other site to the light. The exposure time was 1, 2 and 4 weeks. After the solar treatment, the clay samples were ground for the determination of the moisture content and survival of microorganisms. Microbiological analysis was performed using 1 g of clay that was mixed with 10 mL of sterile distilled water and 100 µL of the suspension was inoculated to the freshly prepared Brilliance *E. coli*/coliform medium (Oxoid, SA) and incubated at 35°C for 48 h; the distinctive colonies identified by colour or shape were counted and expressed as CFU/mL. Individual colonies were subcultured in the fresh Brilliance *E. coli*/coliform medium (Oxoid, SA) and incubated at 35°C for another 48 h and then finally subcultured in the nutrient agar plate ("Lab-Lemco" powder 1.0; yeast extract 2.0; peptone 5.0; sodium chloride 5.0; agar 15.0; pH 7.4 ± 0.2 at 25°C; gram per one liter; Merck Chemicals, SA) under the above conditions. The isolated cultures were sent to Inqaba Biotechnical Industries (Pty) Ltd for the identification of microorganisms through the sequencing of the 16S rDNA.

RESULTS AND DISCUSSION

Chemical and mineralogical composition of the clays

Among the major oxides shown in Table 1 the average SiO_2 values range from 55.62 to 15.56% with the highest value recorded from samples collected at Ikageng source while the samples from Pheramindi source exhibited the lowest value. The concentrations of Al_2O_3 ranged from 5.17 to 33.15% in all the clay samples. Geophagic clay

Table 1. Major oxides (mass %) analysis of samples.

Samples	Oxide concentration (wt %)											
	SiO_2	TiO_2	Al_2O_3	Fe_2O_3	MnO	MgO	CaO	Na_2O	K_2O	P_2O_5	Cr_2O_3	Total
A	55.62	1.04	26.07	12.6	0.008	0.41	0.12	0.53	3.13	0.11	0.03	99.72
B	55.37	0.96	25.55	13.45	0.01	0.39	0.11	0.52	3.02	0.13	0.03	99.59
C	51.79	0.89	28.32	15.23	0.1	0.45	0.02	0.19	2.5	0.24	0.04	99.81
D	47.83	0.92	29.56	18.55	0.03	0.21	0.01	0.2	1.98	0.31	0.03	99.68
E	15.56	0.07	5.17	58.34	0.01	n.d	0.11	0.49	0.73	0.08	0.24	80.86
F	50.72	5.76	33.15	9.03	0.04	0.21	0.09	0.06	0.46	0.11	0.08	99.76

Sample A = Ikageng source; Sample B = Ikageng market; Sample C = Phelandavha source; Sample D = Phelandavha Market; Sample E = Pheramindi source and Sample F = Pheramindi market; n.d = not detected.

Table 2. Mineralogical composition of geophagic clay samples.

Mineral	Ikageng source (%)	Ikageng market (%)	Phelandavha source (%)	Phelandavha market (%)	Pheramindi source (%)	Pheramindi market (%)
Kaolinite			X	X	X	X
Quartz	X	X	X	X	X	X
Muscovite	X	X				
Bentonite		X				

samples were generally characterized by relatively high concentrations of SiO_2, Al_2O_3 and Fe_2O_3. The concentrations of oxides of Ca, Mg, and Na, were relatively low in all geophagic clay samples, less than 0.6%. Lower abundance of MgO and K_2O shows lack of expandable clays (Odewumi, 2013).

X-ray diffraction patterns were obtained for representative geophagic clay samples (Table 2). The most abundant non-clay mineral was quartz in geophagic clay samples (Ikageng samples and Phelandavha samples) and corresponding closely with SiO_2 values from major trace elements and oxides results observed in the XRF. Kaolinite was however the most dominant clay observed in the samples; kaolinite was observed in the market samples from Phelandavha and Pheramindi. This is in accordance with previous studies (Ekosse and Jumbam, 2010) where kaolinite was found in abundance in geophagic clays. Kaolin minerals are used as medicines to treat the causes and the symptoms of gastrointestinal distress (Ekosse et al., 2010). The muscovite was mostly found in the clays from Ikageng; interestingly the mineralogical composition of the market and source clay samples was almost similar confirming the exact source of geophagic clays sold by the vendors.

Bacterial diversity

The phylogenetic distribution results show the amount of different phylum that was detected in all geophagic clay samples (Figure 3a, b and c). The phylum Firmicutes, Actinobacteria, and Proteobacteria were most abundant in almost all the geophagic clay samples. Actinobacteria constituted 49.49% of total bacteria identified in Ikageng market sample and Firmicutes 12.59% of Pheramindi market sample respectively. Unclassified bacterial sequencing could not be linked to existing database and were recorded as unknown (unclassified) bacteria; it was found to be dominant in most of the geophagic clay samples with a significantly high fraction 97.73% for Pheramindi source sample, 96.89% for Ikageng source sample, 95.7% for Phelandavha source sample, 33.52% for Ikageng market sample, 37.56% for Pheramindi market sample and 90.43% for Phelandavha market sample respectively. The Proteobacteria, Firmicutes, Actinobacteria and Tracheophyta phylum were observed in all geoghagic clay samples. The other phylum Firmcutes, Tracheophyta and Chloroflexi were low on Ikagengmarket sample.

