

# Potential of solar treatment to lower the microbial contaminants in geophagic clays

**TL Netshitanini**  
**26980029**

Dissertation submitted in fulfilment of the requirements for the degree *Masters of Engineering* in *Chemical Engineering* at the Potchefstroom Campus of the North-West University

Supervisor: Prof E Fosso-kankeu  
Co-Supervisor: Prof F Waanders  
Co-Supervisor: Dr E Ubomba-Jaswa

May 2017

## **Declaration**

I declare that work presented in this master's dissertation is my own work and that it has not previously been submitted for a degree at any other university. I have acknowledged the sources I have used or quoted by complete reference.

Signature of the Student.....

Signature of the Supervisor .....

Signature of the Co-supervisor.....

## Preface

### Introduction

This dissertation was submitted in article format, as allowed by the North-West University (NWU). Since the dissertation is in article format, chapters on materials and methods, results and discussion are not included as these topics are dealt with in Chapters 3 and 4 in the articles. However, **Chapter 1** is a background and motivation chapter, where the motivation and objectives are discussed and in **Chapter 2** the literature review relevant to the project is included. It also has to be noted that the articles (**Chapters 3, 4 and 5**) have different numbering than the rest of the dissertation because they are included in the form they were submitted to the journals. Additionally, the formatting of the articles is according to journals in which they were published.

**Chapter 6** is a final conclusion or summary of the two articles published and suggestions for further studies. The numbering of tables and figures of these chapters is therefore not consistent with the rest of the dissertation.

### Rationale for selecting this dissertation format

The NWU requires master's candidates to submit at least one full-length research paper to a peer-reviewed journal. In this study the objective of the candidate was therefore to conduct publishable research.

### *Authors and their contribution*

In this section the authors of three articles are presented separately, followed by a section stating each author's contribution.

Chapter 3 (containing Article 1): *Application of solar treatment for the disinfection of geophagic clays from the markets and mining sites*

Fosso-Kankeu E, Netshitanini T.L, Abia A.L.K, Ubomba-Jaswa E, and Waanders F.B

School of Chemical and Minerals Engineering, Faculty of Engineering, North-West University, PO Box 558, Potchefstroom, 2531, South Africa.

Natural Resources and the Environment, CSIR, PO Box 395, Pretoria 0001, South Africa

Department of Environmental, Water and Earth Science, Tshwane University of Technology, 175 Nelson Mandela Drive, Pretoria 0001, South Africa

Chapter 4 (containing Article 2): *Co-effect of leached metals and pH of simulated gastric fluid on the survival of microorganisms in geophagic clays*

Netshitanini T.L, Fosso-Kankeu E, Waanders F.B, Ubomba-Jaswa E and Abia A.L.K

School of Chemical and Minerals Engineering, Faculty of Engineering, North-West University, P.O Box 558, Potchefstroom, 2531, South Africa.

Natural Resources and the Environment, CSIR, PO Box 395, Pretoria 0001, South Africa

Department of Environmental, Water and Earth Science, Tshwane University of Technology, 175 Nelson Mandela Drive, Pretoria 0001, South Africa

Chapter 5 (containing Article 3): *The effect of sunlight on the inactivation of Escherichia coli, Salmonella enterica and naturally occurring microorganisms in edible geophagic clay*

Netshitanini Thembuluwo Lorna, Eunice Ubomba-Jaswa, Elvis Fosso-Kankeu, Frans Waanders, and King Luthern Akebe Abia

Natural Resources and the Environment, CSIR, PO Box 395, Pretoria 0001, South Africa

School of Chemical and Minerals Engineering, Faculty of Engineering, North-West University, PO Box 558, Potchefstroom, 2531, South Africa.

Department of Environmental, Water and Earth Science, Tshwane University of Technology, 175 Nelson Mandela Drive, Pretoria 0001, South Africa

The contributions of the various authors were as follows: the work was accomplished by the master's candidate, Thembuluwo Lorna Netshitanini, with conceptual ideas and recommendation by Prof. E. Fosso-Kankeu (supervisor), Prof F.B. Waanders (co-supervisor) and Dr. E. Ubomba-Jaswa (co-supervisor) on the experimental work, data collection, results, and discussion, as well as on the two articles. Dr. A.L.K Abia assisted with the sample collection, microbiological analysis and made a contribution with regard to the microbiology analysis presented in article 1 (Chapter 3).

## **Status of articles**

Article 1: The article was accepted and published online in African Journal of Biotechnology

Article 2: The article was accepted and published online in International Journal of Science and presented at an International conference

Article 3: The article is intended to be submitted to the journal Science of the Total Environment.

## **Declaration by co-authors**

I, E. Fosso-Kankeu, hereby give my permission that Thembuluwo Lorna Netshitanini may submit the articles/manuscript for degree purposes.

.....

I, F. B. Waanders, hereby give my permission that Thembuluwo Lorna Netshitanini may submit the articles/manuscript for degree purposes.

.....

I, E. Ubomba-Jaswa hereby give my permission that Thembuluwo Lorna Netshitanini may submit the articles/manuscript for degree purposes

.....

I, A.L.K, Abia hereby give my permission that Thembuluwo Lorna Netshitanini may submit the articles/manuscript for degree purposes.

.....

## Academic and technical outputs

### Publication

*Fosso-Kankeu E, Netshitanini TL, Abia ALK, Ubomba-Jaswa E, Waanders FB. 2015. Application of solar treatment for the disinfection of geophagic clays from the markets and mining sites. African Journal of Biotechnology. 14(50):3313-3324*

*Netshitanini T.L, Fosso-Kankeu E, Waanders F.B, Ubomba-Jaswa E and Abia A.L.K. 2016. Co-effect of leached metals and pH of simulated gastric fluid on the survival of microorganisms in geophagic clays. International Journal of Science and Research. 5(4):1107-1116.*

### Conference proceedings

*Elvis Fosso-Kankeu, Frans Waanders, Thembuluwo Lorna Netshitanini, Eunice Ubomba-Jaswa, and King Luthern Akebe Abia. 2015. Identification of metals and microorganisms in geophagic clays: Investigation of their behaviour in simulated gastric fluid. 7<sup>th</sup> International conference on latest Trends in Engineering and technology (ICLTET, 2015), 26-27 November 2015: Irene, Pretoria (South Africa).*

*Netshitanini Thembuluwo Lorna, Elvis Fosso-Kankeu, Frans Waanders, Eunice Ubomba-Jaswa, and King Luthern Akebe Abia. 2016. Inactivation of Escherichia coli, Salmonella Enterica and Naturally Occurring Microorganisms in Edible Geophagic Clay under Sunlight. International Conference on Advances in Science, Engineering, Technology and Natural Resources (ICASETNR-16) Nov. 24-25, 2016 Parys, South Africa.*

### Proceeding article

*Netshitanini Thembuluwo Lorna, Eunice Ubomba-Jaswa, Elvis Fosso-Kankeu, Frans Waanders, and King Luthern Akebe Abia. 2016. The effect of sunlight on the inactivation of Escherichia coli, Salmonella enterica and naturally occurring microorganisms in edible geophagic clay. In process to be submitted to Journal of Science of the Total Environment.*

## Acknowledgements

Firstly, all glory to Him who carried me through this Master's Degree, and gave me the strength to finish the project.

Secondly, a special word of thanks to the following people without whom this dissertation would not have been possible:

- My family and friends for the motivational words of encouragement, inspiration, support and belief in me to finish masters;
- Prof Elvis Fosso-Kankeu, Prof Frans Waanders, Dr Eunice Ubomba-Jaswa and Dr. Abia Akebe Luthern King for the guidance, invested time, and support in all;
- Alusani Manyatshe, Matsie Veronica and Ashley Hlayisani Munyai for assistance with field work;
- Mr. N. Lemmer and Mr. G. Van Rensburg for the provision of laboratory reagents and trace element analysis;
- Mr. E. Malenga and Ms. N. Baloyi from the University of Johannesburg in South Africa for the X-ray diffraction and X-ray fluorescence analyses;
- North-West University and the National Research Foundation (NRF) in South Africa for financial support.

## Abstract

Deliberate consumption of earth materials is called geophagia and is practiced worldwide by humans. The various reasons of geophagia practice have been reported in the literature, i.e. why people consume the clays deliberately. Some believe that it reduces morning sickness in pregnant women remedies gastrointestinal distress and supplements minerals and nutrients. Geophagia improves the health of geophagists because the elements in the clays may have nutritional benefits.

Studies were conducted on geophagist individuals to determine the effect of geophagia on pregnant women and children, microbiological infection, anaemia, deficiency of minerals and heavy metal poisoning. Although much has been written internationally about geophagia practice and the materials found on geophagic clay materials, less has been written regarding the health aspects of geophagia, particularly on the microbiological aspects of geophagic clay materials. Furthermore, very little has been investigated about the disinfection of microorganisms in geophagic clay materials to minimize the risk to human health.

This study attempts to find an application, which is available to all, that can be used to disinfect geophagic clay materials which is solar treatment. Samples (marketed and mined) were collected from markets in Pretoria and Potchefstroom (South Africa) and mined from the respective sources. The results of the trace element contents showed low amounts of toxic constituents such as Mn, Cr, Ni, Co, and Pb. At low pH a large amount of metals leached from the clay samples, including Pb, Ni, Co and Fe. The finding of the study indicated that the microbial content of the geophagic clay varied with the origin of the geophagic clay (marketed clays and mined clays, respectively), indicating that marketed clays had the highest microbial content.

Microorganisms in geophagic clays were also inactivated using sunlight. Sunlight has an effect on the microorganisms found in the edible geophagic clays. *Escherichia coli*, *Salmonella enterica* and naturally occurring microorganisms were completely inactivated under sunlight. No regrowth and reactivation of bacteria were observed from samples that had reached complete inactivation. Marketed and mined samples achieved a complete inactivation after 2 h of sunlight exposure. Increase in clay temperature was observed in all the geophagic clay samples. During cloudy days, the lower temperature and solar irradiance caused the geophagic clay samples to reach non-complete inactivation.

To conclude: The study found that geophagic clays are contaminated with microorganisms and are not safe for consumption. However, solar treatment can be applied to disinfect the contaminated geophagic clay.

**Keywords:** geophagia; human health risk; edible clay; heavy metals; microorganisms; nutritional effect; solar treatment

# Contents

|                                                                            |    |
|----------------------------------------------------------------------------|----|
| Declaration .....                                                          | 2  |
| Preface .....                                                              | 3  |
| Introduction.....                                                          | 3  |
| Rationale for selecting this dissertation format .....                     | 3  |
| Status of articles .....                                                   | 5  |
| Declaration by co-authors .....                                            | 6  |
| Academic and technical outputs .....                                       | 7  |
| Publication.....                                                           | 7  |
| Conference proceedings.....                                                | 7  |
| Proceeding article.....                                                    | 7  |
| Acknowledgements .....                                                     | 8  |
| Abstract .....                                                             | 9  |
| Contents .....                                                             | 11 |
| List of Abbreviation .....                                                 | 13 |
| List of Tables.....                                                        | 14 |
| List of Figures .....                                                      | 15 |
| Chapter 1           Introduction.....                                      | 17 |
| 1.1 Introduction.....                                                      | 17 |
| 1.2 Background and motivation .....                                        | 17 |
| 1.3 Statement of problem. ....                                             | 19 |
| 1.4 Aims and objectives.....                                               | 20 |
| 1.5 Hypotheses.....                                                        | 20 |
| 1.6 Dissertation structure.....                                            | 20 |
| 1.7 References.....                                                        | 22 |
| Chapter 2           Literature review .....                                | 27 |
| 2.1 Introduction.....                                                      | 27 |
| 2.2 Geophagia .....                                                        | 27 |
| 2.2.1 History of geophagia .....                                           | 27 |
| 2.2.2 Geophagia practice.....                                              | 27 |
| 2.2.3 Rationale for geophagia .....                                        | 28 |
| 2.3 Geophagic clays.....                                                   | 29 |
| 2.3.1 Clay minerals .....                                                  | 29 |
| 2.3.2 Properties and preferences of geophagic clays by geophagists.....    | 29 |
| 2.4 Business traits of geophagic clays .....                               | 30 |
| 2.5. Effect of pH and heavy metals on microbes.....                        | 31 |
| 2.6 Heavy Metal contaminants in geophagic clays .....                      | 31 |
| 2.6.1 Sources of heavy metals in geophagic clays.....                      | 32 |
| 2.6.2 Geophagic clays contaminated with heavy metals and human health..... | 32 |

|                                                                                                                                                           |     |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| 2.7 Microbial contaminants in geophagic clays .....                                                                                                       | 33  |
| 2.7.1 Microbial contaminants .....                                                                                                                        | 33  |
| 2.7.2 Risks associated with the microorganism in geophagic clays .....                                                                                    | 34  |
| 2.7.3 Cases of infections associated with geophagia practice .....                                                                                        | 35  |
| 2.8 Geophagia and human health .....                                                                                                                      | 35  |
| 2.8.1 Health benefits of geophagia .....                                                                                                                  | 35  |
| 2.8.2 Health risks associated with geophagia .....                                                                                                        | 36  |
| 2.9 Solar disinfection .....                                                                                                                              | 36  |
| 2.10 The various methods of treatments of the clay .....                                                                                                  | 38  |
| 2.11 Conclusion .....                                                                                                                                     | 39  |
| 2.12 References.....                                                                                                                                      | 40  |
| Chapter 3.....                                                                                                                                            | 54  |
| 3.1 Introduction.....                                                                                                                                     | 55  |
| Application of solar treatment for the disinfection of geophagic clays from the markets and mining sites .....                                            | 56  |
| Chapter 4.....                                                                                                                                            | 77  |
| 4.1 Introduction.....                                                                                                                                     | 78  |
| Co-effect of leached metals and pH of simulated gastric fluid on the survival of microorganisms in geophagic clays .....                                  | 79  |
| Chapter 5.....                                                                                                                                            | 97  |
| 5.1 Introduction.....                                                                                                                                     | 98  |
| The effect of sunlight on the inactivation of, Escherichia coli, Salmonella enterica and naturally occurring microorganisms in edible geophagic clay..... | 99  |
| Chapter 6 Conclusion and recommendations .....                                                                                                            | 121 |
| 6.1 Introduction.....                                                                                                                                     | 121 |
| 6.2 General conclusion .....                                                                                                                              | 121 |
| Appendix A .....                                                                                                                                          | 124 |
| Appendix B .....                                                                                                                                          | 127 |

## List of Abbreviation

|         |                                                          |
|---------|----------------------------------------------------------|
| WHO     | World Health Organisation                                |
| SODIS   | Solar Disinfection                                       |
| UV      | Ultraviolet                                              |
| UVA     | Ultra Violet A                                           |
| UVB     | Ultra Violet B                                           |
| UVC     | Ultra Violet C                                           |
| DNA     | Deoxyribonucleic acid                                    |
| PET     | Polyethylene-terephthalate                               |
| XRD     | X-ray Diffraction                                        |
| XRF     | X-ray Fluorescent                                        |
| ICP-OES | Inductively coupled plasma optical emission spectrometry |
| NGS     | Next Generation Sequence                                 |
| rDNA    | ribosomal deoxyribonucleic acid                          |
| LED     | Light emitting diode                                     |
| HIV     | Human immunodeficiency virus                             |
| DL      | Dilution Limit                                           |
| NWU     | North-West University                                    |
| CNS     | Central Nervous System                                   |

## List of Tables

### Chapter 3

|                                                                                                                   |    |
|-------------------------------------------------------------------------------------------------------------------|----|
| <b>Table 1a:</b> Microorganisms occurring in geophagic clays from Phelandavha and the potential health risks..... | 67 |
| <b>Table 1b:</b> Microorganisms occurring in geophagic clays from Pheramindi and the potential health risks.....  | 67 |
| <b>Table 1c:</b> Microorganisms occurring in geophagic clays from Ikageng and the potential health risks.....     | 68 |
| <b>Table 2:</b> Percentage of organic carbon in the geophagic clays.....                                          | 72 |

### Chapter 4:

|                                                                                                                                                  |    |
|--------------------------------------------------------------------------------------------------------------------------------------------------|----|
| <b>Table 1:</b> Results of major oxides (mass %) analysis of samples.....                                                                        | 83 |
| <b>Table 2:</b> XRD results on geophagic clays.....                                                                                              | 84 |
| <b>Table 3:</b> pH variation during exposure of geophagic clays to simulated gastric fluid.....                                                  | 85 |
| <b>Table 4:</b> Microorganisms occurring in geophagic clays from Ikageng, Phelandavha and Pheramindi samples and the potential health risks..... | 90 |

## List of Figures

### Chapter 2:

- Figure 1:** Geophagic clay materials at the Gauteng local markets. Photograph taken by Thembuluwo (2015). .....31
- Figure 2:** Mining sites where the geophagic clay materials are collected using spade and hands. Photograph taken by Thembuluwo (2015).....39
- Figure 3:** Geophagic clay materials exposed at the open markets at Gauteng local markets. Photograph taken by Thembuluwo (2015). .....39

### Chapter 3:

- Figure 1:** Sampling location in the Gauteng and North West provinces of South Africa.....59
- Figure 2:** Schematic diagram of the solar treatment reactor system.....62
- Figure 3a:** Abundance and diversity of bacterial phylum detected on geophagic clay samples.....64
- Figure 3b:** Microbial classes composition detected in geophagic clay samples.....65
- Figure 3c:** Microbial orders composition detected in geophagic clay samples.....66
- Figure 4:** Effect of solar treatment on the microorganisms in geophagic clays.....70
- Figure 5:** Variation of moisture content during solar treatment.....71

### Chapter 4:

- Figure 1:** Amount of metal (mg/L) leached from the geophagic clays of Phelandavha: (A) Market sample S/L of 1/100; (B) Market sample S/L of 2/100; (C) Source sample S/L of 1/100; (D) Source sample S/L of 2/100.....86
- Figure 2:** Amount of metal (mg/L) leached from the geophagic clays of Ikageng: (A) Market sample S/L of 1/100; (B) Market sample S/L of 2/100; (C) Source sample S/L of 1/100; (D) Source sample S/L of 2/100.....87
- Figure 3:** Amount of metal (mg/L) leached from the geophagic clays of Pheramindi: (A) Market sample S/L of 1/100; (B) Market sample S/L of 2/100; (C) Source sample S/L of 1/100; (D) Source sample S/L of 2/100.....88
- Figure 4:** The metals concentration released and the microorganism that survived.....92

|                                                                                                                   |    |
|-------------------------------------------------------------------------------------------------------------------|----|
| <b>Figure 5:</b> The behaviour of microorganism in the geophagic clay subjected to a simulated gastric fluid..... | 93 |
|-------------------------------------------------------------------------------------------------------------------|----|

## Chapter 5:

|                                                                                                                                                                                                                                                                                      |     |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| <b>Figure 1:</b> Geophagic clay materials sold in the open market in South Africa. Photograph taken by Thembuluwo (2015).....                                                                                                                                                        | 104 |
| <b>Figure 2:</b> Inactivation of <i>Escherichia coli</i> in the geophagic clay exposed directly to sunlight and in dark conditions. Summer season experiment (Figure a) on a sunny day and cloudy day (Figure b). Error bars indicate standard error of triplicate measurements..... | 106 |
| <b>Figure 3:</b> Inactivation of <i>Salmonella enterica</i> in geophagic clay exposed directly to sunlight and dark conditions. Summer season experiment (Figure a) on a sunny day and cloudy day (Figure b). Error bars indicate standard error of triplicate measurement.....      | 107 |
| <b>Figure 4:</b> Inactivation of naturally occurring organisms of marketed clays and mined clays. Error bars indicate standard error of triplicate measurements.....                                                                                                                 | 108 |
| <b>Figure 5:</b> Inactivation of <i>Escherichia coli</i> in geophagic clay exposed directly to sunlight and in dark conditions. Winter season experiment (Figure a) on a sunny day and cloudy day (Figure b). Error bars indicate standard error of triplicate measurements.....     | 109 |
| <b>Figure 6:</b> Inactivation of <i>Salmonella enterica</i> in the geophagic clay exposed directly to sunlight and dark conditions. Winter season experiment (Figure a) on a sunny day and cloudy day (Figure b). Error bars indicate standard error of triplicate measurements..... | 110 |

# Chapter 1

# Introduction

## 1.1 Introduction

An overview of the aim and objectives of the investigation into the solar treatment to lower the microbial contaminants in geophagic clays is provided in this Chapter. In 1.2 background information, together with the motivation for this study, is presented, while problem statement is discussed in 1.3 and general aims and specific objectives are discussed in 1.4. This Chapter is then concluded with a scope in 1.6 that offers the layout of the respective chapters.