The most abundant class was unclassified class in most samples except Ikageng market sample and Pheramindi market sample, whereas Actinobacteria was the most abundant 49.49% in Ikageng market sample followed by 39.27% on Pheramindimarket sample as shown in Figure 3b. The other major classes detected were Bacilli (7.71%) for Pheramindi market sample, Gammaproteobacteria (4.15%) for Phelandavha market sample and Alphaproteobacteria (3.10%) for Ikageng market sample. Figure 3c shows the results of different microbial orders composition detected on geophagic clay samples. The order Actinomycetales belonging to the class Actinobacteria of the phylum Actinobacteria was the

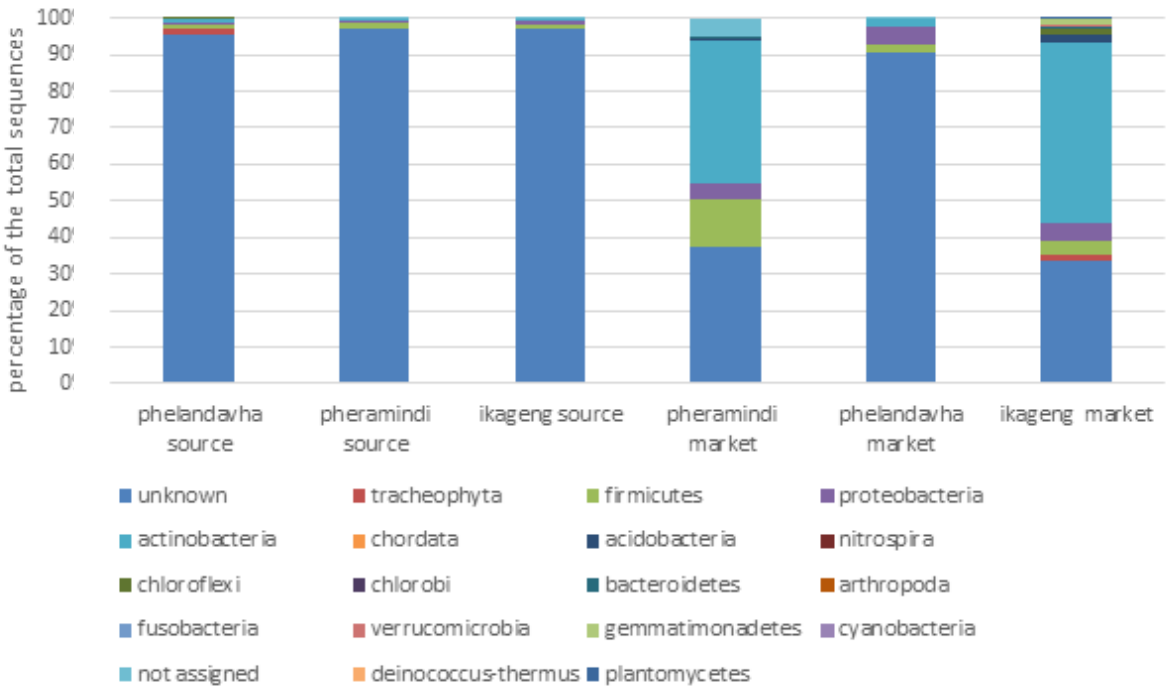

Figure 3a. Abundance and diversity of bacterial phylum detected on geophagic clay samples.

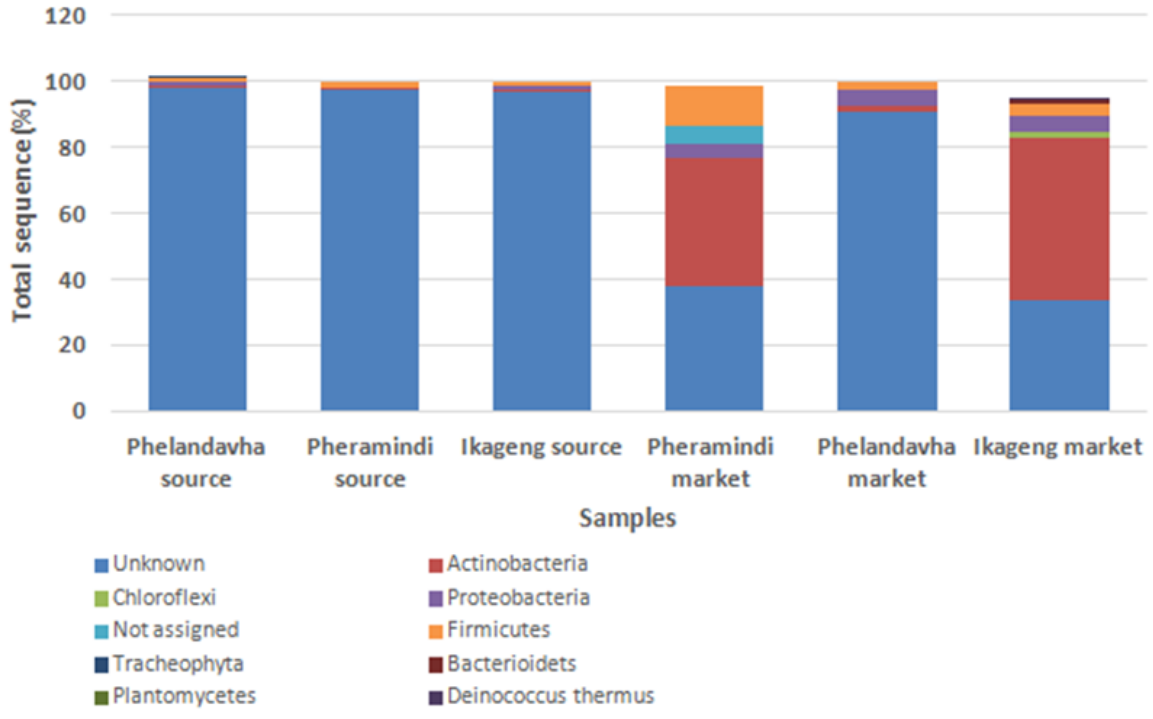

Figure 3b. Microbial classes composition detected in geophagic clay samples.

most abundant in Ikageng market sample, followed by Bifidobacteriales (23.26%) in Pheramindi market sample, then Laurales, Bacilliales and Rhizobiales. Lactobacillales, Bacillales, Costridiales and Aconchulinida

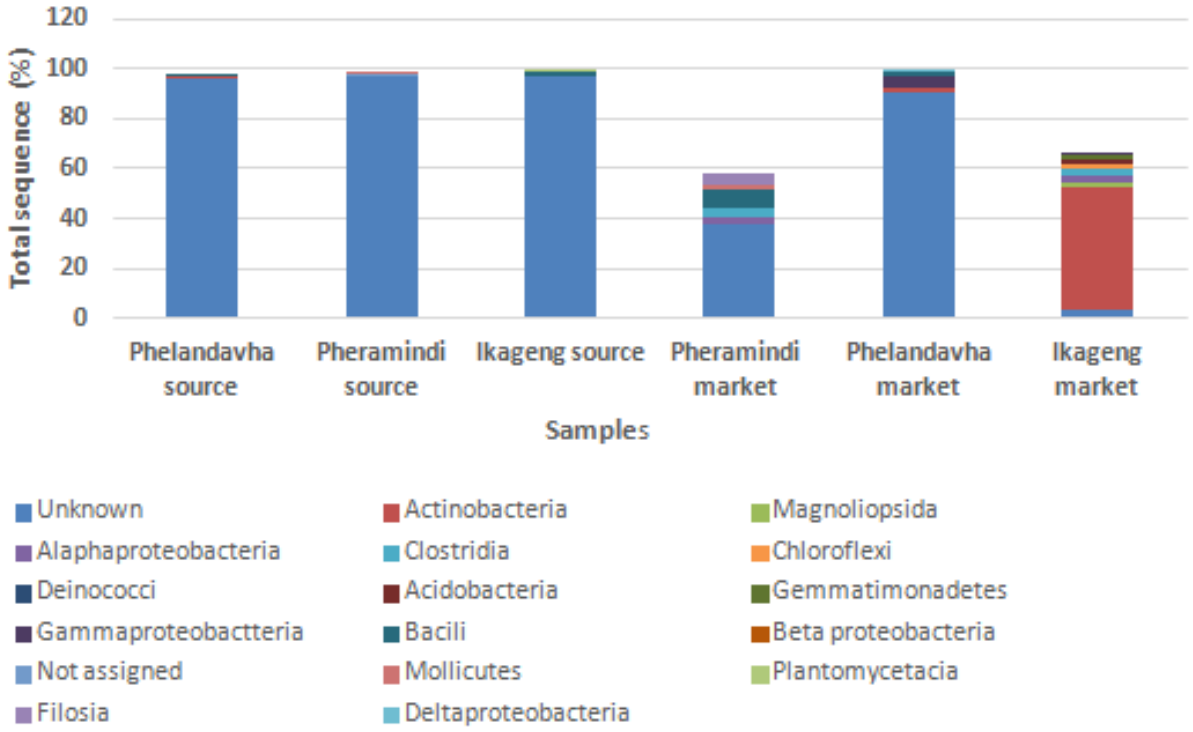

Figure 3c. Microbial orders composition detected in geophagic clay samples.