## 1.2 Background and motivation

The ingestion of clay by humans is a reality and common among many communities, and is more prevalent among pregnant women and children (Brand *et al.*, 2009; Ekosse *et al.*, 2010; Jumbam, 2013). Human geophagia still remains widespread and people are unaware of the negative impacts (WHO, 1996; Songca *et al.*, 2010; Ngole-Jeme & Ekosse, 2015). Geophagia is practiced for various reasons, including supplementary nutrients and minerals (Fosso-Kankeu *et al.*, 2015; Songca & Oluwafemi, 2015).

The consumption of clays could be beneficial as it provides nutrients supplements and minerals, although it may cause drawbacks to human health (Halsted, 1968; Oliver, 1997; Geissler *et al.*, 1998; Reilly & Henry, 2001; Wilson, 2003; Kawai *et al.*, 2009). The geophagic clays could be contaminated by contaminants from collection sites or introduced at the markets (Abrahams, 2002; Fosso-Kankeu & Mulaba-Bafubiandi, 2015). The soils could possibly be contaminated by heavy metals, organic pollutants (pesticides), pathogenic microorganism and potentially harmful elements (Callahan, 2003; Hunter, 2003; Bisi-johnson *et al.*, 2010; Ekosse *et al.*, 2010).

Toxic heavy metals that were found on geophagic clays include Zn (zinc), Cu (copper), Mn (manganese), Ni (nickel), Al (aluminium) and Pb (lead) (Ekosse *et al.*, 2010; Odewumi, 2013), which can impact negatively on human health (Glickman *et al.*, 1981). The amount of geophagic clay consumed determines the severity of the impact on human health. The average amount consumed by pregnant women is reported in some studies to be 20 g/day, and in other studies more than 20 g/day (Vermeer & Frate, 1979; Twenefour, 1999; Tano-Debrah & Bruce-Baiden, 2010; Lar *et al.*, 2015).

Mining activities play a major role in the dispersion of metals in the environment through overflow of mine ponds and the weathering of tailings dumps (Fosso-Kankeu & Mulaba-Bafubiandi, 2015). Mining activities can contaminate streams flowing through the sites where geophagic clays are collected (Fosso-Kankeu & Mulaba-Bafubiandi, 2015). The origin of microorganisms in clays is certainly the contamination at the sites, but mostly during handling by consumers and vendors (Oluma *et al.*, 2012; Fosso-Kankeu & Mulaba-Bafubiandi, 2015).

Water pollution is a major problem in the world for it imposes a risk to human health (Remoundou, & Koundouri, 2009) and most people in rural areas experience a shortage of treated water and end up collecting water from rivers. Moreover, geophagia is most commonly practiced in rural areas and geophagic clays are mainly selected from specific sites, such as riversides, mountains, depth, hills, valleys, and termite mounds (Dean *et al.*, 2004; Ekosse *et al.*, 2010). As the water is polluted, it causes further pollution of the soil, both chemically and biologically and then poses threats to the geophagic individuals.

The surroundings and location of a business (market) can play a role in contamination, for example by heavy metals in exhaust gases originating from vehicles passing by (Ljung *et al.*, 2006). Several incidents of diseases caused by the presence of metals or microorganisms in geophagic clays have been reported (Wong *et al.*, 1991; Cahallan, 2003; Nchito *et al.*, 2004; Kutalek *et al.*, 2010). According to Kutalek *et al.* (2010), when pregnant women consume highly contaminated soil on a regular basis, the implication may be that the unborn baby will be exposed to heavy metals toxic and this may have an impact on the health of unborn baby.

Microorganisms in ingested clays are exposed to adverse conditions in the stomach such as acidity and toxic metals leached from the clays. When heavy metals are present in excessive concentration they can cause toxic effect in the microorganisms (Baath, 1989). When the soil ingested goes through the mouth being chewed or smooth and then to the gastrointestinal tract, there are so many possible reactions that happen. It is however uncertain what could be the combined effect of acidity and metal toxicity on the load of microorganisms in geophagic clays ingested.

Poisoning by lead and other toxic substances in children eating contaminated soils have been observed (Callahan, 2003). Cases of people with diarrhoea associated with geophagia were reported in the Maucho region of Kenya (Shivoga & Moturi, 2009). Several microorganisms classified as either beneficial or harmful have been found in humans consuming clay, and ways to minimize the exposure of humans to microorganisms are not completely known (Ekosse *et al.*, 2010).

Solar disinfection (SODIS) has been shown to disinfect water contaminated with pathogenic bacteria, fungi, viruses and protozoa in water under laboratory conditions using simulated sunlight (McGuigan *et al.*, 1998; McGuigan *et al.*, 1999; Mendez-Hermida *et al.*, 2005; Heaselgrave *et al.*, 2006; Navntoft *et al.*, 2008; Ubomba-Jaswa *et al.*, 2008) and has not thus far been researched in geophagic clays. It is a household water treatment method that millions of people practise through the use of natural sunlight (McGuigan *et al.*, 1998; Rainey & Harding, 2005; Mani, 2006; Ubomba-Jaswa *et al.*, 2010). Soil pasteurization is one of the methods used to kill harmful pathogens in contaminated soil. It involves sterilizing soil by artificially increasing soil temperature or heating the soil by covering it with transparent polyethylene mulching and keeping the soil wet during mulching (Baker & Cook, 1974; Mahrer, 1979).

Therefore, microbial contaminants of geophagic clay can be reduced by the use of solar irradiation and sunlight. It is thus essential to assess the effectiveness of sunlight in different microorganisms, so as to inform and advise communities of any potential health risk and the handling process of clay in between collection sites and markets. The potential use of solar treatment and exposure to sunlight is appropriate for the community because it is freely available.

### **1.3 Statement of problem.**

Through literature review, cases have been reported of people infected by microorganisms after ingestion of soil. Through personal observation, it was found that most women in South Africa engage in geophagia and ingest preferably clayey soils likely to contain metal and microbial contaminants capable to affect their health. Therefore research was required to disinfect geophagic clays using sustainable and environmental method namely sunlight exposure and to investigate co-effect of leached metals and acidity of simulated gastric fluid on the survival of microorganisms in geophagic clays.

## 1.4 Aims and objectives

Compounds in geophagic clays are undesirable and may impact negatively on human health. The aim of this study was, therefore, to identify the contaminants in geophagic clays consumed by the geophagists in South Africa and to reduce the amount of microbial contaminants on the clay by solar irradiation.

The following objectives were devised to address this aim:

- i. To determine the mineralogical and chemical contents of geophagic clays from markets and geophagic clay collection sites.
- ii. To determine the co-effect of leached metals and acidity of simulated gastric fluid on the survival of microorganisms in geophagic clays.
- iii. To inactivate the *Escherichia coli*, *Salmonella enterica* and naturally occurring microorganism in edible geophagic clays under sunlight.

## 1.5 Hypotheses

Geophagic clays contains microorganism that are harmful and beneficial to human health. Sunlight exposure can disinfect microorganisms in geophagic clays.

## 1.6 Dissertation structure

This dissertation is divided into six chapters, each explaining different aspects of the investigation. A summary of each chapter is given below.

### CHAPTER 1: Introduction.

A brief background of geophagia and the solar disinfection water treatment to disinfect water containing pathogenic microbes are explained. The treatment is needed in geophagia practice to minimize the health risk to the soil eaters by the exposure of geophagic clay materials to direct sunlight and solar simulated irradiation. An outline of the aim, objectives, study motivation and study significance is briefly discussed.

### CHAPTER 2: Literature review

An in-depth explanation of geophagia practice, its disadvantages, and history of geophagia, rationale, geophagia clay materials and the preference of geophagists are surveyed. The geophagic clay materials containing various components including metals and

microorganisms, as well as the effect of metals and microorganism contained in the geophagic clays on the health of soil eaters are discussed, and health benefits and risks associated with geophagic practice are reviewed. The handling of clay prior to ingestion and an in-depth explanation of solar disinfection are presented.

**CHAPTER 3:** *Application of solar treatment for the disinfection of geophagic clays from the markets and mining sites.*

This Chapter describes the application of solar treatment to disinfect geophagic clays from markets and mining sites. The study focuses on the microorganisms contaminating the geophagic clay materials. This section also discusses the moisture content, organic carbon, DNA sequence and chemistry of geophagic clay materials

**CHAPTER 4:** *Co-effect of leached metals and pH of simulated gastric fluid on the survival of microorganisms in geophagic clays.*

This Chapter describes the co-effect of metals released from geophagic clay materials and the acidity on the survival of microorganism in geophagic clay materials. The geophagic clay was exposed to simulated gastric fluid (mimicking the gastro-intestinal tract of humans, pH of 2 at 37°C) and the susceptibility of microorganisms to low pH and metals released was investigated. The chemical and mineralogical compositions of the studied clayey materials are also reported in this section.

**CHAPTER 5:** *The effect of sunlight on the inactivation of Escherichia coli, Salmonella enterica and naturally occurring microorganisms in edible geophagic clay.*

This Chapter describes the inactivation of *Escherichia coli*, *Salmonella enterica* and naturally occurring microorganisms in edible geophagic clay under sunlight. The purpose of the study is to investigate the ability of sunlight to inactivate microorganisms that are detrimental to human health. These microorganisms can either be on the surface of clay samples or inside the layers of the clay.

**CHAPTER 6: Conclusion and recommendations**

This is the last Chapter of the dissertation. It presents the findings and discussion of the results of this study. It also makes recommendations for further research

## 1.7 References

- Abrahams, P.W. 2002. Soils: Their implications to human health. *Science of Total Environment*, 291:1-32.
- Baath, E. 1989. Effects of heavy metals in soil on microbial processes and populations (a review). *Water Air Soil Pollution*, 47:335–379.
- Baker, K.F. & Cook, J.R. 1974. *Biological Control of Plant Pathogens*. 1<sup>st</sup> ed. San Francisco, Freeman.
- Bisi-Johnson, M.A., Obi, C.L. & Ekosse, G-I.E. 2010. Microbiological and health related perspectives of geophagia: An overview. *African Journal of Biotechnology*, 9(19): 5784 – 5791.
- Brand, C.E., de Jager, L. & Ekosse, G-I.E. 2009. Possible Health Effects Associated With Human Geophagic Practice: An Overview. *Medical Technology SA*, 23(1): 11 - 13.
- Callahan, G.N. 2003. Eating Dirt. *Emerging Infect Dis*, 9(8): 1016-1021.
- Dean, J.R., Deary, M.E., Gbafa, B.K. & Scott, W.C. 2004. Characterization and analysis of persistent organic pollutants and major, minor and trace elements in calabash chalk. *Chemosphere*, 57: 21–25.
- Ekosse, G-I.E., de Jager, L. & Ngole, V. 2010. Traditional mining and mineralogy of geophagic clays from Limpopo and Free State provinces, South Africa. *African Journal of Biotechnology*, 9(47): 8058 – 8067.
- Fosso-Kankeu, E. & Mulaba-Bafubiandi, A.F. 2015. Sources of bacteria contaminating geophagic clays. (In: GE Ekosse, VM Ngole, L de Jager, E Fosso-Kankeu., ed. *Human and enzootic geophagia: Ingested soils and practice*. South Africa: Lambert Academic Publishing. p. 247-265).

Fosso-Kankeu, E., Netshitanin, T.L., Abia, A.L.K., Ubomba-Jaswa, E. & Waanders F.B. 2015. Application of solar treatment for the disinfection of geophagic clays from markets and mining sites. *African Journal of Technology*, 14(50): 3313-3324.

Geissler, P.W., Mwaniki, D., Thiong'o, F. & Friis, H. 1998. Geophagy as a risk factor for geohelminth infections: a longitudinal study of Kenyan primary school children. *Transactions Royal Society Tropical Medicine & Hygiene*, 92: 7-11.

Glickman, L.T., Chaudry, I.H., Costantino, J., Clack, F.B., Cypress, R.H. & Winslow, L. 1981. Pica patterns, toxocariasis and elevated blood lead in children. *Journal of Tropical Medical Hygiene*, 30: 191-195.

Halsted, J.A. 1968. Geophagia in Man: Its Nature and Nutritional Effects. *The American Journal of Clinical Nutrition*, 21(12): 1384 - 1391.

Heaselgrave, W., Patel, N., Kilvington, S., Kehoe, S.C. & McGuigan, K.G. 2006. Solar disinfection of poliovirus and *Acanthamoeba polyphaga* cysts in water - a laboratory study using simulated sunlight. *Letters Applied Microbiology*, 43(2): 125-30.

Hunter, B.T. 2003. The widespread practice of consuming soil. Consumer's Research Magazine. [http://goliath.ecnext.com/coms2/gi\\_0199-626795](http://goliath.ecnext.com/coms2/gi_0199-626795) Date of access: 5 April. 2015.

Jumbam, N.D. 2013. Geophagic materials: the possible effects of their chemical composition on human health. *Transactions of the Royal Society of South Africa*, 68(3): 177-182.

Kawai, K., Saathoff, E., Antelman, G., Msamanga, G. & Fawzi, W.W. 2009. Geophagy (Soil-eating) in Relation to Anemia and Helminth Infection among HIV-Infected Pregnant Women in Tanzania. *The American Journal of Tropical Medicine and Hygiene*, 80(1): 36 – 43.

Kutalek, R., Wewalka, G., Gundacker, C., Nuier, H., Wilson, J., Haluza, D., Huhulescu, S., Hillier, S., Jager, M. & Prinz, A. 2010. Geophagy and potential health implication: Geohelminths, microbes and heavy metals. *Transaction of the royal society of tropic medicine and hygiene*, 104: 787-795.

Lar, U.A., Agene, J.I. & Umar, A.I. 2015. Geophagic clay materials from Nigeria: a potential source of heavy metals and human health implication in mostly women and children who practice it. *Environmental Geochemical Health*, 37: 363-375.

Ljung, K., Selinus, O., Otabbong, E. & Berglund, M. 2006. Metal and arsenic distribution in soil particle sizes relevant to soil ingestion by children. *Applied Geochemistry*, 21:1613–1624.

Mahrer, Y. 1979. Predictions of soil temperature of a soil mulched with transparent polyethylene. *Journal of Applied Meteorology*, 18: 1263-1267.

Mani, S.K. 2006. Development and evaluation of small-scale systems for solar disinfection of contaminated drinking water in India. Newcastle-upon-Tyne, UK: Northumbria University. (Thesis-PhD).

McGuigan, K.G., Joyce, T.M., Conroy, R.M., Gillespie, J.B. & Elmore-Meegan, M. 1998. Solar disinfection of drinking water contained in transparent plastic bottles: characterizing the bacterial inactivation process. *Journal of Applied Microbiology*, 84:1138–1148.

McGuigan, K., Joyce, T. & Conroy, R. 1999. Solar disinfection: use of sunlight to decontaminate drinking water in developing countries. *Journal of Medical Microbiological*, 48: 785-787.

Mendez-Hermida, F., Castro-Hermida, J.A., Ares-Maraz, E., Kehoe, S.C. & McGuigan, K.G. 2005. Effect of batch-process solar disinfection on survival of *Cyprosporidium parvum* oocysts in drinking water. *Applied and Environmental Microbiology*, 71: 1653-1654.

Navntoft, C., Ubomba-Jaswa, E., McGuigan, K.G. & Fernández-Ibañez, P. 2008. Effectiveness of solar disinfection using batch reactors with non-imaging aluminium reflectors under real conditions: natural well water and solar light. *Journal Photochemical and Photobiology B: Biology*, 93 (3): 155–161.

Nchito, M., Geissler, P.W., Mubila, L., Friis, H. & Olsen, A. 2004. Effects of iron and multi-micronutrient supplementation on geophagy: a two-by-two factorial study among Zambian schoolchildren in Lusaka. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 98(4): 218 – 227.

Ngole-Jeme, V.M. & Ekosse, G-I.E. 2015. A Comparative Analyses of Granulometry, Mineral Composition and Major and Trace Element Concentrations in Soils Commonly Ingested by Humans. *International journal of environmental research and public health*, 12: 8933-8955.

Odewumi, S.C. 2013. Mineralogy and Geochemistry of Geophagic Clays from Share Area, Northern Bida Sedimentary Basin, Nigeria. *Journal Geology Geoscience*, 2: 108.

Oliver, M.A. 1997. Soil and human health: a review. *European Journal of Soil Science*, 48: 573– 592.

Oluma, H.O.A., Akande, T., Ebah, E.E. & Godwin, O.C. 2012. Prevalence of airborne bacteria in markets in Markurdi Metropolis. *Journal of biological and bio conservation*, 4:6-13.

Rainey, R.C. & Harding, A.K. 2005. Drinking water quality and solar disinfection: effectiveness in peri-urban households in Nepal. *Journal of Water Health*, 3:239–248.

Reilly, C. & Henry, J. 2001. Geophagia: why do humans consume soil? *British Nutrition Foundation*, 25(2): 141–144.

Remoundou, K. & Koundouri, P. 2009. Environmental Effects on Public Health: An Economic Perspective. *International journal of environmental research and public health*, 6(8): 2160-2178.

Songca, S. & Oluwafemi, O. 2015. Human and enzootic geophagic soils-Potential Chemical and Biochemical Implications. (In: GE Ekosse, VM Ngole, L de Jager, E Fosso-Kankeu., ed. Human and enzootic geophagia: Ingested soils and practice. South Africa: Lambert Academic Publishing. p. 57-70).

Songca, S.P., Ngole, V.M., Ekosse, G.E. & De Jager, L. 2010. Demographic characteristics associated with consumption of geophagic clays among ethnic groups in the Free State and Limpopo provinces. *Indilinga: African Journal of Indigenous Knowledge System*, 9: 110–123.

Shivoga, W.A. & Moturi, W.N. 2009. Geophagia as a risk factor for diarrhoea. *Journal Infect Developing Countries*, 3(2): 94-98.

Tano-Debrah, K. & Bruce-Baiden, G. 2010. Microbiological characterization of dry white clay, a pica element in Ghana. *Nature Science Report and Opinion*, 2(6): 77-81.

Twenefour, D. 1999. A study of clay eating among lactating and pregnant women and associated motives and effects. Legon: University of Ghana. (Dissertation- B.Sc.).

Ubomba-Jaswa, E., Boyle, M. A. R. & McGuigan, K. G. 2008. Inactivation of enteropathogenic *E. coli* by solar disinfection (SODIS) under simulated sunlight conditions. *Journal of Physics: Conference Series*, 101: 1-4.

Ubomba-Jaswa, E., Fernánde z-Ibañ ez, P., Navntoft, C., Polo-Lo´pez, M. & McGuigan, K. G. 2010. Investigating the microbial inactivation efficiency of a 25L batch solar disinfection (SODIS) reactor enhanced with a compound parabolic collector (CPC) for household use. *Journal Chemistry Technology Biotechnology*, 85: 1028–1033.

Vermeer, D.E. & Frate, D.A. 1979. Geophagia in rural Mississippi; Environmental and cultural context and nutritional implications. *Journal of Clinical Nutrition*, 32: 2129-2133.

WHO (World Health Organisation). 1996. Trace Elements in Human Nutrition and Health; World Health Organisation: Geneva Switzerland. (WHO 1996).

Wilson, M.J. 2003. Clay Mineralogical and Related Characteristics of Geophagic materials. *Journal of Chemical Ecology*, 29 (7): 1525–1547.

Wong, M.S., Bundy, D.A.P. & Golden, M.H.N. 1991. The rate of ingestion of *Ascaris lumbricoides* and *Trichuris trichiura* eggs and its relationship to infection in two children's homes in Jamaica. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 85: 89-91.

# Chapter 2

# Literature review

## 2.1 Introduction

In this chapter a review of published literature relevant to this study is presented. This includes mainly the background of geophagia practice, the history of geophagia, reasons for geophagia practice, the occurrence of metals and microorganisms in geophagic clay, the risk associated with toxic metals and microorganisms in geophagia, solar disinfection, and pre-treatments of geophagic clays are also included. Finally, a brief summary is provided to conclude the chapter.

## 2.2 Geophagia

The term “pica” refers to the craving for non-food substance including chalk, paper, ink, ice and soil for periods of a month or longer (Halsted, 1968; Brand *et al.*, 2009). Geophagia falls under subgroups of pica as each is defined by the materials that are ingested; commonly described types of pica include ingestion of ice (pagophagia) or starch (amylophagia) (Ferguson & Keaton, 1950; Edward *et al.*, 1959; Keith *et al.*, 1968; Vermeer & Frate, 1979). Geophagia is a complex term, and refers to the deliberate habit of consuming soil, clay, and earth-like substance (Ngole-Jeme & Ekosse, 2015).

### 2.2.1 History of geophagia

Geophagia has a long history. It has occurred for many thousands of years and it remains widespread (Ekosse & Jumbam, 2010). According to Ghorbani (2008) geophagia dates back to between 40 BC and 1 000 AD. Soil ingestion was mentioned by various philosophers and travellers and it was regarded as a symptom of some other disorder (Tayie, 2004; Ghorbani, 2008). The origin of the practice of geophagia was considered to be the continent of Africa. As a result of human migration it spread to most other continents (Abrahams, 2002; Hooda & Henry, 2009).

### 2.2.2 Geophagia practice

Geophagia is the eating of clay and soil (Abrahams, 2002). Geophagia is practiced almost everywhere in the world, including China, Australia, Europe, Sweden, South America, South

Africa, the Middle East, Botswana, Ghana, Cameroon and Swaziland by different races, age groups and gender (Vermeer & Frate, 1979; Abrahams & Parsons, 1997; Walker *et al.*, 1997; Woywodt & Kiss, 2002; Ekosse & Jumbam, 2010).

Geophagia is reported among women (especially pregnant women) and children under the age of two (Jumbam, 2013), particularly in Africa (Brand *et al.*, 2009), and also among people with psychiatric disorders (Ekosse *et al.*, 2010). Geophagia occurs either deliberately or involuntarily; young children under the age of two have the tendency to eat non-food materials such as soil through the exploration of their surroundings (Hawley, 1985; Fessler & Abrams, 2004; Jumbam, 2013). Prevalence of geophagia among black South African women is reported as 37% among pregnant women (Grace & Adams, 2012).

### **2.2.3 Rationale for geophagia**

Geophagia is practised for various reasons, such as cultural, medicinal, psychological, traditional, and nutritional needs and mineral deficiency (Young *et al.*, 2008; Ekosse *et al.*, 2010; Grace & Adams, 2012; Diko & Diko, 2013). Pregnant women claim that the taste of the clay reduces nausea, discomfort, and vomiting during morning sickness (Hunter, 1973; Diamond, 1998; Tayie & Lartey, 1999). Moreover, pregnant women in the southern USA believe that the consumption of clay helps the unborn children to develop well, reduce vomiting and also helps with the swollen legs experienced by pregnant women (Hunter, 2003; Bisi-Johnson *et al.*, 2010). Pregnant women including in Georgia also claim that it makes them feel better and reduces morning sickness (Hunter, 2003; Kawai *et al.*, 2009; Bisi-Johnson *et al.*, 2010).

In Malawi it was reported that it is surprising if a pregnant woman does not consume soil, because they believe that geophagia is how a woman knows that she is pregnant (Shinondo & Mwikuma, 2008). Studies have shown that young women believe that the consumption of soil and clay enhances their beauty by softening the skin and making it lighter (Woywodt & Kiss, 2002; Hunter, 2003; Ekosse & Jumbam, 2010). It was reported that African black slaves in the new world used to commit suicide by engaging in geophagia due to the notion that after death they would return to their homes spiritually (Abrahams, 2002).

Other individual geophagists consider clay a delicacy and it is added as an additional ingredient to food (Brand *et al.*, 2009). In the 19th century in Sweden, some people mixed earth (clay, soil) and flour to bake bread (Halsted, 1968). The consumption of clay has been

associated with diets (nutrients or minerals) that are low in zinc, iron and calcium (Reilly & Henry, 2000). According to Thompson (1913) clays are anti-poison and food detoxifier to counteract toxins (Diamond, 1998). Some individual geophagists in Ghana claim that they consume clay because of its flavour or the smell of the clay (Twenefour, 1999).

## **2.3 Geophagic clays**

Earthy materials that are consumed differ in several aspects, including physical, chemical and biological properties (Ngole, 2015). Geophagic clays are clays that humans and animals ingest.

### **2.3.1 Clay minerals**

Clay refers to earthy materials composed mainly of small particles, which is mud with suitable water amount and hardened when dehydrated (Guggenheim & Martin, 1995, 1996). Clays are primary inorganic materials that contain large quantities of organic materials including organism that impact negatively on human health (Tano-Debrah & Bruce-Baiden, 2010). Clay varies in colour: red, yellow, brownish, whitish, blackish, and grey (Woywodt & Kiss, 2002; Ekosse *et al.*, 2010). Clay minerals are characteristic minerals of the earth near the surface and are present in the majority of earth-like materials consumed (Maisanaba *et al.*, 2015).

Geophagic clays containing smectite, goethite, calcite, quartz, illite, sepiolite, and kaolinites are dominant (Ekosse *et al.*, 2010). Kaolinite and smectite impact positively on human health (Guarino *et al.*, 2001; Narkeviciute *et al.*, 2002; Hooda & Henry, 2009). Kaoline mineral is used to treat diarrhoea and enhances bioactivities, because of its medicinal properties (Mpuchane *et al.*, 2010). The quartz particles have a negative impact on human health due to the fact that they could cause damage to teeth tissues and cause rupture of the gastrointestinal tract (Ngole *et al.*, 2010).

### **2.3.2 Properties and preferences of geophagic clays by geophagists**

Geophagic clays are usually selected from specific sites, such as termite mounds, pits and river banks, hills, mountains and valleys (Reilly & Henry, 2000; Shinondo & Mwikuma, 2008). Geophagists prefer a specific type of clay dependent on colour, smell and taste

properties (Reilly & Henry, 2000). Geophagists mostly prefer clay from 25-75 cm below the surface because it is less contaminated by pathogens and other harmful substances than the top soil (Vermeer & Ferrell, 1985; Hunters, 2003).

The textures of geophagic clays vary with geographical area: those from the Free State province (South Africa) are soft and fine while those from Limpopo province (South Africa) are sandy and powdery (Ekosse *et al.*, 2010). Geophagists, including South African geophagists, generally prefer geophagic clay which has a soft, smooth and powdery consistency (Ekosse *et al.*, 2010). Several soil types are ingested by geophagic individuals around the world including whitish, creamy, greyish, khaki, yellowish, red, black, white, brown clay types, and termite mounds (Woywodt & Kiss, 2002; Ekosse *et al.*, 2010).

White clay usually contains kaolin, while yellowish clay contains yellow iron oxide (goethite) and reddish clay also contains iron, which could be a source of iron supplementation because the colour of the soil depends upon iron oxides/ hydroxides (Abrahams & Parsons, 1997; Young *et al.*, 2008). The blackish clay contains higher levels of organic matter (Ekosse *et al.*, 2010).

## **2.4 Business traits of geophagic clays**

The human geophagia practice is popular in business, especially in African rural communities and **Figure 1** shows the geophagic clays being sold in South Africa, Gauteng local markets. Thus traditional miners collect geophagic clay from selected areas and sell it to the retailers (Vermeer & Ferrell, 1985; Ekosse *et al.*, 2010). On most continents, such as Africa and Asia, clays are sold in markets (Nyanza *et al.*, 2014). In the south of the USA human geophagia practice is so prevalent that clays are purchased in small bags and large sacks for various reasons, such as for relatives who stay behind, convenience of travellers (Lourie *et al.*, 1963; Edward *et al.*, 1994). Furthermore, clays are mined in Nigeria in huge amounts and sold to other markets (Hunters, 1973).



**Figure 1:** Geophagic clay materials at Gauteng local markets. Photograph taken by Thembuluwo (2015)

## 2.5. Effect of pH and heavy metals on microbes

Microorganisms need some trace metals to grow, however, even low concentration of certain metals can be toxic for microorganisms. High heavy metals content in soil impact negatively to soil microbial population which may impact negatively to the soil properties (Ahmad *et al.*, 2005). Studies reported that other microorganisms may adapt to high content of toxic heavy metals by developing various mechanisms to resist heavy metals content (Kozdroj, 1995; Zhu *et al.*, 2006; Rathnayake *et al.*, 2010). Microbes such as *Helicobacter pylori* and *Clostridium botulinum* can survive the acidic environment (Zhu *et al.*, 2006). However, microbes exposed to pretreatment at pH 1.0 or 2.0 failed to survive the pretreatment (Zhu *et al.*, 2006).

According to Impellitteri and Allen (2001) pH is the most important parameter controlling distribution of heavy metals in soils because pH directly affects the solubility of elements; other metals becomes more soluble and available with increased toxicity. When pH is alkaline other metals becomes unavailable to organism. High content of heavy metals together with low pH may cause decrease in microbial load (Kikovic, 1997; Šmejkalová *et al.*, 2003). Soil contamination can cause change in diversity of soil microflora (Zaguralskaya, 1997) and microbes require more organic matter and nutrient content to survive unfavourable conditions (Fosso-Kankeu & Mulaba-Bafubiandi, 2015). Availability of nutrients in soil can be affected by pH.

## 2.6 Heavy Metal contaminants in geophagic clays

Geophagic clays can be contaminated by metals attached to the clays or trapped as small particles. Metal contaminants can adversely impact on the health of humans when they are

ingested. The occurrence of metals and the risks associated with the toxic metals in geophagic clays are discussed next:

### **2.6.1 Sources of heavy metals in geophagic clays**

Wastewater from mining, industrial areas, sewage sludge, spillage of petrochemicals, disposal of high metal waste and from manufacturing contaminate the environment and cause risk to the health of people (Khan *et al.*, 2008; Wuana & Okieimen, 2011; Padilla-Ortega *et al.*, 2013). The use of synthetic products such as paints, fertilizers and pesticides play a role in the dispersion of heavy metals in the environment (Khan *et al.*, 2008; Zhang *et al.*, 2010; Wuana & Okieimen, 2011; Padilla-Ortega *et al.*, 2013). Wastewater from industrial areas normally contains lead, copper, cadmium, nickel and zinc (Chuah *et al.*, 2005) and in addition, mining activities generate huge volumes of waste that contribute to the pollution of waste that contribute to the pollution of soil.

Groundwater, soil chemistry, and local transport mechanisms influence the amount, absorption and dispersal of metal contaminants (Evanko & Dzombak, 1997). Once these metals are dispersed in the soil it is contaminated and those heavy metals remain in the soil. However, few metals, such as mercury and selenium, can be transformed by microorganisms (Clarkson & Magos, 2006). Lead, chromium, arsenic, zinc, cadmium, copper, mercury, and nickel are dominant metals in the contaminated sites such as pits, river banks, hills, mountains and valleys (USEPA, 1996; Evanko & Dzombak, 1997). Fertilizers and pesticides are regularly used widely in agriculture and horticulture and these compounds contain traces of toxic metals, such as Cd, Co, Cu, and Pb (Jones & Jarvis, 1981; Raven *et al.*, 1998; Wuana & Okieimen, 2011).

### **2.6.2 Geophagic clays contaminated with heavy metals and human health.**

Geophagic clay soil contains toxic heavy metals which may negatively influence the human health, depending on the type and quantity of metals, as well as the toxicity to human health. Clays have net negative surface charges causing the toxic metals to bind to their surfaces (Otto & Haydel, 2013). Ingestion of clay contaminated with heavy metals by pregnant woman and children can cause damage to the brain and kidney (Kutalek *et al.*, 2010).

The toxic metals (As, Cd, Pb, Se and Sb) found in geophagic clays may negatively affect human health (Lar *et al.*, 2015). Studies showed that lead absorption in the human body at

any concentration is poisonous and lead is toxic in any amount of exposure (Lanphear *et al.*, 1996). Acute and chronic diseases on human health can be caused by ingestion of large quantities of heavy metals (As, Pb and Cd) (Otto & Haydel, 2013; Lar *et al.*, 2015).

High levels of zinc intake is considered toxic and causes nausea, dehydration, vomiting, electrolyte imbalance, lack of muscular coordination and dermatitis (Alloway & Ayres, 1993). Nickel causes dermatitis, eczema, vertigo and dyspnoea and can be carcinogenic (WHO, 1993). Furthermore, Cr can cause cancer to organs (Alloway & Ayres, 1993). An exposure to high levels of Cu (copper) can cause severe kidney damage and respiratory failure (WHO, 1993).

## **2.7 Microbial contaminants in geophagic clays**

### **2.7.1 Microbial contaminants**

Micro-organisms in clay can be introduced through natural contamination and the handling of the clay (Ekosse *et al.*, 2010). Sometimes the mined geophagic clays are consumed directly before they undergo some processing, such as pounding, sieving, slurring, and grinding (Ekosse *et al.*, 2010) or the geophagic clay can be ingested as a drink or powder (Kutalek *et al.*, 2010).

Heat treatment of the mined geophagic clays is used to reduce and/or completely remove potential pathogens and to improve the texture, taste and colour (Ekosse *et al.*, 2010, Hunter, 2003; Reilly & Henry, 2001; Young *et al.*, 2010). It is accomplished with other treatments such as boiling, oven baking, and sun drying, and burning (Reilly & Henry, 2001; Hunter, 2003; Ekosse *et al.*, 2010; Young *et al.*, 2010). Furthermore, pregnant women prefer smoked, roasted or baked clay soils (Shinondo & Mwikuma, 2008).

According to Tano-Debrah and Bruce-Baiden (2010) soil sold in the market of Uganda could be contaminated with microorganisms and constitute sources of microbial infection. Moreover, the presence of contaminants on the surface of geophagic clays indicates unhygienic handling of the clay (Tano-Debrah & Bruce-Baiden, 2010). Microorganisms require high water activities and moisture to grow (Kawai *et al.*, 2009; Tano-Debrah & Bruce-Baiden, 2010).

Tano-Debrah & Bruce-Baiden (2010) observed that low water content in the clay resulted to unsuitable environment for microorganisms. The isolated microorganisms found in dry

white clay from the markets in Uganda include Coliform bacteria, *Staphylococcal spp*, *Alcaliyers spp*, *Candida spp* and yeast, and these microorganisms in the samples could be a risk to the health of consumers (Tano-Debrah & Bruce-Baiden, 2010).

### **2.7.2 Risks associated with the microorganism in geophagic clays**

Pathogenic microorganisms in geophagic clay may negatively influence human health (Bisi-Johnson *et al.*, 2010). Unbaked materials contain high levels of microbial contamination in geophagic materials (Kutalek *et al.*, 2010). This is a concern because clay is usually consumed uncooked. These pathogenic microorganisms include *Clostridium perfringens*, *C. tetani*, *C. botulinum* (Ekosse *et al.*, 2010), *Aspergillus spp* (Bisi-Johnson *et al.*, 2013), *Francisella spp.* (Kugeler & Mead, 2008), *Cryptococcus neoformans* (Emmons, 1951), and *Histoplasma capsulatum* (Emmons, 1949).

The microorganism groups often present in soil are bacteria, prions, viruses, microalgae, and prokaryotic actinomycetes (Ekosse *et al.*, 2010). The microbial ecology of clay includes harmful and beneficial microorganisms which differ in their functions and their impact on animal and human health (Ekosse *et al.*, 2010). *Clostridium perfringens* and *C. tetani* contaminate the top soil and produce toxins that cause infections by contamination of wounds (Abrahams, 2002). The microorganism infection can actually result in anaemia in geophagists (Abrahams, 2002; Ekosse *et al.*, 2010). Furthermore, a study showed that anaemia is more prevalent in western Kenyan school children who were geophagists (Gessler *et al.*, 1998). In addition, studies have shown the association between iron-deficiency anaemia and geophagia (Woywodt & Kiss, 2002; Von Garnier *et al.*, 2008).

There is a high prevalence of geohelminths infection worldwide (Chan, 1997). Prions primarily affect the cardiovascular and nervous system (Prusiner, 1998) and also cause the onset development of brain disorder (Caughey 2003, Colling *et al.*, 2006). According to Kawai *et al.* (2009) HIV-infected women who ingest soils are infected with geohelminth infection and it is associated with iron deficiency. The eggs of *Ascaris lumbricoides* and *Trichuris trichiura* can be transmitted with ingestion of clay and resulted to ascariasis which is characterized by abdominal pain, nausea and trichiuriasis (Abrahams, 2002). However, the prevalence of *A. lumbricoides* infection is found to be lower in geophagists who prefer eating tree termite soil and a risk factor to those who prefer termite mounds soil (Saathoff *et al.*, 2002).

### **2.7.3 Cases of infections associated with geophagia practice**

Geophagia is associated with helminthic diseases and especially linked to *Ascaris lumbricoides* and *Trichuris trichiura* infection among pregnant women (Hunter, 1973; Geissler *et al.*, 1999). The ingestion of pathogenic nematodes (geohelminth infection) results in the disease helminthiasis and it includes infection caused by a range of different geohelminthic species, including *Ascaris lumbricoides* and *Trichuris trichiura* and the hookworms and moreover, helminthiasis is more prevalent worldwide (Holland & Kennedy, 2002; Bethony *et al.*, 2006; WHO, 2008). Tanzanian pregnant women who ingest soils were reported infected with *Ascaris lumbricoides* infection (Kawai *et al.*, 2009).