were all detectable at above 1% in the sample from Pheramindi market. All samples were dominated by the uncultured bacterium constituting 95.04% of microorganisms in Phelandavha source sample, 96.30% in Ikageng source sample, 96.79% in Pheramindi source sample, 28.47% in Ikageng market sample, 32.78% in Pheramindi market sample, 89.41% in Phelandavha market sample. The other genera detected were all below 0.9%. Most of the species identified were uncultured species such as uncultured Actinobacteria (14.35%), uncultured Rubrobacteria (4.48%), uncultured Achiromyces (3.49%) and others.

Microorganisms and potential health risks

A blast output allowed identifying the microbial species with the potential to affect the health of geophagist; the results in Tables 3a, b and c only show the selected microorganisms with the potential to harm clay consumers. It can be observed that more potentially harmful microorganisms occurred in the samples obtained from the market, indicating that storage and/or handling may have contributed to the contamination of clays or the clays were not exactly from the sampling points at the mining sites. Although the microorganisms identified in the samples were mostly opportunistic microorganisms, it is however important to mention that immunocompromised individuals such as children, unborn babies (exposed through pregnant women), HIV infected individuals and elderly are among the geophagists and are therefore likely to be infected by such microorganisms.

Inactivation of microorganisms by solar treatment

The exposition of geophagic clays to simulated solar treatment was considered in this study to inhibit the microorganisms. A general trend in Figure 4 indicates a decrease of the number of microorganisms as the exposure time increases. Using pulsed-light, Anderson et al. (2000) also achieved the inactivation of microorganisms occurring in food. However, for the samples obtained from the market in Pheramindi, an increase of the number of microorganisms was observed. The effectiveness of bacterial deconta-mination using solar treatment has been reported (Malato et al., 2009) to be proportional to the intensity of radiation or duration of exposure and the temperature, while being inversely proportional to the depth of supporting material or the porosity of the material. In the case of pieces of geophagic clays used in this study, the radiation was most likely to affect the microorganisms at the surface, given that the clays were relatively compact; while the temperature was likely to mostly reduce the moisture

Table 3a. Microorganisms occurring in geophagic clays from Phelandavha and the potential health risks.

Sampling area	Mining site		Market site	
	Microorganisms	**Potential disease**	**Microorganisms**	**Potential disease**
Phelandavha	*Propionibacterium acnes*	Acnes, chronic blepharitis and endophthalmitis	*Propionibacterium acnes*	Acnes, chronic blepharitis and endophthalmitis
	*Spiroplasma*sp.	Creutzfeldt-Jakob disease (CJD)	*Pseudomonas* sp.	Pneumonia, Septic shock, Urinary tract infection, Gastrointestinal infection, Skin and soft tissue infections
	Streptococcus pneumoniae	Pneumonia	*Escherichia coli*	Gastroenteritis, urinary tract infection, neonatal meningitis
	Pseudomonas aeruginosa	Pneumonia, Septic shock, Urinary tract infection, Gastrointestinal infection, Skin and soft tissue infections	*Spiroplasma*sp.	Creutzfeldt-Jakob disease (CJD)
	Alpha proteobacterium	Bacteraemia	*Shigellasonnei*	Dysentery
	Escherichia coli	Gastroenteritis, urinary tract infection, neonatal meningitis	*Mycoplasma amphoriforme*	Bronchitis
	Neisseria meningitidis	Meningitis	*Klebsiellaoxytoca*	Colitis and sepsis
	Synechococcus sp.	Cyanotoxins	*Mycobacterium avium*	Lungs infection (Lady Windermere syndrome)

Table 3b. Microorganisms occurring in geophagic clays from Pheramindi and the potential health risks.

Sampling area	Mining site		Market site	
	Microorganisms	**Potential disease**	**Microorganisms**	**Potential disease**
Pheramindi	*Escherichia coli*	Gastroenteritis, urinary tract infection, neonatal meningitis	*Spiroplasma*sp.	Creutzfeldt-Jakob disease (CJD)
	*Spiroplasma*sp.	Creutzfeldt-Jakob disease (CJD)	*Gemellahaemolysans*	Involved in pulmonary exacerbations of cystic fibrosis patients
	P. acnes	Acnes, chronic blepharitis and endophthalmitis	*Corynebacterium pseudogenitalium*	Urinary tract infection
	Streptococcus agalactiae	Neonatal sepsis	*Finegoldia magna*	Septic arthritis
	Streptococcus mitis	Endocarditis	*Peptoniphilus*sp.	Soft tissue, bone and joints infection
			Streptococcus salivarius	Sepsis
			Escherichia coli	Gastroenteritis, urinary tract infection, neonatal meningitis
			Streptococcus mitis	Endocarditis
			Propionibacterium acnes	Acnes, chronic blepharitis and endophthalmitis
			Pseudomonas sp.	Pneumonia, Septic shock, Urinary tract infection, Gastrointestinal infection, Skin and soft tissue infections

Table 3b. Contd.

	Staphylococcus sp.	Sialadenitis and other diseases
	*Leptotrichia*sp.	Neutropenia
	Abiotrophiapara-adiacens	Bacterial endocarditis
	*Veillonella*sp.	Osteomyelitis and endocarditis
	Mycobacterium fortuitum	Skin infection and osteomyelitis
	Citrobacteramalonaticus	Neonatal meningitis and gastroenteritis

Table 3c. Microorganisms occurring in geophagic clays from Ikageng and the potential health risks.

Sampling area	Mining site		Market	
	Microorganisms	**Potential disease**	**Microorganisms**	**Potential disease**
Ikageng	*Propionibacterium acnes*	Acnes, chronic blepharitis and endophthalmitis	*Mycobacterium* sp.	Tuberculosis
	Pseudomonas aeruginosa	Pneumonia, Septic shock, Urinary tract infection, Gastrointestinal infection, Skin and soft tissue infections	*Spiroplasma*sp.	Creutzfeldt-Jakob disease (CJD)
	*Spiroplasma*sp.	Creutzfeldt-Jakob disease (CJD)	*Tsukamurella*sp.	Pneumonia
	Escherichia coli	Gastroenteritis, urinary tract infection, neonatal meningitis	*Faecalibacteriumprausnitzii*	Crohn's disease and obesity
	Citrobacteramalonaticus	Neonatal meningitis and gastroenteritis	*Propionibacterium acnes*	Acnes, chronic blepharitis and endophthalmitis
			Escherichia coli	Gastroenteritis, urinary tract infection, neonatal meningitis
			Klebsiellaoxytoca	Colitis and sepsis
			Pseudomonas aeruginosa	Pneumonia, Septic shock, Urinary tract infection, Gastrointestinal infection, Skin and soft tissue infections
			Shigelladysenteriae	Dysentery

content. The damaging effect of lights on microorganisms may be explained by the fact that, reactive oxygen species resulting from the adsorption of UV-A light by intracellular chemophores interact with and break the DNA, modify the nucleic base and death may therefore occur. The synergetic effect of mild heat and UV-A light has been suggested as being responsible of the inactivation of microorganisms during exposure to sun light (Acra et al., 1980). The susceptibility of microorganisms to solar irradiation has however been reported to vary with the type of microbial group or species; Gram-negative bacteria and *E. coli* are said to be more susceptible to the solar disinfection than Gram-positive bacteria, protozoa and fungi (Malato et al., 2009); this partially explains why the reduction of the number of microorganisms was inconsistent among the clay samples. The identification of microorganisms through sequencing of 16S rDNA showed that the more resistant species included *Bacillus subtilis*, *Paenibacillus*, *Bacillus cereus*, *Bacillus thuringiensis*, *Bacillus humi* and *Arthrobacterarilaitensis* which are all Gram-positive bacteria.

Moisture content of the geophagic clays

Water availability is one of the most important parameters regulating biological activities in soil. According to Schnurer et al. (1986), changes in water availability will influence soil organisms through complex interactions with nutrient conditions, soil temperature and pore size distribution. To better understand the effect of solar light on the inactivation of microorganisms in geophagic clays, the water content of the clays was monitored after the period of exposures; Figure 5 shows that the moisture percentage decreased with exposure time, the same

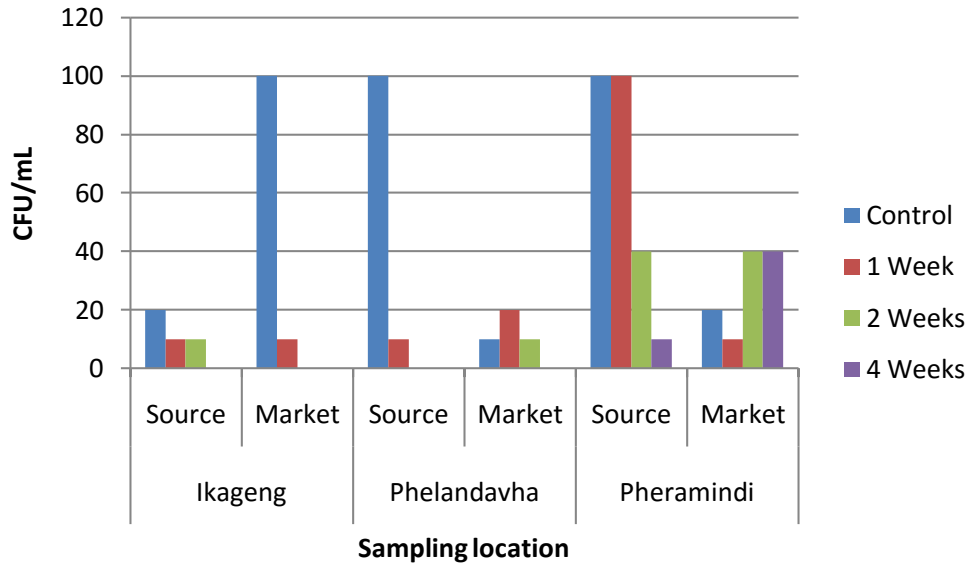

Figure 4. Effect of solar treatment on the microorganisms in geophagic clays.