Studies have shown that pregnant women who ingest soil from Kenya are at risk of geohelminth infection and especially *A. lumbricoides* re-infection (Luoba *et al.*, 2004; Luoba *et al.*, 2005). High prevalence of *A. lumbricoides* infection was reported among school children from Kwazulu-Natal (South Africa) who collect their soil from termitaria (Saathoff *et al.*, 2002). The parasitic infection associated with childhood geophagia is *baylisascaris*. A case has been reported where the raccoon roundworm (*Baylisascaris procyonis*) infected two children at separate sites resulting in central nervous system (CNS) damage and the death of one (Centres for disease control, 2002). *Ascaris* infections have been reported in high number of people and ascariasis infection is mostly common in children who ingest soil (Ozumba & Ozumba, 2002). Furthermore, an ingestion of soil is associated with the parasitic infection of toxocariasis commonly found in the United States and caused by *Toxocara canis* (Laufer, 2002).

## **2.8 Geophagia and human health**

Among different reasons given by people practicing geophagia, is its impact on health. Studies have found that many geophagists consider the practice has a positive impact on health, believing that clayey materials contain supplements of minerals and nutrients (Abrahams, 2005), relief from gastro-intestinal distress, the alleviation of excessive acidity in the digestive tract and detoxification of noxious substances (Hood *et al.*, 2004; Walker *et al.*, 1997).

### **2.8.1 Health benefits of geophagia**

Studies have found that the mineral kaolin (a form of clay) impacts positively on human health (Guarino *et al.*, 2001; Narkeviciute *et al.*, 2002; Hooda & Henry, 2009). Kaolin is beneficial in its use to relieve gastro-intestinal distress (Bisi-Johnson *et al.*, 2010). Pregnant women practise geophagia because they believe it contains calcium, iron, magnesium, and zinc that would supply minerals (Hood *et al.*, 2004; Abrahams, 2005; Abrahams, 2005). Clay contains constituents that create a barrier to toxins and microorganisms (Callahan, 2003; Bisi-Johnson *et al.*, 2010). Clay minerals such as kaolin and smectite have the potential to reduce vomiting (nausea), the severity and duration of diarrhoea, and gastro-intestinal upset (Guarino *et al.*, 2001; Narkeviciute *et al.*, 2002; Hooda & Henry, 2009).

### **2.8.2 Health risks associated with geophagia**

Geophagic clays contain excessive and harmful compounds, including minerals (potassium and zinc); heavy metals (lead), bacteria, viruses; hookworm, fungi, protozoa, and protozoa (Saathoff *et al.*, 2002; Garg *et al.*, 2004; Bisi-Johnson *et al.*, 2010; Khan *et al.*, 2011). Geophagia can also be a risk factor for developing diarrhoea as a result of poor sanitation (Shivoga & Moturi, 2009). However, geophagia can also be a treatment for diarrhoea (Hooda & Henry, 2009).

The mineral quartz has a negative impact on human health and may cause tooth abrasion, cracks and erosion, and tooth decay (Johnson *et al.*, 2007). Geophagia is associated with geohelminths infection and other microbial infections (Abrahams, 2002). Lead poisoning was observed in children who practiced geophagia (Cahallan, 2003). Studies have shown that some geophagists experience severe constipation and even intestinal obstruction (Woywodt & Kiss, 1999; Ye *et al.*, 2004). Hyperkalaemia is associated with geophagia because of contamination of potassium in clay (Ghorbani, 2008).

## **2.9 Solar disinfection**

Solar disinfection (SODIS) is a water treatment method that uses UV solar radiation and mild heat (Conroy *et al.*, 1996; McGuigan *et al.*, 1998; Conroy *et al.*, 2001; Solar water disinfection, 2002; Mani, 2006; Ubomba-Jaswa *et al.*, 2010; McGuigan *et al.*, 2012). SODIS inactivate waterborne pathogens because microorganisms are sensitive to heat (McGuigan *et al.*, 2012). SODIS is currently used daily by more than 45 million people, mostly in developing countries (McGuigan *et al.*, 2012).

SODIS containers can be glass or plastic bags or discarded plastic (PET) bottles (Walker *et al.*, 2004; Byrne *et al.*, 2011). At its simplest the method involves filling transparent containers with contaminated water and then placing it in direct sunlight for several hours or at least 6 hours and 24-48 hours in cloudy weather (Reed *et al.*, 2005; McGuigan *et al.*, 2012). SODIS can destroy microorganisms or disinfects water through two effects 1) optical inactivation; and 2) thermal inactivation (Wegelin *et al.*, 1994).

SODIS germicidal effect is a combination of optical inactivation effect and thermal heating effect (McGuigan *et al.*, 2012) and this germicidal effect means that the inactivation of microorganism occurs at fast rate. The optical inactivation process involves the use of the ultraviolet (UV) and visible wavelength of sunlight that can be absorbed by molecules (Wegelin *et al.*, 1994; Reed *et al.*, 2005). Polyethylene plastic bottles enhance the optical inactivation (Reed *et al.*, 2005; McGuigan *et al.*, 2012).

Reflectors are used on cloudy days to concentrate the available ultra violet A (UVA) radiation (Duffie & Beckman, 2006; McGuigan *et al.*, 2012). UV radiation, visible light and infrared radiation are the solar radiation wavelength (Jagger, 1985; Wegelin *et al.*, 1994; Solar water disinfection, 2002). UVB and UVC light do not reach the earth and mostly UVA radiation reaches earth (Solar water disinfection, 2002). UVA radiation has a harmful effect on human pathogens and damages the cells (Solar water disinfection, 2002).

The thermal inactivation process involves the solar infrared radiation and black-painted containers or solar cookers (Wegelin *et al.*, 1994; Duffie & Beckman, 2006). Bottles or tubes painted with conventional black paint are used, converting sunlight to heat (Duffie & Beckman, 2006; McGuigan *et al.*, 2012). Absorption of solar infrared radiation may raise the temperature of illuminated water to the point where pathogens are inactivated (Reed *et al.*, 2005; Ubomba-Jasma *et al.*, 2010; McGuigan *et al.*, 2012). Placing filled bottles on the reflective surface increases the amount of energy absorbed by the bottles (Mani, 2006) and painting the underside of the container black or the use of black plastic enhances solar heating (Sommer *et al.*, 1997).

SODIS is not a recent technology, but not yet demonstrated for clay treatment. SODIS meets certain criteria, making it suitable for use in communities (Solar water disinfection, 2002). These criteria include low cost, ease to use and sustainability to become effective within the household (Solar water disinfection, 2002). SODIS depends on the weather and

climatic condition for it requires sufficient solar radiation (Sommer *et al.*, 1997). During cloudy days there is less radiation energy available, thus the exposure has to be 2 days to ensure there is complete inactivation (Sommer *et al.*, 1997). Moreover, limitations of SODIS include that no modification of chemical quality and the volume is limited to less than 3ℓ (Solar water disinfection, 2002). Exposing geophagic clay to sunlight or UVA radiation may inactivate pathogenic microorganisms.

## **2.10 The various methods of treatments of the clay**

Mined clays are collected using different techniques including hand grabbing, scraping, and knives (Ekosse *et al.*, 2010), see **Figure 2**. The geophagic clays are prepared in different ways by geophagist individuals before ingestion (Ekosse *et al.*, 2010). Mined soil contains visible contaminants and unwanted soil material which are removed using hand grabbing (Ekosse *et al.*, 2010). Heat treatment and boiling geophagic clays are used as treatment in Limpopo Province and Free State Province (Hunter, 2003; Ekosse *et al.*, 2010).

To sterilize the soil, geophagist individuals use heating, baking, boiling, and burning (Reilly & Henry, 2001; Hunter, 2003; Ekosse *et al.*, 2010; Young *et al.*, 2010). Sterilization of soil improve texture, colour and taste of geophagic clays (Ekosse *et al.*, 2010). Geophagic clays can be exposed to sunlight during business time (Fosso-Kankeu *et al.*, 2015). The processing of geophagic clays before consumption by grinding, pounding, sieving, and slurring, concentrates fine clay particles, reduces organic matter content and assists ingestion (Gosselain & Smith, 2005; Ekosse *et al.*, 2010).

According to Fosso-Kankeu & Mulaba-Bafubiandi (2015) low amount of bacteria present in the clay resulted from pre-treatment such as drying and baking of clay. Soil pasteurization is one of the methods used to kill harmful pathogens in contaminated soil. It involves sterilizing soil by artificially heating the soil by covering it with transparent polyethylene, and keeping the soil wet during mulching is effective to disinfect soil (Baker & Cook, 1974; Mahrer, 1979).



**Figure 2:** Mining sites where the geophagic clay materials are collected using spades and hands. Photograph taken by Thembuluwo (2015).



**Figure 3:** Geophagic clay materials exposed at open Gauteng local markets. Photograph taken by Thembuluwo (2015).

## 2.11 Conclusion

Geophagia practice is widespread around the world (Ngole-Jeme & Ekosse, 2015). Geophagic clay and soils are the materials that the geophagist individuals deliberately ingest (Ngole-Jeme & Ekosse, 2015). The reasons for geophagia practice are unclear, but researchers reported some factors which include hunger, mineral supplementation, relieving gastro-intestinal distress and removal of toxins and pathogens (Hooda *et al.*, 2004; Young *et al.*, 2010). There are health drawbacks from ingestion of soil, including microbiological infection, mental retardation, brain damage, heavy metal poisoning and dental damage (Abrahams, 2002; Bethony *et al.*, 2006; Ellis & Pataki, 2012).

Some geophagists believe soil ingestion benefits their health by mineral supplementation, relieving of gastro-intestinal distress and removal of toxins (Walker *et al.*, 1997; Cahallan, 2003; Wilson, 2003; Hooda *et al.*, 2004). Geophagic soils and clays are processed before ingestion by grinding, heating, slurring and baking (Ekosse *et al.*, 2010). In the following chapter a detailed paper on the application of solar treatment to reduce microorganisms is presented.

## 2.12 References

- Abrahams, P.W. 2002. Soils: Their implications to human health. *Science of Total Environment*, 291:1-32.
- Abrahams, P.W. 2005. Geophagy and involuntary ingestion of soil. (In Selinus O, Alloway B, Centeno J A., eds. *Essentials of Medical Geology: Impact of the Natural Environment on Public Health*. Amsterdam, Netherlands: Elsevier. p. 435-458).
- Abrahams, P.W. & Parsons, J. A. 1997. Geophagy in the Tropics: an appraisal of three geophagical materials. *Environmental of Geochemical Health*, 19 (1): 19-22.
- Ahmad, I., Hayat, S., Ahmad, A., Inam, A. & Samiullah, I. 2005. Effect of heavy metal on survival of certain groups of indigenous soil microbial population. *Journal Applied Science Environment Management*, 9:115–121.
- Alloway, B.J. & Ayres, D.C. 1993. Chemical principles of environment pollution. 1<sup>st</sup> ed. Glasgow, U.K: Blackie academic & professional.
- Baker, K.F. & Cook, J.R. 1974. Biological Control of Plant Pathogens. 1st ed. San Francisco, Freeman.
- Bethony, J., Brooker, S., Albonico, M., Geiger, S.M., Loukas, A., Diemert, D. & Hotez, P.J. 2006. Soil transmitted helminth infections: *ascariasis*, *trichuriasis*, and hookworm. *Lancet*, 367: 1521– 1532.
- Bisi-Johnson, M.A., Obi, C.L. & Ekosse, G-I.E. 2010. Microbiological and health-related perspectives of geophagia: An overview. *African Journal of Biotechnology*, 9(19): 5784 – 5791.
- Bisi-Johnson, M.A., Oyelade, H.A., Adediran, K.A. & Akinola, S.A. 2013. Microbial Evaluation of Geophagic and Cosmetic Clays from Southern and Western Nigeria: Potential Natural Nanomaterials. *International Journal of Environmental, Chemical, Ecological, Geological and Geophysical Engineering*, 7(12): 832-835.

Brand, C.E., de Jager, L. & Ekosse, G-I.E. 2009. Possible Health Effects Associated With Human Geophagic Practice: An Overview. *Medical Technology SA*, 23(1): 11 - 13.

Byrne, J.A., Fernandez-Ibañez, P.A., Dunlop, P.S.M., Alrousan, D.M.A. & Hamilton, J.W.J. 2011. Photocatalytic Enhancement for Solar Disinfection of Water: A Review. *International Journal of Photoenergy*, 1-12.

Callahan, G.N. 2003. Eating Dirt. *Emerging Infection Diseases*, 9(8): 1016-1021.

Caughey, B. 2003. Prion protein conversions: insight into mechanisms, TSE transmission barriers and strains. *British Medical Bulletin*, 66(1): 109-120.

Centers for Disease Control for Prevention. 2002. Raccoon roundworm encephalitis—Chicago, Illinois, and Los Angeles, California, 2000. *MMWR Morb Mortal Weekly Rep*; 50:1153-5.

Chan, M.S. 1997. The global burden of intestinal nematode infections-Fifty years on. *Parasitol. Today*, 13: 438-443.

Chuah, T.G., Jumasiah, A., Katayon, S. & Thomas Choong, S.Y. 2005. Rice husk as a potentially low-cost bio sorbent for heavy metal and dye removal: an overview. *Desalination*, 175: 305–316.

Clarkson, T. W. & Magos, L. 2006. The toxicology of mercury and its chemical compounds.

*Critical Reviews in Toxicology*, 36(8): 609-662.

Colling, J., Whitfield, J., Mckintosh, E., Beck, J., Mead, S., Thomas, D.J. & Alpers, M.P. 2006. Kuru in the 21st century-an acquired human prion disease with very long incubation periods. *Lancet*, 367(9528): 2068- 2074.

Conroy, R.M., Elmore-Meegan, M., Joyce, T., McGuigan, K.G. & Barnes, J. 1996. Solar disinfection of drinking water and diarrhoea in Maasai children: a controlled field trial. *Lancet*, 348: 1695–1697.

Conroy, R.M., Meegan, M.E., Joyce, T., McGuigan, K.G. & Barnes, J. 2001. Solar disinfection of drinking water protects against cholera in children under 6 years of age. *Archives of disease in childhood*, 85: 293–295.

Diamond, J. 1998. "Eat dirt". *Discover*: 70-75.

Diko, C. N. S. é. & Diko, M.L. 2013. Geophagia among female adolescents as a culturally driven practice. *Indilinga African journal Indigenous Knowledge System*, 12 (2): 242–251.

Duffie, J.A. & Beckman, W. 2006. *Solar Engineering of Thermal Processes*. 4th Ed. New York: John Wiley & Sons.

Edwards, C.H., Johnson, A.A., Knight, E.M., Oyemade, U.J., Cole, O.J., Westney, O.E., Johns, S., Laryea, H. & Westney, L.S. 1994. African American women and their pregnancies. *Journal of Nutrition*, 124(6): 9545–9625.

Edwards, C.H., McDonald, S., Mitchell, J.R., Jones, L., Mason, L., Kemp, A.M., Laing, D. & Trigg, L. 1959. Clay and corn starch-eating women. *Journal of American Dietetics*, 35:810-805.

Ekosse, G.I. & Jumbam, N.D. 2010. Geophagic clays: Their mineralogy, chemistry and possible human health effects. *African Journal of Biotechnology*, 9(40): 6755 – 6767.

Ekosse, G-I.E., de Jager, L. & Ngole, V. 2010. Traditional mining and mineralogy of geophagic clays from Limpopo and Free State provinces, South Africa. *African Journal of Biotechnology*, 9(47): 8058 – 8067.

Ellis, C.R. & Pataki, C. 2012. Pica. Medscape. <<http://emedicine.medscape.com/article/914765-overview>> Date of access: 15 Sep 2016.

Emmons, C.W. 1949. Isolation of *Histoplasma capsulatum* from soil. *Public Health Service Report*, 64: 892-896.

Emmons, C.W. 1951. Isolation of *Cryptococcus neoformis* from soil. *Public Health Service Report*, 62: 685-690.

Evanko, C.R. & Dzombak, D.A. 1997. Remediation of metals contaminated soils and groundwater. *Technology evaluation, GWRTAC*, 50-52.

Ferguson, J.H. & Keaton, A. 1950. Studies on the clients of pregnant women in Mississippi: ingestion of clay and laundry starch. *New Orleans Medical and Surgical Journal*, 102:460-463.

Fessler, D.M.T. & Abrams, E.T. 2004. Infant mouthing behavior: the immunocalibration hypothesis. *Med Hypothesis*, 63: 925-932.

Fosso-Kankeu, E. & Mulaba-Bafubiandi, A.F. 2015. Sources of bacteria contaminating geophagic clays. (In: GE Ekosse, VM Ngole, L de Jager, E Fosso-Kankeu., ed. Human and enzootic geophagia: Ingested soils and practice. South Africa: Lambert Academic Publishing. p. 247-265).

Garg, M., Shaver, M.J. & Easom, A. 2004. Pica: An underappreciated cause of electrolyte abnormalities. *Nephrology News and Issues*, 18: 28-29.

Geissler, P.W., Mwaniki, D., Thiong'o, F. & Friis, H. 1998. Geophagy as a risk factor for geohelminth infections: a longitudinal study of Kenyan primary school children. *Transactions Royal Society Tropical Medicine & Hygiene*, 92: 7-11

Geissler, P.W., Prince, R.J., Levene, M., Poda, C., Beckerleg, S.E., Muteni, W. & Shulman, CE. 1999. Perceptions of soil-eating and anaemia among pregnant women on the Kenyan coast. *Social Science and Medicine*, 48(8): 1069-1079.

Ghorbani, H. 2008. Geophagia, a Soil- Environmental Related Disease. *International Meeting on Soil Fertility, Land Management and Agro climatology*, Turkey: 957-967.

Grace, G. & Adams, A. 2012. Physiological effects of geophagy (soil eating) with reference to iron nutritional status in pregnant women: a study in selected antenatal clinics in KSD

municipal area of the Eastern Cape, South Africa. (*In* 2012 3rd International Conference on Biology, Environment and Chemistry: IPCBEE 46(21). Singapore: IACSIT Press. p. 96-98).

Gosselain, O.P & Smith, L.A. 2005. The source: Clay selection and processing practice in Sub-Saharan Africa. (*In*: Livingstone Smith, A., Bosquet. D. and Martineau. R., eds. Pottery manufacturing processes: Reconstruction and interpretation. Oxford: British Archaeological Reports International Series. p. 33-47).

Guarino, A., Bisceglia, M., Castellucci, G., Lacono, G., Casali, L.G., Bruzzese, E., Musetta, A. & Greco, L. 2001. Smectite in the treatment of acute diarrhoea: A nationwide randomised controlled study of the Italian Society of Paediatric Gastroenterology and Hepatology (SIGEP) in collaboration with primary care paediatricians. SIGEP study group for Smectite in Acute Diarrhea. *Journal of Paediatric Gastroenterology and Nutrition*, 32(1): 71-75.