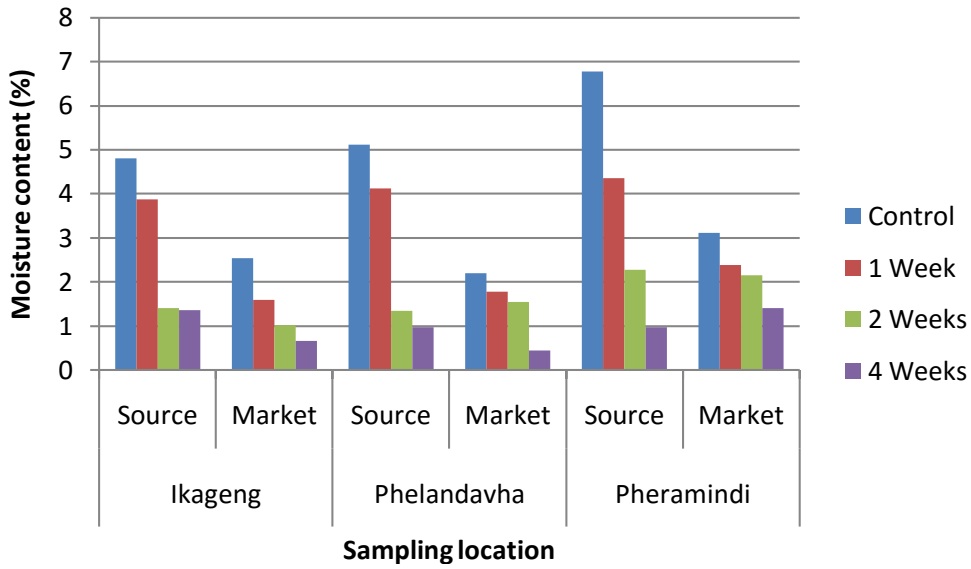

Figure 5. Variation of moisture content during solar treatment.

trend was observed in almost all the samples, this is due to the increase of temperature under solar irradiation. Biological life certainly requires water to live, but the amount of water required also varies among the group of microorganisms. A decrease of the moisture may imply that the clay becomes more compact and the porosity is reduced hence lower oxygen is available for microorganisms; some microorganisms may therefore easily die under lower moisture percentage. However, such conditions may be suitable for the growth of other microorganisms. Dunn et al. (1985) found that mild heating increased germination of dormant forms yielding significantly higher counts than those of unheated soils. Furthermore, the diversity of microorganisms is likely to decrease as the temperature increase, but heat-shock fungi and bacteria, whose spores require heat to germinate. It was also observed that microorganisms number increased in the samples obtained from the market of Pheramindi after two weeks of exposure to solar radiation.

Organic carbon in the geophagic clays

Nutrients requirement vary among bacteria which to survive needs energy and organic carbon that will be

Table 4. Percentage of organic carbon in the geophagic clays.

Sampling area		Clay organic carbon content (%)
Ikageng	Field	1.26
	Market	1.33
Phelandavha	Field	1.06
	Market	1.33
Pheramindi	Field	1.33
	Market	1.5

converted to new cells. The content of organic matter in the soil determines the speed of decomposition by bacteria; sugars and proteins are easily digestible, while the decomposition of lignin is very slow. Continuous recycling and renewing of organic matters in the soil influence the composition of microorganisms populations which is determined by the amount, the type and availability of the organic matter (Fosso-Kankeu and Mulaba-Bafubiandi, 2015).

Clay organic carbon content results are shown in Table 4. All the samples of geophagic clays show relatively higher percentage of organic carbon contents with an average of organic carbon of 1.30%. This shows that there are nutrients available to support the growth of microorganisms in the geophagic clays from the field or from the market.