Guggenheim, S. & Martin, R.T. 1995. Definition of clay and clay minerals: Joint report of the AIPEA nomenclature and CMS nomenclature committees. *Clays and Clay Minerals*, 43(2): 255-256.

Guggenheim, S. & Martin, R.T. 1996. Reply to the comment by D.M. Moore on Definition of clay and clay mineral: Joint report of the AIPEA and CMS Nomenclature Committees. *Clays and Clay Minerals*, 44:713-715.

Habold, C., Reichardt, F., Le Maho, Y., Angel, F., Liewig, N., Lignot, J.H. & Oudart, H. 2009. Clay ingestion enhances intestinal triacylglycerol hydrolysis and non-esterified fatty acid absorption. *British Journal of Nutrition*, 102(2): 249-257.

Halsted, J.A. 1968. Geophagia in Man: Its Nature and Nutritional Effects. *The American Journal of Clinical Nutrition*, 21(12): 1384 - 1391.

Hawley, J.K. 1985. Assessment of Health Risk from Exposure to Contaminated Soil. *Risk analysis*, 5(4): 289-302.

Holland, C.V. & Kenned, M.W. 2002. The geohelminths: Ascaris, Trichus and hookworm. *World class parasites*, 2: 11-14.

Hooda, P. & Henry, J. 2009. Geophagia and Human Nutrition. 1st ed. USA: Berghahn Books.

Hooda, P.S., Henry, C.J.K., Seyoum, T.A., Armstrong, L.D.M. & Fowler, M.B. 2004. The potential impact of soil ingestion on human mineral nutrition. *Science of the Total Environment*, 333: 75-87.

Hunter, J.M. 1973. Geophagy in Africa and in the United States: a culture-nutrition hypothesis. *Geographical Review*, 63: 170–195.

Hunter, B.T. 2003. The widespread practice of consuming soil. Consumer's Research Magazine. [http://goliath.ecnext.com/coms2/gi\\_0199-626795](http://goliath.ecnext.com/coms2/gi_0199-626795) Date of access: 5 April 2015.

Impellitteri, Ch. A. & Allen, H.E. 2001. Organic matter control of soil-soil solution metal partitioning. Biogeochemistry of trace elements, ICOBTE.

Jagger, J. 1985. Solar –UV actions on living cells. New York: Prager.

Jones, L.H.P. & Jarvis. S.C. 1981. The fate of heavy metals. (*In*: Greenland. D.J. and Hayes M.HB., eds. The chemistry of soil processes. New York; John Wiley. p. 593-620).

Johnson, C.D., Shynett, B., Dosch, R. & Paulson, R. 2007. An unusual case of tooth loss, abrasion, and erosion. *General Dentistry*, 55(5): 445-448.

Jumbam, N.D. 2013. Geophagic materials: the possible effects of their chemical composition on human health. *Transactions of the Royal Society of South Africa*, 68(3): 177-182.

Kawai, K., Saathoff, E., Antelman, G., Msamanga, G. & Fawzi, W.W. 2009. Geophagy (Soil-eating) in Relation to Anemia and Helminth Infection among HIV–Infected Pregnant Women in Tanzania. *The American Journal of Tropical Medicine and Hygiene*, 80(1): 36 – 43.

Keith, L., Evenhouse, H. & Webster, A. 1968. Amylophagia during pregnancy. *Obstetrics and Gynecology*, 32:415-418.

Khan, D.A., Ansasri, W.M., & Khan, F.A. 2011. Synergistic effects of iron deficiency and lead exposure on blood lead levels in children. *World Journal of Paediatrics*, 7(2): 150-154.

Khan, S., Cao, Q., Zheng, Y. M., Huang, Y. Z. & Zhu Y. G. 2008. Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. *Environmental Pollution*, 152(3): 686–692.

Kikovic, D.D. 1997. Influence of heavy metals emitted by thermoelectrical power plants and chemical industry on Kosovo soils microflora. *Rev Res Wk Faculty Agriculture*, Belgrade, 42: 61– 75.

Kozdrój, J. 1995. Microbial responses to single or successive soil contamination with Cd or Cu. *Soil Biology Biochemical*, 27:1459–1465.

Kugeler, K.J. & Mead, P.S. 2008. Isolation and Characterization of a Novel *Francisella* sp. from Human Cerebrospinal Fluid and Blood. *Journal Clinical Microbiology*, 46(7): 2428-2431.

Kutalek, R., Wewalka, G., Gundacker, C., Nuier, H., Wilson, J., Haluza, D., Huhulescu, S., Hillier, S., Jager, M. & Prinz, A. 2010. Geophagy and potential health implication: Geohelminths, microbes and heavy metals. *Transaction of the royal society of tropic medicine and hygiene*, 104: 787-795.

Lanphear, B. P., Weitzman, M. & Winter, N. L. 1996. Lead contaminated house dust and urban children's blood lead levels. *American Journal of Public Health*, 86: 1416–1421.

Lar, U.A., Agene, J.I. & Umar, A.I. 2015. Geophagic clay materials from Nigeria: a potential source of heavy metals and human health implication in mostly women and children who practice it. *Environmental Geochemical Health*, 37: 363-375.

Laufer, M. 2002. Toxocariasis. <http://emedicine.medscape.com/article/999850-overview>  
Date assessed: 5 May 2015.

Lourie, R.S., Layman, E.M. & Millican, F.K. 1963. Why children eat the things that are not food. *Children*, 10:143.

Luoba, A.I., Geissler, P.W., Estambale, B., Ouma, J.H., Magnussen, P., Alusala, D., Ayah, R., Mwaniki, D. & Friis, H. 2004. Geophagy among pregnant and lactating women in Bodo District, western Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 98(12): 734 - 741.

Luoba, A.I., Geissler, P.W., Estambale, B., Ouma, J.H., Alusala, P., Ayah, R., Mwaniki, D., Magnussen, P. & Friis, H. 2005. Earth-eating and reinfection with intestinal helminths among pregnant and lactating women in western Kenya. *Tropical Medicine and International Health*, 10(3): 220 – 227.

Mahrer, Y. 1979. Predictions of soil temperature of a soil mulched with transparent polyethylene. *Journal of Applied Meteorology*, 18: 1263-1267.

Maisanaba, S., Prieto, A., Puerto, M., Gutierrez-Praena, D., Demir, E., Marcos, R. & Camean, A.M. 2015. In vitro genotoxicity testing of carvacrol and thymol using the micronucleus and mouse lymphoma assays. *Mutant Res*, 784-785.

Mani, S.K. 2006. Development and evaluation of small-scale systems for solar disinfection of contaminated drinking water in India. Newcastle-upon-Tyne, UK: Northumbria University. (Thesis-PhD).

McGuigan, K.G., Conroy, R.M., 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. *Journal of hazardous materials*, 235-236, 29-46.

McGuigan, K.G., Joyce, T.M., Conroy, R.M., Gillespie, J.B. & Elmore-Meegan, M. 1998. Solar disinfection of drinking water contained in transparent plastic bottles: characterizing the bacterial inactivation process. *Journal Applied Microbiology*, 84: 1138–1148.

Mpuchane, S.F, Ekosse, G., Gashe, B.A., Morobe, I. & Coetzee, S.H. 2010. Microbiological characterisation of Southern African medicinal and cosmetic clays. *International Journal of Environmental Health Research*, 20(1):27-41.

Mulaba-Bafubiandi, A.F. & Fosso-Kankeu, E. 2011. Indigenous microorganisms in littoral clayey soils and the possible route of human exposure. (In: GIE Ekosse, L de Jager & VM Ngole. eds. An Innovative Perspective on the Role of clays and clay minerals and Geophagia on Economic development. Book of Conference Proceeding of the 1st International Conference of Clays and Clay Minerals in Africa and 2nd International Conference on Geophagia in Southern Africa. South Africa: Lambert Academic Publishing. p. 124-130).

Narkeviciute, I., Rudzeviciene, O., Leviniene, G., Mociskiene, K. & Eidukevicius, R. 2002. Management of Lithuanian children's acute diarrhoea with Gastrolit solution and dioctahedral smectite. *European Journal of Gastroenterology and Hepatology*, 14: 419-424.

Navntoft, C., Ubomba-Jaswa, E., McGuigan, K.G. & Fernández-Ibañez, P. 2008. Effectiveness of solar disinfection using batch reactors with non-imaging aluminium reflectors under real conditions: natural well water and solar light. *Journal Photochemical and Photobiology B: Biology*, 93 (3): 155–161.

Nchito, M., Geissler, P.W., Mubila, L., Friis, H. & Olsen, A. 2004. Effects of iron and multi-micronutrient supplementation on geophagy: a two-by-two factorial study among Zambian schoolchildren in Lusaka. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 98(4): 218 – 227.

Ngole, V.M. 2015. Chemical characteristics of geophagic soils. (In: GE Ekosse, VM Ngole, L de Jager, E Fosso-Kankeu., ed. Human and enzootic geophagia: Ingested soils and practice. South Africa: Lambert Academic Publishing. p. 57-70).

Ngole-Jeme, V.M. & Ekosse, G-I.E. 2015. A Comparative Analyses of Granulometry, Mineral Composition and Major and Trace Element Concentrations in Soils Commonly Ingested by Humans. *International journal of environmental research and public health*, 12: 8933-8955.

Ngole, M.V., Ekosse, G.E., de Jager, L. & Songca, S.P. 2010. Physicochemical characteristics of geophagic clayey soils from South Africa and Swaziland. *African Journal of Biotechnology*, 9(36): 5929-5937.

Nyanza, E.C., Joseph, M., Premji, S.S., Thomas, D.S. & Mannion, C. 2014. Geophagy practices and the content of chemical elements in the soil eaten by pregnant women in artisana and small scale gold mining communities in Tanzania. *BMC Pregnancy Childbirth*, 14 (1): 1.

Otto, C.C. & Haydel, S.E. 2013. Microbial clays: composition, activity, mechanisms of action and therapeutic applications. *Microbial pathogens and strategies for combating them: science, technology and education*, 2(4): 1169-1180.

Ozumba, U.C. & Ozumba, N. 2002. Patterns of helminth infection in the human gut at the University of Nigeria Teaching Hospital, Enugu, Nigeria. *Journal of Health Science*, 48: 263-268.

Padilla-Ortega, E., Leyva-Ramos, R. & Flores-Cano, J.V. 2013. Binary adsorption of heavy metals from aqueous solution onto natural clays. *Chemical Engineering Journal*, 225: 535–546.

Prusiner, S.B. 1998. The prion diseases. *Brain pathology*, 8:499-513.

Rathnayake IVN, Megharaj M, Bolan N, Naidu R. Tolerance of heavy metals by Gram positive soil bacteria. *Int J Civil Environ Eng*. 2010;2: 191–195.

Raven, K.P., Jain. A. & Loeppert, R.H. 1998. Arsenite and arsenate adsorption on ferrihydrite: kinetics, equilibrium and adsorption envelopes. *Environmental science technology*, 32: 344-349.

Reed, R.H., Bright Singh, I.S., Mani, S., Kanjur, R., Simon, N. & Mol, S. 2005. Making the most of a scarce resource – solar disinfection of drinking water in the arid zone of western Rajasthan. *Arid Lands Newsletter* 57 at: <http://ag.arizona.edu/OALS/ALN/aln57/reed.html>  
Date of access: 15 October 2016.

Reilly, C. & Henry, J. 2000. Why do humans consume soil? *Nutrition Bulletin*, 25: 141–144.

Reilly, C. & Henry, J. 2001. Geophagia: why do humans consume soil? *British Nutrition Foundation*, 25(2): 141–144.

Saathoff, E., Olsen, A., Kvalsvig, J.D. & Geissler, P.W. 2002. Geophagy and its association with geohelminth infection in rural schoolchildren from Northern KwaZulu Natal, South Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 96: 485-490.

Shinondo, C. J. & Mwikuma, G. 2009. Geophagy as a risk factor for helminth infections in pregnant women in Lusaka, Zambia. *Medical Journal of Zambia*, 1(35): 48-52.

Shivoga, W.A. & Moturi, W.N. 2009. Geophagia as a risk factor for diarrhoea. *Journal Infect Developing Countries*, 3(2): 94-98.

Šmejkalová, M., Mikanova, O. & Boruvka, L. 2003. Effects of heavy metal concentrations on biological activity of soil micro-organisms. *Plant soil environmental*, 49(7): 321–326.

Solar water disinfection. 2002. A guide for the application of SODIS. *SANDEC report*, 6(02):1-88.

Sommer, B., Marinõ, A., Solarte, Y., Salas, M.L., Dierolf, C. & Valiente, C. 1997. SODIS – an emerging water treatment process. *Journal Water Supply Res Technol.-Aqua*, 46 (3): 127–137.

Tano-Debrah, K. & Bruce-Baiden, G. 2010. Microbiological characterization of dry white clay, a pica element in Ghana. *Nature Science Report and Opinion*, 2(6): 77-81.

Tayie, F. 2004. Motivating factors and health issues. *African Journal of Food, Agriculture, Nutrition and Development*, 4 (1).

Tayie, F.A.K. & Lartey, A. 1999. Pica practice among pregnant Ghanaians: Relationship with infant birth-weight and maternal haemoglobin level. *Ghana Medical Journal*; 33:67-76.

Thompson, C.J.S. 1913. Terra sigillata, a famous medicament of ancient times: 17th International Medical Congress, section 23. *History of Medicine*, 23: 433-444.

Twenefour, D. 1999. A study of clay eating among lactating and pregnant women and associated motives and effects. Legon: University of Ghana. (Dissertation- B.Sc.).

Ubomba-Jaswa, E., Fernández-Ibañez, P., Navntoft, C., Polo-López, M. & McGuigan, K. G. 2010. Investigating the microbial inactivation efficiency of a 25L batch solar disinfection (SODIS) reactor enhanced with a compound parabolic collector (CPC) for household use. *Journal Chemistry Technology Biotechnology*, 85: 1028–1033.

USEPA (U.S. Environmental Protection Agency). 1996. Report: recent Developments for In Situ Treatment of Metals contaminated Soils. <https://www.epa.gov/remedytech/recent-developments-situ-treatment-metal-contaminated-soils> Date of access: 15 November 2015.

Vermeer, D.E. & Frate, D.A. 1979. Geophagia in rural Mississippi; Environmental and cultural context and nutritional implications. *Journal of Clinical Nutrition*, 32: 2129-2133.

Vermeer, D.E. & Ferrell, R.E. 1985. Nigerian geophagical clay: a traditional anti-diarrheal pharmaceutical. *Science*, 227: 634-636.

Von Garnier, C., Stünitz, H., Decker, M., Battegay, E. & Zeller, A. 2008. Pica and refractory iron deficiency anaemia: a case report. *Journal of Medical Case Reports*, 200 (82): 324.

Walker, D.C., Len, S.V. & Sheehan, B. 2004. Development and evaluation of a reflective solar disinfection pouch for the treatment of drinking water. *Applied and Environmental Microbiology*, 70 (4): 2545-2550.

Walker, A.R.P., Walker, B.F., Sookaria, F.I., Canaan, R.J. 1997. Pica. *Journal of health*, 997(117): 280-284.

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. *Journal Water SRT-Aqua*, 43: 154-169.

WHO (World Health Organization). 1993. Guidelines for drinking water quality. Geneva.

WHO (World Health Organization). 2008. World prevalence of anaemia. Geneva.

Wilson, M.J. 2003. Clay Mineralogical and Related Characteristics of Geophagic materials. *Journal of Chemical Ecology*, 29 (7): 1525–1547.

Wong, M.S., Bundy, D.A.P. & Golden, M.H.N. 1991. The rate of ingestion of *Ascaris lumbricoides* and *Trichuris trichiura* eggs and its relationship to infection in two children's homes in Jamaica. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 85: 89-91.

Woywodt, A. & Kiss, A. 1999. Perforation of the Sigmoid colon due to geophagia. *Archa Surgeon*, 134: 88-89.

Woywodt, A. & Kiss, A. 2002. Geophagia: The history of earth-eating. *Journal of the Royal Society of Medicine*, 95, 143 – 146.

Wuana, R.A. & Okieimen, F.E. 2011. Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation. *International Scholarly Research Network Ecology*, 2011: 1-20.

Ye, D., Kam, K., Sanou, F., Traore, S.S., Kambou, S., Yonoba, C., Dao, F. & Sawadogo, A. 2004. Intestinal obstruction and geophagia in a 14-year old child. *Archives de Pediatrie*, 11:461-462.

Young, S.L., Wilson, J.M., Miller, D. & Hillier, S. 2008. Toward a Comprehensive Approach to the Collection and Analysis of Pica Substance, with Emphasis on Geophagic Materials. *Plos one*, 3(9): 1 - 11.

Young, S.L., Wilson, J.M., Hillier, S., Delbos, E., Ali, S.M. & Stoltzfus, R.J. 2010. Differences and Commonalities in Physical, Chemical and Mineralogical Properties of Zanzibari Geophagic Soils. *Journal of Chemical Ecology*, 36: 129 – 140.

Zhang, M.K., Liu, Z.Y. & Wang, H. 2010. Use of single extraction methods to predict bioavailability of heavy metals in polluted soils to rice. *Communications in Soil Science and Plant Analysis*, 41(7): 820–831.

Zhu, H., Hart, C.A., Sales, D. & Roberts N.B. 2006. Bacterial killing in gastric juice – effect of pH and pepsin on *Escherichia coli* and *Helicobacter pylori*. *Journal of Medical Microbiology*, 55:1265– 1270.

Zaguralskaya, L.M. 1997. Microbiological monitoring of forest ecosystems in the northern taiga subzone in conditions of anthropogenic impact. *Lesovedenie*, 5: 3–12

# Chapter 3

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

This article was accepted and published by African Journal of Biotechnology and it was added in exact format in which it was published in journal named above.

### 3.1 Introduction

This paper innovates by considering for the first time solar treatment for the inactivation of microorganisms in geophagic clay. As microbial contamination is widespread, it is important to pre-treat geophagic clays before consumption in view of the alleviation of adverse effects related to contaminants present in the clay. Geophagists obtained their clays from the mining site or the markets where microbial contamination is likely to occur. This paper aims to establish the risk of microbial infection from clay's consumption and assesses the potential of solar irradiation as an effective sustainable method for the treatment of geophagic clay.

Samples were collected from the open markets and the respective sources (clay mining sites). Samples were analysed for chemical composition, mineralogical composition, moisture content and organic carbon. Samples were exposed to solar treatment simulated in a chamber for 8 h a day. High numbers of microorganisms were found in both source (mined) and open market clays, which also contain large amounts of organic carbon and moisture.

Results showed a reduction in the number of microorganisms when exposed to solar simulated irradiation for 2 weeks. Complete inactivation of microorganisms in Ikageng and Pheramindi samples was achieved after 2 weeks with decreasing water content. 16S rDNA sequencing showed the dominance of *Bacillus Subtilis*, *Paenibacillus cereus*, *Bacillus thuringiensis*, *Bacillus humi* and *Arthrobacterarilaitensis* in clay samples.