DISCUSSION

The geophagic clay samples from all the different sources contain large diversity of microorganisms which occur in the clays during contamination in the field and possibly during handling and storage. Among these microorganisms some were identified to have the potential to harm the geophagists, but mostly immuno-compromised individuals. The relatively high moisture content of the clays as well as the presence of organic carbon, show that nutrients are available to allow the microorganisms to easily survive in the clays if no pretreatment is applied for their inactivation. The application of solar treatment allows the inactivation of microorganisms at the surface of the clays through photo oxidative process which mostly results into microbial cell membrane damage and breakage of DNA which may be lethal (Ubomba-Jaswa et al., 2009). The second effect of solar treatment as a result of temperature increase may be less significant as it is reported that significant synergetic effects between radiation and temperature are observed when temperature rises to 50°C. It is however important to mention that in this study the relative increase in temperature plays the role of reducing water availability and the porosity (oxygen availability) of clays, resulting in the inactivation of microorganisms; although most of the pathogenic microorganisms are likely to be inactivated under such conditions, it was found that after two to four weeks the conditions become suitable for the germination of dormant microorganisms which were in the form of spores. Dunn et al. (1985) have categorized these microorganisms as heat-shock fungi and bacteria, whose spores require heat to germinate.

Conclusion

Irrespective of their sources, the geophagic clays considered in this study contain large amount of microorganisms with the potential to affect human health. Additional microorganisms observed in the clays obtained from the vendors, indicates that handling and storage may contribute to further contamination of the clays collected in the field. The moisture content and the presence of organic carbon in the clays imply that microorganisms in the clay will survive as long as no specific treatment is applied to inactivate them. The solar treatment has shown the potential to reduce the level and the diversity of microorganisms in the clays, however, enhancement methods must be considered to ensure that the pathogenic microorganisms are reduced to safer level during exposure to sun light.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors are grateful to the sponsor from the North-West University and the National Research Foundation (NRF) in South Africa. The contributions of Dr. O. Ntwampe, Mr N. Lemmerand Mr G. Van Rensburgare really appreciated.

REFERENCES

Acra A, Karahagopian Y, Raffoul Z, Dajani R (1980). Disinfection of oral rehydration solution by sunlight. Lancet 2:1257-1258.

Anderson JG, Rowan NJ, MacGregor SJ, Fouracre RA (2000).Inactivation of food-borne enteropathogenic bacteria and spoilage fungi using pulsed-light. Plasma Science, IEEE Transact. 28(1):83-88.

Bisi-Johnson MA, Obi CL,Ekosse G-I (2015).Microbiological Underpinnings of Geophagia and Geophagicmaterials.In: GE Ekosse, VM Ngole, L de Jager, E Fosso-Kankeu (Eds). 2015. Human and Enzootic Geophagia: Ingested Soils and Practices. Lambert Academic Publishing.ISBN 978-0-620-64308-5.

Bisi-Johnson MA, Oyelade HA, Adediran KA, Akinola SA (2013).Microbial Evaluation of Geophagic and Cosmetic Clays from Southern and Western Nigeria: Potential Natural Nanomaterials. Int. J. Environ. Chem. Ecol. Geol. Geophys. Eng. 7:12.

Conroy RM, Elmore-Meegan M, Joyce T, McGuigan KG, Barnes J (1996). Solar disinfection of drinking water and diarrhoea in Maasai children: A controlled field trial. Lancet 348:1695-1697.