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

Fosso-Kankeu E<sup>1\*</sup>, Netshitanini TL<sup>1</sup>, Abia ALK<sup>2</sup>, Ubomba-Jaswa E<sup>3</sup>, Waanders FB<sup>1</sup>

<sup>1</sup>School of Chemical and Minerals Engineering, Faculty of Engineering, North-West University, PO Box 558, Potchefstroom 2531, South Africa

<sup>2</sup>Department of Environmental, Water and Earth Science, Tshwane University of Technology, 175 Nelson Mandela Drive, Pretoria 0001, South Africa

<sup>3</sup>Natural Resources and the Environment, CSIR, PO Box 395, Pretoria 0001, South Africa

## **Abstract**

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 the 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. 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.

**Keywords:** 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 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) have 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 parasites geohelminths. Cases of people infected with geohelminths such *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; this may have also contributed 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). Solar disinfection (SODIS) is currently applied for water treatment by millions of people around the world and allows reducing outbreaks related to waterborne diseases in developing countries (Conroy

*et al.*, 1996 McGuigan *et al.*, 1998; Conroy *et al.*, 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) (Wegelin *et 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 on 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 for reflectors to enhance the optical inactivation 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; McGuigan *et 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) and UV-A radiation reaches the surface of the earth (Solar water disinfection, 2002). 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.

In this study the survivals of indigenous microorganisms as well as the contribution of handling on the microbial contamination of geophagic clays were investigated; for the first time solar treatment was considered for the disinfection of geophagic clays.

## **Methodology**

### **Study area**

The study was conducted in two locations of the Gauteng province and the North-West province. 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. The markets 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).

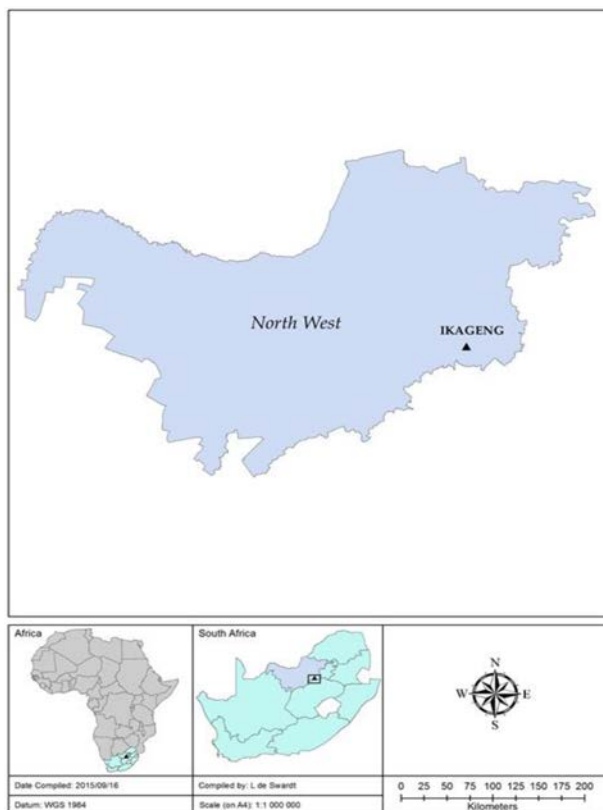


**Figure 1:** Sampling location in two provinces of South Africa

## **Sample collection**

Geophagic clay samples were purchased from two (2) different daily markets in Pretoria and Potchefstroom and others 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 vendor's 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 were in company of the vendors who assisted to localize 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 in the laboratory for analysis.

## **DNA extraction**

Samples from the market and mining sites were grounded and homogenized into fine powders using mortar and pestle. Genomic DNA was extracted from  $\pm 250$  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).

## **Soil moisture**

The samples were weighed ("wet weight") and then placed in a thermostatically controlled oven. Samples were dried at a temperature of  $100^{\circ}\text{C} \pm 5^{\circ}\text{C}$  for 24 hours. The samples were then weighed again ("dry weight") then their soil moisture percentage could be calculated according to the following equation:

$$\%W = \frac{W_1 - W_2}{W_1}$$

Where:

%W = Percentage of the moisture in the sample

W1 = Initial weight of the sample

W2 = Weight of dried sample

### Carbon analysis

Clay organic carbon content was determined according to the modified Walkley-Black chromic acid wet oxidation method: 1g of dried clay (ground to < 60 mesh) was mixed with 10 ml 1N K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> in a 250 ml Erlenmeyer flask, then 20 ml of concentrated H<sub>2</sub>SO<sub>4</sub> 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 200ml with distilled water after 30 minutes and four drops of Ferroin was added then titrated with 0.4 N FeSO<sub>4</sub> till an end point at which the organic content could be calculated.

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

$$\%C = \frac{0.003g \times N \times 10 \text{ ml} \times \left(1 - \frac{T}{S}\right) \times 100}{ODW}$$

Where N is the normality of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> solution, T is the volume of FeSO<sub>4</sub> used in sample titration (mL), S is the volume of FeSO<sub>4</sub> 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 a wavelength in the range 315 - 400 nm; to deliver the power within the visible spectrum a LED is 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 was fitted at the back of the chamber and linked to the laptop for instant recording of the temperature in the photocatalytic chamber.

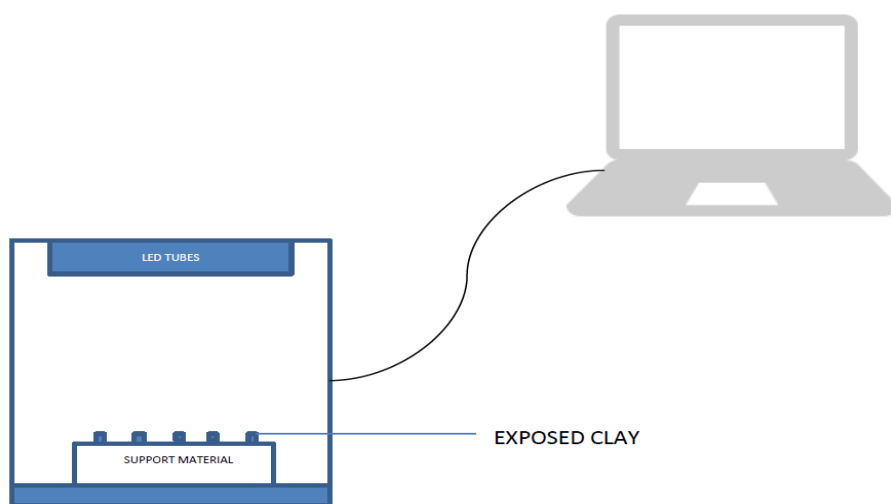


**Figure 2:** Schematic diagram of the solar treatment reactor system

Clay samples were prepared by careful cutting into square pieces of 2 cm x 2 cm with a thickness of 0.3 cm. Solar treatment was applied by exposing the cut clay samples to UV-A lights for 8 hours a day in the photocatalytic chamber linked to the laptop. The exposure time was 1 week, 2 weeks and four weeks. After the solar treatment, the clay samples were grinded 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  $\mu$ l of the suspension was inoculated to the freshly prepared Brilliance *E. coli*/coliform medium (Oxoid, SA) and incubated at 35°C for 48 hours; 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 hours 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  $\pm$  0.2 at 25°C; g per 1L; 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

### Bacterial diversity

The phylogenetic distribution results showed the amount of different phylum that were detected in all geophagic clay samples in Figure 3a. The phylum Firmicutes, Actinobacteria, and Proteobacteria were most abundant in almost all the geophagic clay samples. Actinobacteria constituting 49.49 % of total bacteria identified in Ikageng M sample and Firmicutes 12.59% of Pheramindi M sample respectively. Unclassified bacterial sequencing could not be linked to existing database and were recorded as unknown (unclassified) bacteria, it was found to be dominated in most of the geophagic clay samples with a significant number of the sequences; 97.73% for Pheramindi S sample, 96.89 % for Ikageng S sample, 95.7 % for Phelandavha S sample, 33.52% for Ikageng M sample, 37.56% for Pheramindi M sample and 90.43% for Phelandavha M sample respectively. The Proteobacteria, Firmicutes, Actinobacteria and Tracheophyta phylum were observed in all geophagic clay samples. Actinobacteria was the most abundant phylum in Ikageng M sample (49.49%). The other phylum Firmicutes, Tracheophyta and Chloroflexi were low on Ikageng M sample.

The most abundant class was unclassified class in most samples except Ikageng M sample and Pheramindi M sample, whereas Actinobacteria was the most abundant 48.99% on Ikageng M sample followed by 39.27% on Pheramindi M sample shown in Figure 3b. The other major classes detected Bacilli (7.71%) for Pheramindi M sample, gammaproteobacteria (4.15%) for Phelandavha M sample and Alphaproteobacteria (3.10%) for Ikageng M 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 most abundant on Ikageng M sample, followed by Bifidobacteriales (23.26%) of Pheramindi M sample, Laurales, Bacilliales and Rhizobiales. Lactobacillales, Bacillales, Costriidiales and Aconchulinida were all detectable at above 1% for sample Pheramindi M sample. All samples are dominated by the uncultured bacterium (95.04%) Phelandavha S sample, 96.30% Ikageng S sample, 96.79% Pheramindi S sample, 28.47% Ikageng M sample, 32.78% Pheramindi M sample, 89.41% Phelandavha M 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.

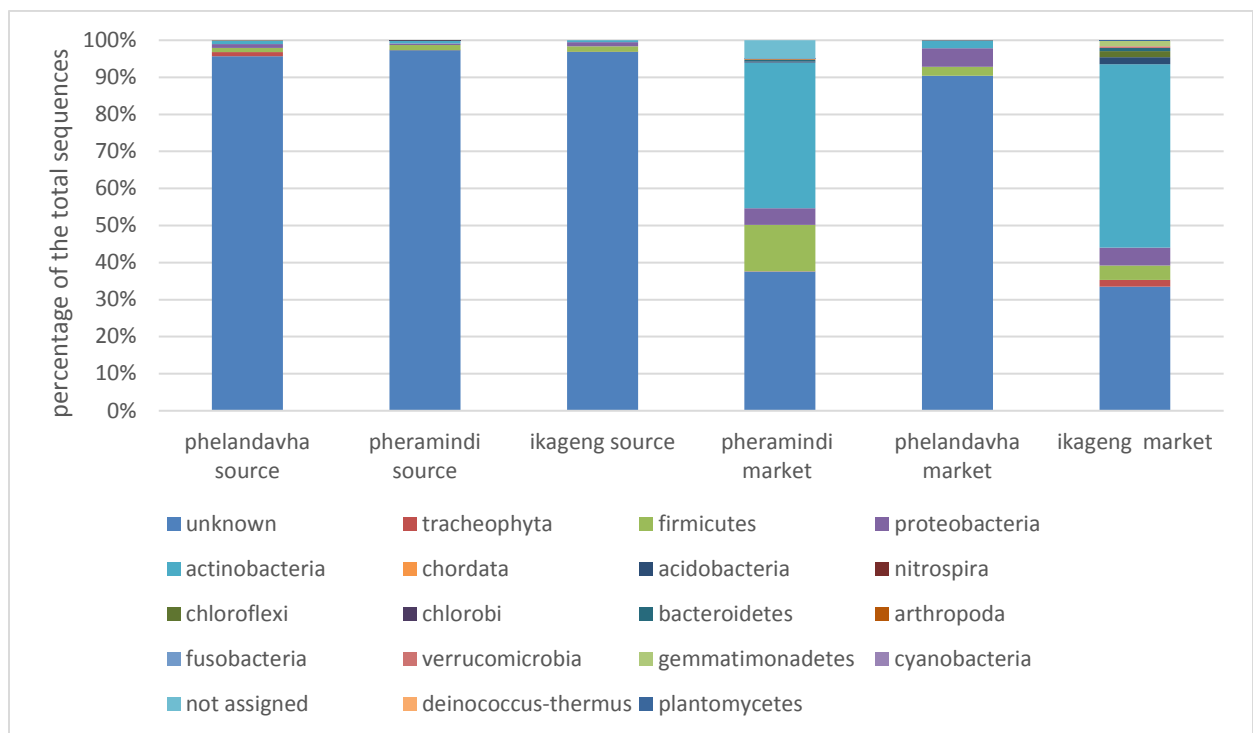


**Figure 3a:** Abundance and diversity of bacterial phylum detected on geophagic clay samples

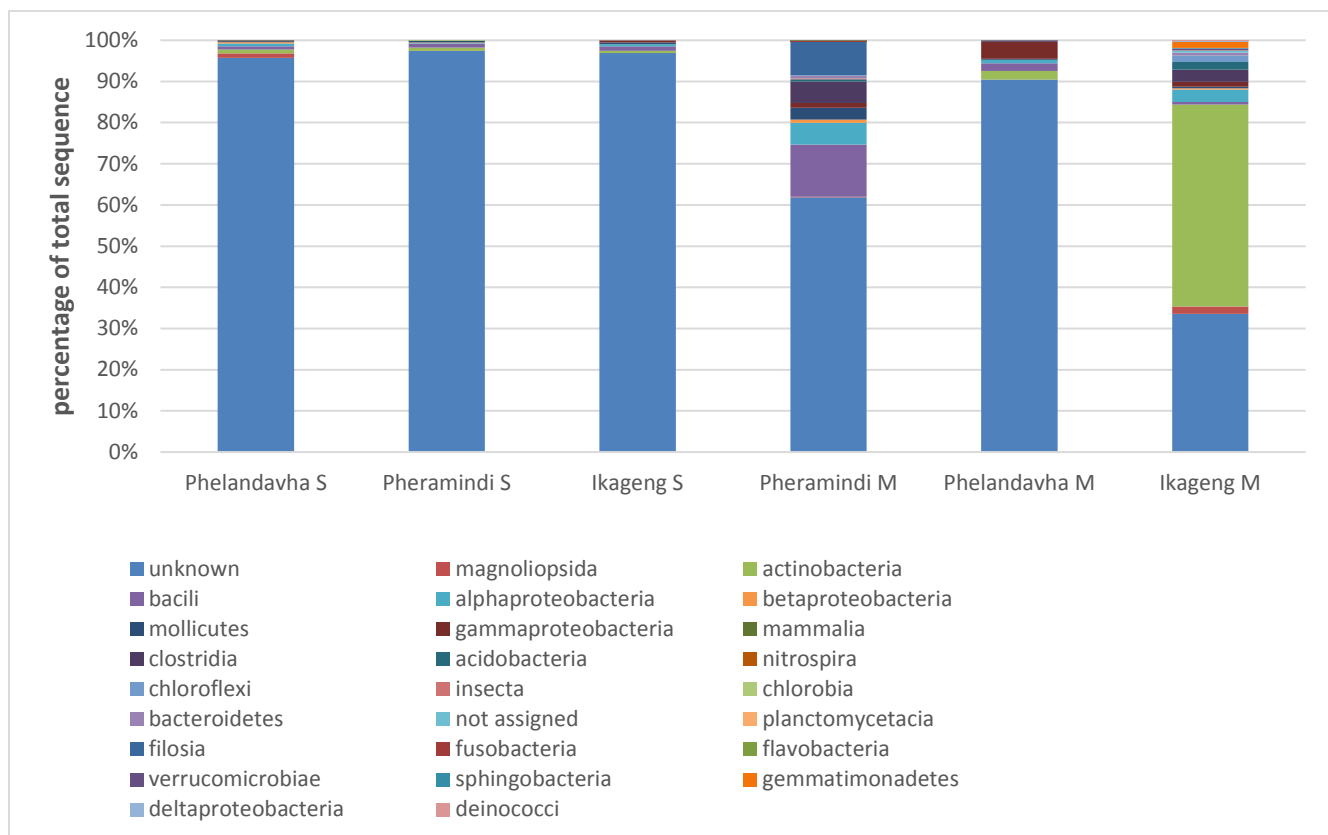


**Figure 3b:** Microbial classes' composition detected in geophagic clay samples

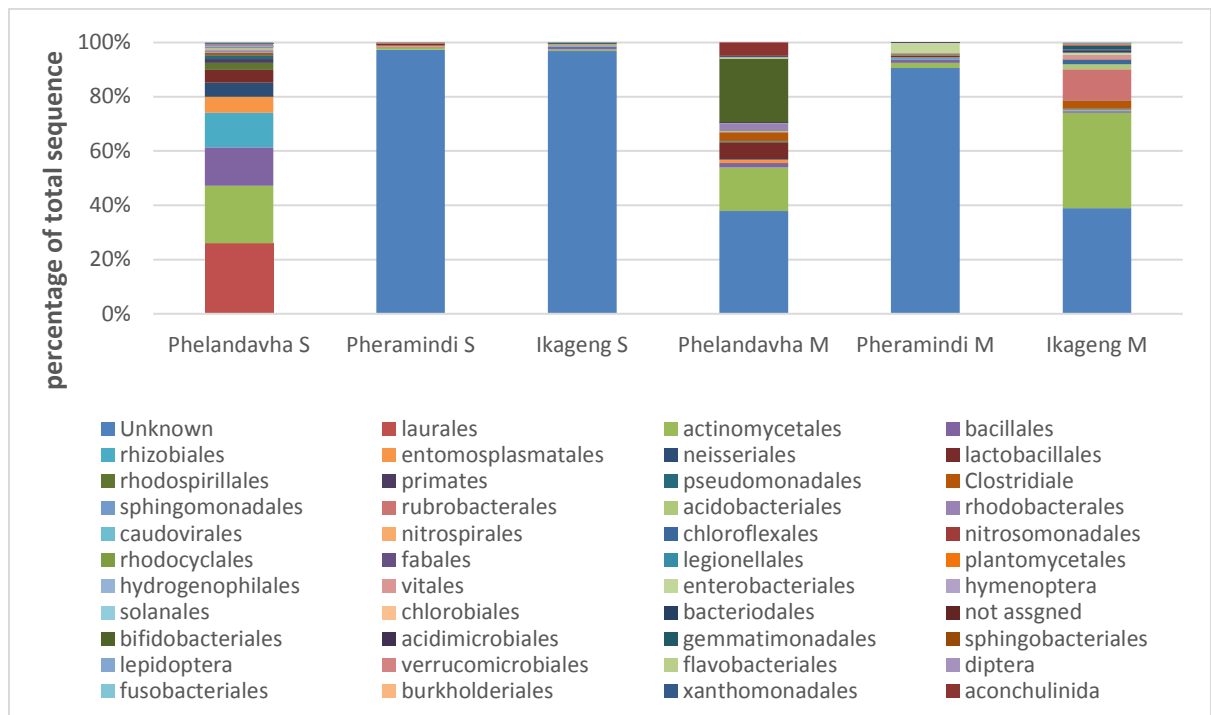


**Figure 3c:** Microbial orders composition detected in geophagic clay samples

### 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 1a, 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 mostly opportunistic microorganisms were identified in the samples, 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.

Table 1a : 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   | <i>Propionibacterium acnes</i>  | Acnes, chronic blepharitis and endophthalmitis                                                                | <i>Propionibacterium acnes</i> | Acnes, chronic blepharitis and endophthalmitis                                                                |
|               | <i>Spiroplasma sp.</i>          | Creutzfeldt-Jakob disease (CJD)                                                                               | <i>Pseudomonas sp</i>          | Pneumonia, Septic shock, Urinary tract infection, Gastrointestinal infection, Skin and soft tissue infections |
|               | <i>Streptococcus pneumoniae</i> | Pneumonia                                                                                                     | <i>Escherichia coli</i>        | Gastroenteritis, urinary tract infection, neonatal meningitis                                                 |
|               | <i>Pseudomonas aeruginosa</i>   | Pneumonia, Septic shock, Urinary tract infection, Gastrointestinal infection, Skin and soft tissue infections | <i>Spiroplasma sp</i>          | Creutzfeldt-Jakob disease (CJD)                                                                               |
|               | <i>alpha proteobacterium</i>    | Bacteraemia                                                                                                   | <i>Shigella sonnei</i>         | Dysentery                                                                                                     |
|               | <i>Escherichia coli</i>         | Gastroenteritis, urinary tract infection, neonatal meningitis                                                 | <i>Mycoplasma amphoriforme</i> | Bronchitis                                                                                                    |
|               | <i>Neisseria meningitidis</i>   | Meningitis                                                                                                    | <i>Klebsiella oxytoca</i>      | Colitis and sepsis                                                                                            |
|               | <i>Synechococcus sp.</i>        | Cyanotoxins                                                                                                   | <i>Mycobacterium avium</i>     | Lungs infection (Lady Windermere syndrome)                                                                    |

Table 1b : 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    | <i>Escherichia coli</i>         | Gastroenteritis, urinary tract infection, neonatal meningitis | <i>Spiroplasma sp</i>                   | Creutzfeldt-Jakob disease (CJD)                                 |
|               | <i>Spiroplasma sp.</i>          | Creutzfeldt-Jakob disease (CJD)                               | <i>Gemella haemolysans</i>              | Involved in pulmonary exacerbations of cystic fibrosis patients |
|               | <i>Propionibacterium acnes</i>  | Acnes, chronic blepharitis and endophthalmitis                | <i>Corynebacterium pseudogenitalium</i> | Urinary tract infection                                         |
|               | <i>Streptococcus agalactiae</i> | Neonatal sepsis                                               | <i>Fingoldia magna</i>                  | Septic arthritis                                                |
|               | <i>Streptococcus mitis</i>      | Endocarditis                                                  | <i>Peptoniphilus sp</i>                 | Soft tissue, bone and joints infection                          |
|               |                                 |                                                               | <i>Streptococcus salivarius</i>         | Sepsis                                                          |

|  |                                       |                                                                                                               |
|--|---------------------------------------|---------------------------------------------------------------------------------------------------------------|
|  | <i>Escherichia coli</i>               | Gastroenteritis, urinary tract infection, neonatal meningitis                                                 |
|  | <i>Streptococcus mitis</i>            | Endocarditis                                                                                                  |
|  | <i>Propionibacterium acnes</i>        | Acnes, chronic blepharitis and endophthalmitis                                                                |
|  | <i>Pseudomonas sp</i>                 | Pneumonia, Septic shock, Urinary tract infection, Gastrointestinal infection, Skin and soft tissue infections |
|  | <i>Staphylococcus sp.</i>             | Sialadenitis and other diseases                                                                               |
|  | <i>Leptotrichia sp.</i>               | Neutropenia                                                                                                   |
|  | <i>Abiotrophia para-<br/>adiacens</i> | Bacterial endocarditis                                                                                        |
|  | <i>Veillonella sp.</i>                | Osteomyelitis and endocarditis                                                                                |
|  | <i>Mycobacterium<br/>fortuitum</i>    | Skin infection and osteomyelitis                                                                              |
|  | <i>Citrobacter<br/>amalonaticus</i>   | Neonatal meningitis and gastroenteritis                                                                       |

Table 1c : Microorganisms occurring in geophagic clays from Ikageng and the potential health risks

| Sampling area | Mining site                     |                                                                                                               | Market                              |                                                                                                               |
|---------------|---------------------------------|---------------------------------------------------------------------------------------------------------------|-------------------------------------|---------------------------------------------------------------------------------------------------------------|
|               | Microorganisms                  | Potential disease                                                                                             | Microorganisms                      | Potential disease                                                                                             |
| Ikageng       | <i>Propionibacterium acnes</i>  | Acnes, chronic blepharitis and endophthalmitis                                                                | <i>Mycobacterium sp.</i>            | Tuberculosis                                                                                                  |
|               | <i>Pseudomonas aeruginosa</i>   | Pneumonia, Septic shock, Urinary tract infection, Gastrointestinal infection, Skin and soft tissue infections | <i>Spiroplasma sp.</i>              | Creutzfeldt-Jakob disease (CJD)                                                                               |
|               | <i>Spiroplasma sp.</i>          | Creutzfeldt-Jakob disease (CJD)                                                                               | <i>Tsukamurella sp.</i>             | Pneumonia                                                                                                     |
|               | <i>Escherichia coli</i>         | Gastroenteritis, urinary tract infection, neonatal meningitis                                                 | <i>Faecalibacterium prausnitzii</i> | Crohn's disease and obesity                                                                                   |
|               | <i>Citrobacter amalonaticus</i> | Neonatal meningitis and gastroenteritis                                                                       | <i>Propionibacterium acnes</i>      | Acnes, chronic blepharitis and endophthalmitis                                                                |
|               |                                 |                                                                                                               | <i>Escherichia coli</i>             | Gastroenteritis, urinary tract infection, neonatal meningitis                                                 |
|               |                                 |                                                                                                               | <i>Klebsiella oxytoca</i>           | Colitis and sepsis                                                                                            |
|               |                                 |                                                                                                               | <i>Pseudomonas aeruginosa</i>       | Pneumonia, Septic shock, Urinary tract infection, Gastrointestinal infection, Skin and soft tissue infections |
|               |                                 |                                                                                                               | <i>Shigella dysenteriae</i>         | Dysentery                                                                                                     |
|               |                                 |                                                                                                               |                                     |                                                                                                               |

## Inactivation of microorganisms by solar treatment

Exposition of geophagic clays to simulated solar treatment was considered in this study to inhibit the microorganisms. The results in Figure 4 show a general trend as of the number of microorganisms in most of the clays decrease with an increase of exposure time. However, for the samples obtained from the market in Pheramindi, an increase of the number of microorganisms was observed. The effectiveness of bacterial decontamination 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 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 occurs. 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 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 *Arthrobacter arilaitensis* which are all Gram-positive bacteria.

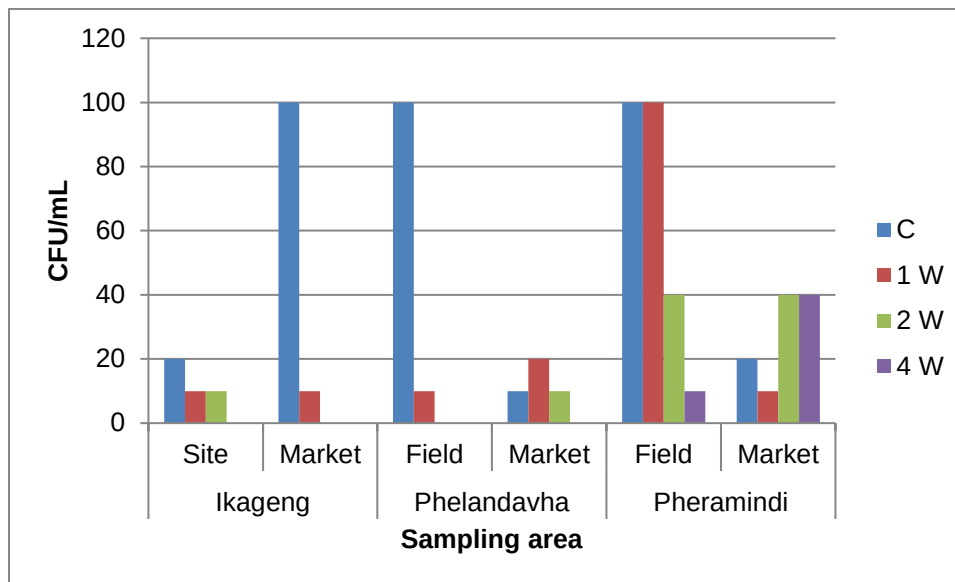


**Figure 4:** Effect of solar treatment on the microorganisms in geophagic clays

#### **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 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 also implies 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 exposure.

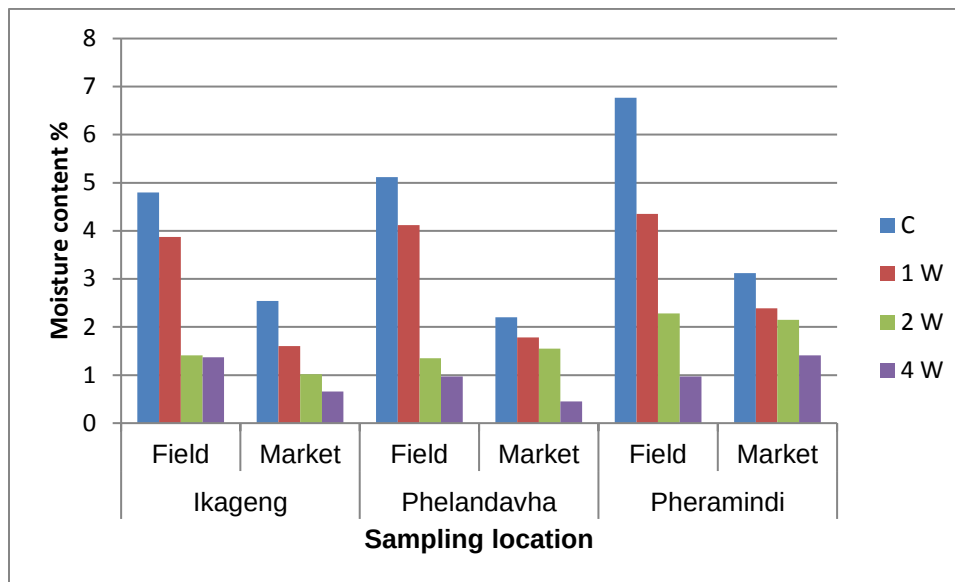


**Figure 5:** Variation of moisture content during solar treatment

### Organic carbon in the geophagic clays

Nutrients requirement vary amount bacteria which to survive needs energy and organic carbon that will be converted to new cells. The content of organic matters 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 2. 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.

**Table 2:** Percentage of organic carbon in the geophagic clays

| Sampling area |        | Clay organic carbon content<br>% |
|---------------|--------|----------------------------------|
| Ikageng       | Field  | 1.26                             |
|               | Market | 1.33                             |
| Phelandavha   | Field  | 1.06                             |
|               | Market | 1.33                             |
| Pheramindi    | Field  | 1.33                             |
|               | Market | 1.5                              |

## 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 photooxidative process which mostly result 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 relatively increase of temperature play 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 most of the pathogenic microorganisms are destroyed during exposure to sun light.

## **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 and Mr N. Lemmer are really appreciated.

## **References**

Bisi-Johnson MA, Obi CL and Ekosse G-I. 2015. Microbiological Underpinnings of Geophagia and Geophagic materials. 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.

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, Ngole-Jeme 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.

Fosso-Kankeu E, Mulaba-Bafubiandi AF. 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 rhizosphere ecosystems. *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, pp. 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. of hazardous materials. 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, et al. 1993. The chemoprophylaxis and chemotherapy of opportunistic infections. *Antibiotiki i Khimioterapiia*. 38, 44-48.

Nchito M, Geissler PW, Mubila L, Friss H, Olsen A. 2004. Effects of iron and multivitamin supplementation on geophagy: a two-by-two factorial study among Zambian schoolchildren in Lusaka. Royal Society of Tropical Medicine Hygiene 98 (4), 218-227.

Ngole-Jeme VM and 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.

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. Microbial Ecology. 12(2): 217-230.

Smirnov W, Reznik SR, V'iunitskaia VA, Sorokulova IB, Samgorodskaja NV and 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 and Oluwafemi O. 2015. Human and enzootic geophagic soils-Potential Chemical and Biochemical Implications. 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.

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. J Water SRT-Aqua 43, 154-169.

Wong MS, Bundy DAP, Golden MHN. 1991. The rate of ingestion of *Ascaris lumbricoides* and *Trichuris trichiura* eggs and its relationship to infection in two children's homes in Jamaica. Trans. R. Soc. Trop. Med. Hyg. 85, 89-91.

# Chapter 4

**Co-effect of leached metals and pH of simulated gastric fluid on the survival of microorganisms in geophagic clays.**

This article was accepted and published by International Journal of Science  
and Research

## 4.1 Introduction

This paper describes the effect of metals released from geophagic clay in the acidic environment of simulated gastric fluid at a pH value of 2 on the microorganisms in the geophagic clay. Metal and microbe contamination of soil originate from soil and open markets. Clay samples were collected from open markets and source sites. Geophagic clay materials were exposed to simulated gastric fluid to simulate conditions in human gastric system; and the susceptibility of microorganisms under such conditions assessed using plate counting. The objective of the study was to determine co-effect of leached metals and pH on the microorganisms contained in the geophagic clays when the soil is ingested.

The concentrations of metals leached from geophagic clays were high, especially lead (Pb), nickel (Ni), copper (Cu) and Iron (Fe) at low pH. Initially the geophagic clays were contaminated with large amounts of microorganisms. At low pH large amounts of metals were released and microorganisms growths were inhibited. This could be beneficial or harmful to the health of the geophagists. Further investigation is required to correlate metals released with human health.

# Co-effect of leached metals and pH of simulated gastric fluid on the survival of microorganisms in geophagic clays

Netshitanini T.L.<sup>1</sup>, Fosso-Kankeu E<sup>1\*</sup>, Waanders F.B<sup>1</sup>, Ubomba-Jaswa E<sup>2</sup> and Abia A.L.K<sup>3</sup>

School of Chemical and Minerals Engineering, Faculty of Engineering, North-West University, PO Box 558, Potchefstroom 2531, South Africa

Natural Resources and the Environment, CSIR, PO Box 395, Pretoria 0001, South Africa

Department of Environmental, Water and Earth Science, Tshwane University of Technology, 175 Nelson Mandela Drive, Pretoria 0001, South Africa

## Abstract

In this study, the bacterial activity was investigated following exposure of geophagic clays to simulated gastric fluid. The inhibitory effects of either acidity or metals released were considered. The geophagic clay samples were characterized using XRD and XRF techniques; the bacteria were identified using 16S rDNA sequencing and their survival in simulated gastric overtime was monitored using plate count. The results showed that heavy metals were released in the gastric fluid overtime while the pH remained acid over the same period of time. The mobility of the metals from the geophagic clays decreased in the order Pb>Ni>Co>Fe>Cr>Cu>Zn. The count of cell number under such conditions showed that both acidity and metal concentrations had contributed to the decrease of cell number in the simulated gastric fluid. Total metal concentration increase from 25 to 35 mg/L contributed to a 50% decrease of bacterial number after 150 min; a decrease of bacteria count overtime was observed in samples containing mostly Gram-negative bacteria; while no significant change was observed in samples dominated by Gram-positive bacteria. The results have shown the decrease of bacterial activity and increase of metals released at low pH. The acidity inhibits the growth of bacteria and also increases metal release which further enhances bacteria inhibition.

**Keywords:** Geophagic clay, heavy metals, micro-organisms, simulated gastric fluid, health risk

## Introduction

The consumption of non-food materials is generally called pica and there are different forms of pica, examples of pica as described in [1] include geophagia (dirt or clay eating) and pagophagia (ice eating). Geophagia is the most encountered form of pica, which is defined as the deliberate consumption of soil and related materials [2]. It is a very extensive practice in all continents of the world, and has been reported in Africa, Asia, South America, North America [3, 4]. Geophagia has been reported commonly in pregnant women and children [5]. Soil ingestion can be anything, however, eating dropped food, contacting dirty hands and mouthing cannot be considered as geophagia since they are accidental. Study has reported the prevalence of pica among urban and rural South Africa women to be 38.3% and 44% respectively compared with 2.2% and 1/6% among Indians and white women respectively [6]. Geophagic individuals believe that geophagia provides health benefits as supplementation of mineral and nutrients, ailment of diarrhea, reduce nausea and reduce pregnant sickness [7; 8; 9; 10]. The consumption of soil and clay may cause mineral deficiency [5, 11] because geophagic materials are capable to adsorb elements such as iron [12]. Geophagia is associated with dirt because of the natural source of the clay [13]. Geophagia clays could be contaminated with microorganisms, metals and other contaminants from collection sites or resulting from exposure to the polluted atmosphere at the markets. Studies have shown that the geophagic clays contained harmful materials such as heavy metals, pathogenic bacteria, viruses and parasites [7, 13]. The regular ingestion of highly contaminated soil could result in brain and renal system impairment in the unborn baby due to lead poisoning [14]; apart from lead poisoning other toxicities have been observed in children eating contaminated soils [15]. Several studies have linked geohelminths infection with soil consumption, for the eggs of parasitic worm (geohelminths) consumed with soil [2; 16] and moreover, geohelminths infection has also been linked to iron deficiency among HIV-infected women who consumed soil [17]. Studies have shown that geophagic pregnant women from Kenya are at risk for geohelminth infection and especially *A. lumbricoides* re-infection [18, 19]. Bacteria tend to thrive in the environment where they can either get nutrients or be away from the predators [13] and can survive under harsh conditions [20]. For the bacteria to survive in any environment they need nutrients, energy and organic carbon that will be converted to new cells [13]. Many microbes can enter the stomach, but some of them do not survive the low pH acidity of the stomach in human. In the study of [21], micro-organisms *Klebsiella*, *Salmonella*, *Shigella flexneri*, *Proteus*, *Enterobacter*, *Enterococcus faecalis*, *Enterococcus faecium*, *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Candida albicans*, did not survive pretreatment at

pH 1.0 or 2.0, whereas at pH 4.0, all bacteria tested survived. *Helicobacter pylori*, and *Clostridium botulinum* are microbes that can survive the acidic environment of the stomach of the host [21]. Microorganisms in ingested clays are exposed to adverse conditions in the stomach such as acidity and toxic metals leached from the clays; When heavy metals are present in excessive concentration they can cause toxic effect in the microorganisms [22]. When the soil ingested goes through the mouth being chewed or smoothed and then to the gastrointestinal tract there are so many possible reactions that happen. It is however uncertain what could be the combined effect of acidity and metal toxicity on the load of microorganisms in geophagic clays ingested. In this study, there is an attempt to predict the survival of microorganisms following concurrent exposure to acidic gastric fluid and leached metals.

## **Methodology**

### *Materials*

Samples were purchased from markets in Pretoria and Potchefstroom and mined from the respective sources. The collected clay samples were packed into sterile plastic bags. The bags were sealed and labelled and then transported to the laboratory for analysis. Samples were kept in the refrigerator at 4°C until analyzed.

### *DNA sequence*

Samples from the market and mining sites were ground and homogenized into fine powders using mortar and pestle. Genomic DNA was extracted from  $\pm 250$  mg of each of the six 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 a commercial biotechnical company for sequencing using Next Generation Sequencing (NGS).

### *Leaching of metals using simulated gastric fluid*

The simulated gastric fluid was prepared by dissolving 60.06 g glycine in 1.9 L of deionized water and adjusting the pH to 1.54 by adding concentrated HCl. The solution was then brought to a volume of 2 L and incubated at 37°C for 30, 60, 90, 120 and 150 mins. Hundred milliliter (100 mL) of the simulated gastric fluid was added into a bottle containing 1 g of clay sample, the mixture was placed in an incubator with shaker set at a speed of 120 rpm at

37°C. Subsequently, 5 mL samples were collected at 30, 60, 90, 120 and 150 mins intervals using a 45 µm nitrocellulose syringe filter. The pH of the mixture was simultaneously determined at each sampling period. The filtered solution was diluted by adding 15 mL of deionized water prior to analysis. The metal concentrations in the leachates were then determined using an inductively coupled plasma optical emission spectrometer (ICP-OES).