Conroy RM, Meegan ME, Joyce T, McGuigan KG, Barnes J (2001). Solar disinfection of drinking water protects against cholera in children under 6 years of age, Arch. Dis. Child. 85:293-295.
Duffie JA, Beckman W (2006). J.A. Duffie (Ed.), Solar Engineering of Thermal Processes, Wiley, New York.
Dunn PH, Barro SC, Poth M (1985). Soil moisture affects survival of microorganisms in heated chapparal soil. Soil Biology and Biochemistry. 17(2):143-148.
Ekosse GI and Jumbam ND (2010).Geophagic clays: Their mineralogy, chemistry and possible human health effects. Afr. J. Biotechnol. 9(40):6755-6767.
Ekosse GI, Ngole VM, de Jager L, Fosso-Kankeu E (2015). Human and Enzootic Geophagia: Ingested Soils and Practices. Lambert Academic Publishing.ISBN 978-0-620-64308-5.
Ekosse G-IE, de Jager L, Ngole V (2010). Traditional mining and mineralogy of geophagic clays from Limpopo and Free State provinces, South Africa. Afr. J. Biotechnol. 9(47):8058-8067.
Fosso-Kankeu E, Mulaba-BafubiandiAF(2015).Sources of bacteria contaminating geophagic clays. In: GE Ekosse, VM Ngole, L de Jager, E Fosso-Kankeu (Eds). 2015. Human and Enzootic Geophagia: Ingested Soils and Practices. Lambert Academic Publishing.ISBN 978-0-620-64308-5.
Geissler PW, Mwaniki D, Thiong'o F, Friis H (1998). Geophagy as a risk factor for geohelminth infections: a longitudinal study of Kenyan primary school children. Trans. R. Soc. Trop. Med. Hyg. 92:7-11.
Kent A, Triplett EW (2002). Microbial communities and their interactions in soil and rhizosphereecosystems.Annu. Rev. Microbiol. 56:211-236.
Lamps LW, Madhusudhan KT, Havens JM, Greenson JK, Bronner MP, Chiles MC, Dean PJ Scott MA (2003). Pathogenic Yersinia DNA is detected in bowel and mesenteric lymph nodes from patients with Crohn's disease. Am. J. Surg. Pathol. 27:220-227.
Liu Y, Van Kruiningen HJ, West AB, Cartun RW, Cortot A Colombel JF (1995).Immunocytochemical evidence of *Listeria*, *Escherichia coli*, and *Streptococcus* antigens in Crohn's disease. Yeast 108:1396-1404.
Malato S, Fernandez-Ibanez P, Maldonado MI, Blanco J, Gernjak W (2009). Decontamination and disinfection of water by solar photocatalysis: Recent overview and trends. Catalysis Today 147:1-59.
Mani SK (2006). Development and evaluation of small-scale systems for solar disinfection of contaminated drinking water in India, School of Applied Sciences, PhD thesis, Northumbria University, Newcastle-upon-Tyne, UK, P 727.
McGuigan KG, Conroy RM, Mosler H-J, Du-preez M, Ubobwa-Jaswa E, Fernandez-Ibanez P (2012). Solar water disinfection (SODIS): A review from bench –top to roof-top. J. Hazard. Mater. 235-236:29-46.
McGuigan KG, Joyce TM, Conroy RM, Gillespie JB, Elmore-Meegan M (1998). Solar disinfection of drinking water contained in transparent plastic bottles: characterizing the bacterial inactivation process. J. Appl. Microbiol. 84:1138-1148.
Mel'nikova VM, Gracheva NM, Belikov GP, Blatun LA, Shcherbakova EG (1993). Thechemoprophylaxis and chemotherapy of opportunistic infections. Antibiot. Khimioter 38:44-48.
Nchito M, Geissler PW, Mubila L, Friss H, Olsen A (2004). Effects of iron and multinutrient supplementation on geophagy: a two-by-two factorial study among Zambian schoolchildren in Lusaka. Trans. R. Soc. Trop. Med. Hyg. 98(4):218-227.
Ngole-Jeme VM, Ekosse G-I (2015).A comparative analysis of granulometry, mineral composition and major and trace element concentrations in soils commonly ingested by humans. Int. J. Environ. Res. Public Health 12:8933-8955.
Odewumi SC (2013). Mineralogy and Geochemistry of Geophagic Clays from Share Area, Northern Bida Sedimentary Basin, Nigeria. J. Geol. Geosci. 2:108.
Rainey RC, Harding AK (2005). Drinking water quality and solar disinfection: effectiveness in peri-urban households in Nepal. J. Water Health 3:239-248.
Rubery E (2002). A review of the evidence for a link between exposure to *Mycobacterium paratuberculosis* (MAP) and Crohn's disease (CD) in humans.FSA/MAP/Report. Food Standards Agency. Ref Type: Report.
Schnurer J, Clarholm M, Bostrom S, Rosswall T (1986). Effects of moisture on soil microorganisms and nematodes: A field experiment. Microb. Ecol. 12(2):217-230.
Smirnov W, Reznik SR, V'iunitskaia VA, Sorokulova IB, Samgorodskaia NV,Tofan AV (1993).The current concepts of the mechanisms of the therapeutic-prophylactic action of probiotics from bacteria in the genus Bacillus. Mikrobiol Z. 55(4):92-112.
Solar water disinfection (2002). Aguide for the application of SODIS. SANDEC report No 6/02.
Songca S, Oluwafemi O (2015). Human and enzootic geophagic soils-Potential Chemical and Biochemical Implications. In: Ekosse GE, Ngole VM, de Jager L, Fosso-Kankeu E (Eds). Human and Enzootic Geophagia: Ingested Soils and Practices. Lambert Academic Publishing.ISBN 978-0-620-64308-5.
Ubomba-Jaswa E, Navntoft C, Polo-López MI, Fernandez-Ibáñez P, McGuigan KG (2009). Solar disinfection of drinking water (SODIS): An investigation of UV-A dose on inactivation efficiency. Photochem. Photobiol. Sci. 8:587-595.
Walkey A, Black IA (1934). An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci. 37:29-37.
Wegelin M, Canonica S, Mechsner K, Fleischmann T, Pesaro F, Metzler A (1994). Solar water disinfection: scope of the process and analysis of radiation experiments. Aquat. J. Water Supply Res. Technol. 43:154-169.
Wong MS, Bundy DAP, Golden MHN (1991). The rate of ingestion of Ascarislumbricoides and Trichuristrichiura eggs and its relationship to infection in two children's homes in Jamaica. Trans. R. Soc. Trop. Med. Hyg. 85:89-91.