#### *Characterization of clay*

Mineralogical studies of the geophagic clays were carried out using an X-ray diffractometer (XRD). The samples were ground and homogenized to a fine powder and small amounts of powdered samples were loaded on sample holders and mounted in the Philips PW 3710 XRD X-ray diffractometer system for identification of the mineral phases. The XRD equipment, which operated at 40 kV and 45 mA, was equipped with a Cu-K $\alpha$  radiation tube and a graphite monochromator. Samples were scanned at a speed of 1°2 $\theta$ /min; at a covering range of 2°2 $\theta$  to 70°2 $\theta$ . A PW 1877 automated powder diffraction (APD). X'PERT Data Collector software package was employed to capture raw data and a Philips X'PERT Graphics and Identity software package was used for qualitative identification and semi-quantitative analyses of the minerals. Concentrations of major and trace metals was determined using an X-ray fluorescent spectrophotometer.

#### *Microbial analysis*

To determine the subsistence of microorganisms in the simulated gastric fluid, 1 mL of solution was collected from the mixture of clay samples and simulated gastric fluid after 30, 60, 90, 120 and 150 mins then aseptically transferred into a test tube containing 9 mL of sterile distilled water and homogenized using a vortex. Serial dilution was done up to 10<sup>-5</sup> dilution. Hundred µL of the diluted samples as well as the undiluted samples 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 color or shape were counted and expressed as CFU/mL. Individual colonies were sub-cultured in the fresh Brilliance *E. coli*/coliform medium (Oxoid, SA) and incubated at 35°C for another 48 h and then finally sub-cultured 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  $\pm$  0.2 at 25°C; gram per one liter; Merck Chemicals, SA) under the above conditions. The isolated cultures were sent to a commercial biotechnical laboratory for the identification of microorganisms through the sequencing of the 16S rDNA.

## Results and discussion

### *Chemical composition*

Results obtained for the major oxides (Table 1) show the average  $\text{SiO}_2$  values ranged from 15.56% - 55.62% with the highest value recorded from samples collected at Ikageng source (sample A) while the samples from Pheramindi source (sample E) exhibited the lowest value. The concentrations of  $\text{Al}_2\text{O}_3$  ranged from 5.17% - 33.15% in the clay samples. Oxides of Si, Al, and Fe made up the greatest percentage of major element oxides in all the geophagic clay samples (Table 1). Results presented in Table 1 indicate that the concentration of  $\text{Al}_2\text{O}_3$  was lower in sample E than other samples. The concentrations of oxides of Ca, Mg, and Na, were relatively low in all the geophagic clay samples and were less than 0.6%. Low abundance of MgO and  $\text{K}_2\text{O}$  shows a lack of expandable clays [7]. A high ratio of  $\text{SiO}_2/\text{Al}_2\text{O}_3$  is related to the quartz content of the sample.

**Table 1:** Results of major oxides (mass %) analysis of samples

| Oxide<br>concentration<br>(wt %) | Samples           |                   |                        |                        |                       |                       |
|----------------------------------|-------------------|-------------------|------------------------|------------------------|-----------------------|-----------------------|
|                                  | Ikageng<br>source | Ikageng<br>market | Phelandavh<br>a source | Phelandavh<br>a Market | Pheramin<br>di source | Pheramin<br>di market |
| $\text{SiO}_2$                   | 55.62             | 55.37             | 51.79                  | 47.83                  | 15.57                 | 50.72                 |
| $\text{TiO}_2$                   | 1.04              | 0.96              | 0.89                   | 0.92                   | 0.076                 | 5.76                  |
| $\text{Al}_2\text{O}_3$          | 26.07             | 25.55             | 28.32                  | 29.56                  | 5.18                  | 33.15                 |
| $\text{Fe}_2\text{O}_3$          | 12.6              | 13.45             | 15.23                  | 18.55                  | 58.35                 | 9.03                  |
| MnO                              | 0.008             | 0.01              | 0.1                    | 0.03                   | 0.012                 | 0.04                  |
| MgO                              | 0.41              | 0.39              | 0.45                   | 0.21                   | n.d                   | 0.21                  |
| CaO                              | 0.12              | 0.11              | 0.02                   | 0.01                   | 0.17                  | 0.09                  |
| $\text{Na}_2\text{O}$            | 0.53              | 0.52              | 0.19                   | 0.2                    | 0.5                   | 0.06                  |
| $\text{K}_2\text{O}$             | 3.13              | 3.02              | 2.5                    | 1.98                   | 0.74                  | 0.46                  |
| $\text{P}_2\text{O}_5$           | 0.11              | 0.13              | 0.24                   | 0.31                   | 0.08                  | 0.11                  |
| $\text{Cr}_2\text{O}_3$          | 0.03              | 0.03              | 0.04                   | 0.03                   | 0.25                  | 0.08                  |
| $\text{Co}_2\text{O}_3$          | 0                 | 0                 | 0.01                   | 0.013                  | 0.044                 | 0                     |
| NiO                              | 0.009             | 0.009             | 0.009                  | 0.011                  | 5.5                   | 0.01                  |
| CuO                              | 0.006             | 0.008             | 0.013                  | 0.009                  | 0.011                 | 0.01                  |
| ZnO                              | 0                 | 0                 | 0.008                  | 0.008                  | 0                     | 0.007                 |
| PbO                              | 0                 | 0                 | 0                      | 0                      | 1.12                  | 0.01                  |

### *Mineralogical studies of clays*

X-ray diffraction patterns were obtained for representative geophagic clay samples (Table 2). The most abundant of the non-clay minerals was quartz (Ikageng samples and Phelandavha samples) and corresponded closely with SiO<sub>2</sub> values from major trace elements and oxides results. The sample from Pheramindi source contained the lowest amounts of quartz. The results show that kaolinite is a dominant clay mineral in the geophagic clay samples (except the samples from Ikageng). Kaolin minerals are used as medicines to treat diarrhea [2]. Others minerals in low abundance present in the samples were muscovite, gypsum, anatase and illite. The presence bentonite minerals in the sample from Ikageng market and not in the sample from the source might be due to contamination.

**Table 2:** XRD results on geophagic clays

| Sample ID          | Minerals |           |           |           |
|--------------------|----------|-----------|-----------|-----------|
|                    | Quartz   | Kaolinite | Muscovite | Bentonite |
| Ikageng source     | X        |           | X         |           |
| Ikageng market     | X        |           | X         | X         |
| Phelandavha source | X        | X         |           |           |
| Phelandavha market | X        | X         |           |           |
| Pheramindi source  | X        | X         |           |           |
| Pheramindi market  | X        | X         |           |           |

### *pH of extraction fluid over specified time*

The mobility of metals can be affected by certain factors such as pH, soil physical properties, mineralogical and chemical composition and fluid composition [12]. However, pH is the most essential parameter affecting the mobility of heavy metal in the soil. Geophagic clays have chemical properties which include pH, cation exchange and ion exchange which affect the interaction in the gastrointestinal tract [23]. Constant shaking was used in the present experiments to simulate the movement within the stomach. After two and a half hours, there was no difference in the pH of the mixture compared with the initial pH of the extractive fluid (Table 3). The noticeable change of pH observed in the leachate was from pH 1.32 to 1.67, which could be ascribed to the electrostatic neutralization of protons in solution by the negatively charged groups in the clays [24]. When there is a decrease in soil pH, metal solubility and availability increases [12]. The variation

of the pH of the extracted fluid overtime was inconsistent and negligible (Table 3). The change of pH could be also explained by the mineralogical composition of clay.

**Table 3:** pH variation during exposure of geophagic clays to simulated gastric fluid

| Samples ID | Time (minutes) |        |        |        |         |         |
|------------|----------------|--------|--------|--------|---------|---------|
|            | Initial pH     | 30 min | 60 min | 90 min | 120 min | 150 min |
| M1A        | 1.39           | 1.66   | 1.67   | 1.55   | 1.55    | 1.56    |
| M2B        | 1.39           | 1.53   | 1.65   | 1.59   | 1.56    | 1.57    |
| S1A        | 1.46           | 1.5    | 1.58   | 1.51   | 1.5     | 1.48    |
| S2B        | 1.48           | 1.49   | 1.56   | 1.48   | 1.49    | 1.56    |
| I1A        | 1.35           | 1.39   | 1.36   | 1.4    | 1.34    | 1.35    |
| I2B        | 1.37           | 1.38   | 1.36   | 1.38   | 1.39    | 1.4     |
| SI1A       | 1.41           | 1.44   | 1.38   | 1.37   | 1.34    | 1.43    |
| SI2B       | 1.4            | 1.41   | 1.42   | 1.41   | 1.42    | 1.42    |
| P1A        | 1.44           | 1.44   | 1.45   | 1.44   | 1.4     | 1.39    |
| P2B        | 1.45           | 1.4    | 1.44   | 1.47   | 1.43    | 1.44    |
| SP1A       | 1.36           | 1.4    | 1.41   | 1.39   | 1.41    | 1.4     |
| SP2B       | 1.34           | 1.34   | 1.42   | 1.42   | 1.43    | 1.48    |

Results are expressed as mean  $\pm$ SE (n=2). I1A=Ikageng market; SI1A= Ikageng source; M1A=Phelandavha market; S1A= Phelandavha source P1A=Pheramindi market; SP1A=Phelandavha source

#### *Dissolution of geophagic clays in simulated gastric fluid and subsequent behavior of metals*

Previous studies [25, 26] showed that metal bio-accessibility under low pH (acidic conditions) is consistent with an increased metal mobility in soils at low pH. Metals such as Pb, Ni, Co and Fe showed a high bio-accessibility with an increased mobility. The mobility of the metals was at a different pace during the leaching period and decreased in the order Pb>Ni> Co>Fe>Cr>Cu>Zn Zn (Fig 1a, 1b,1c, 1d, 2a, 2b,2c, 2d, 3a, 3b, 3c and 3b).

At the solid-liquid 2:100 higher concentration of metals compare to the solid-liquid 1:100 where there was the low amount of metals leached. For example, Fe concentration ranged from 2.44 to 6.04 mg/L at the solid-liquid 1:100, while an increase of the amount of Fe leached (4.85- 13.75 mg/L) was recorded at the solid-liquid 2:100 from the sample collected at Pheramindi field. The solid-liquid ratio therefore has a significant effect on the quantity of metal release. The average amount reported consumed by the pregnant women is 20g/day [27]. It ensues from the above that a once off ingestion of large amount of geophagic clays may expose the consumer to relatively high concentration of heavy metals.

Metals were released in various concentrations at different time periods of the leaching experiment. In all samples, Iron (Fe) was released in higher amounts as time increased. The effect of time on the release of metals from the geophagic clays shows that there is a relative increase in the release over time (Figures 1, 2 and 3). Most metals were released from the first 30 minutes while Cr was initially released after an hour in the Pheramindi samples.

Higher metals concentrations were released from the samples collected in Phelandavha (1A) and Pheramindi (P1A) as compared to the samples collected from Ikageng (I1A). For example, the average concentration of Fe and Cu released from 1A was 5.13 mg/L and 0.51 mg/L respectively, while from P1A the average concentration was 5.20 mg/L and 0.60 mg/L for Fe and Cu respectively. Comparatively lower amounts of metal was released from the I1A; this was likely due to the presence of bentonite clay which has a high binding affinity for the metals and tends to immobilize the metals.

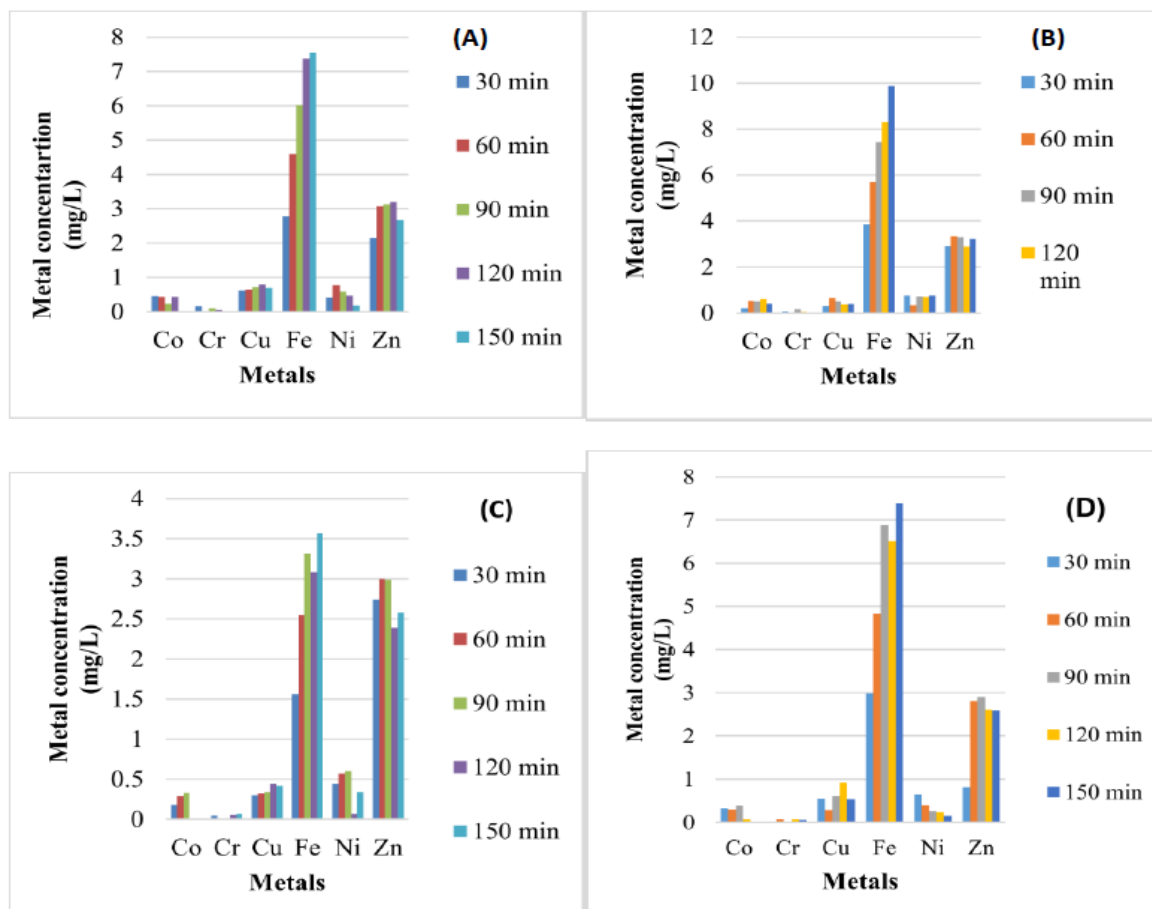


**Figure 1:** Amount of metal (mg/L) leached from the geophagic clays of Phelandavha: (A) Market sample S/L of 1/100; (B) Market sample S/L of 2/100; (C) Source sample S/L of 1/100; (D) Source sample S/L of 2/100.

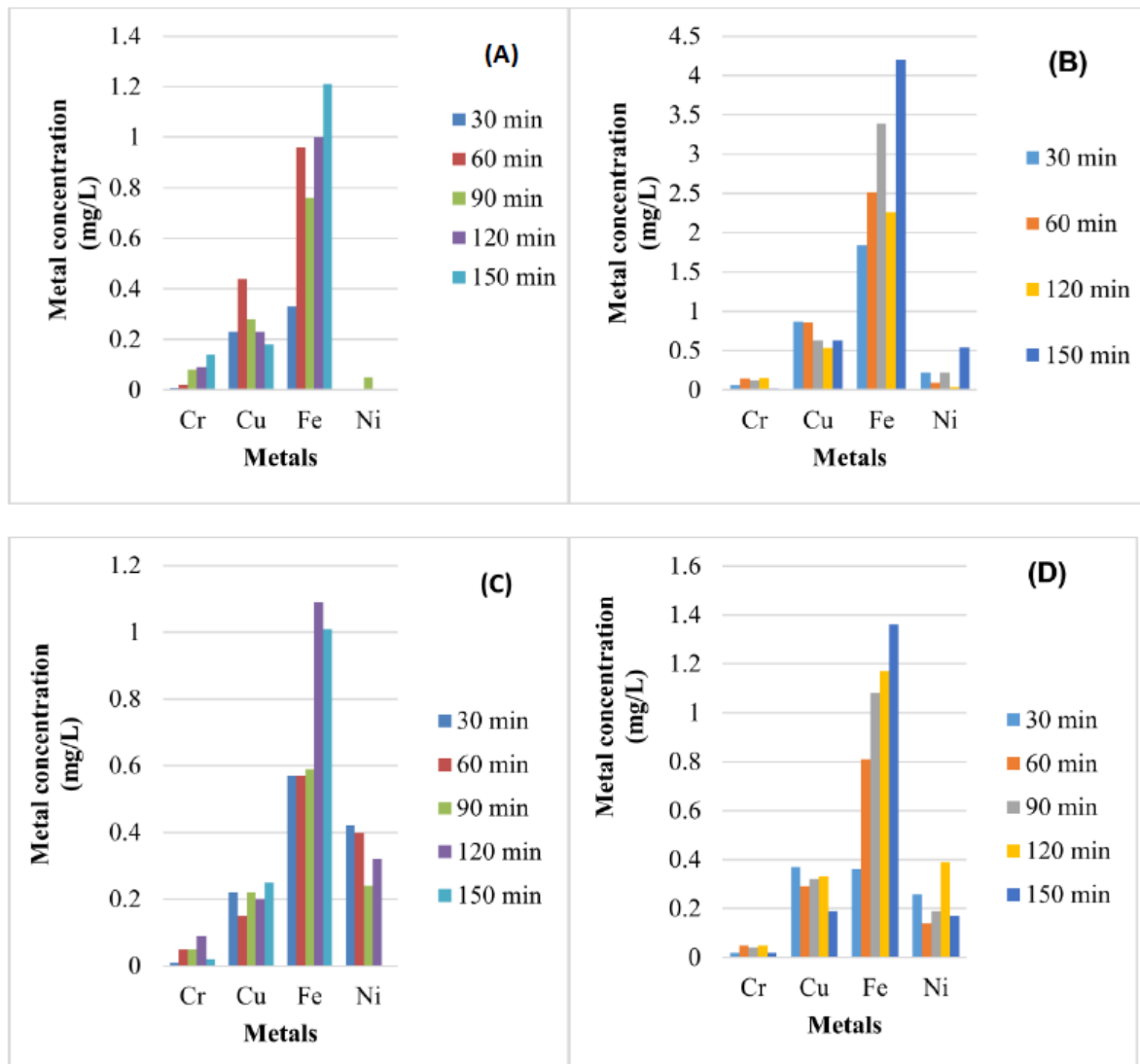


**Figure 2:** Amount of metal (mg/L) leached from the geophagic clays of Ikageng: (A) Market sample S/L of 1/100; (B) Market sample S/L of 2/100; (C) Source sample S/L of 1/100; (D) Source sample S/L of 2/100.

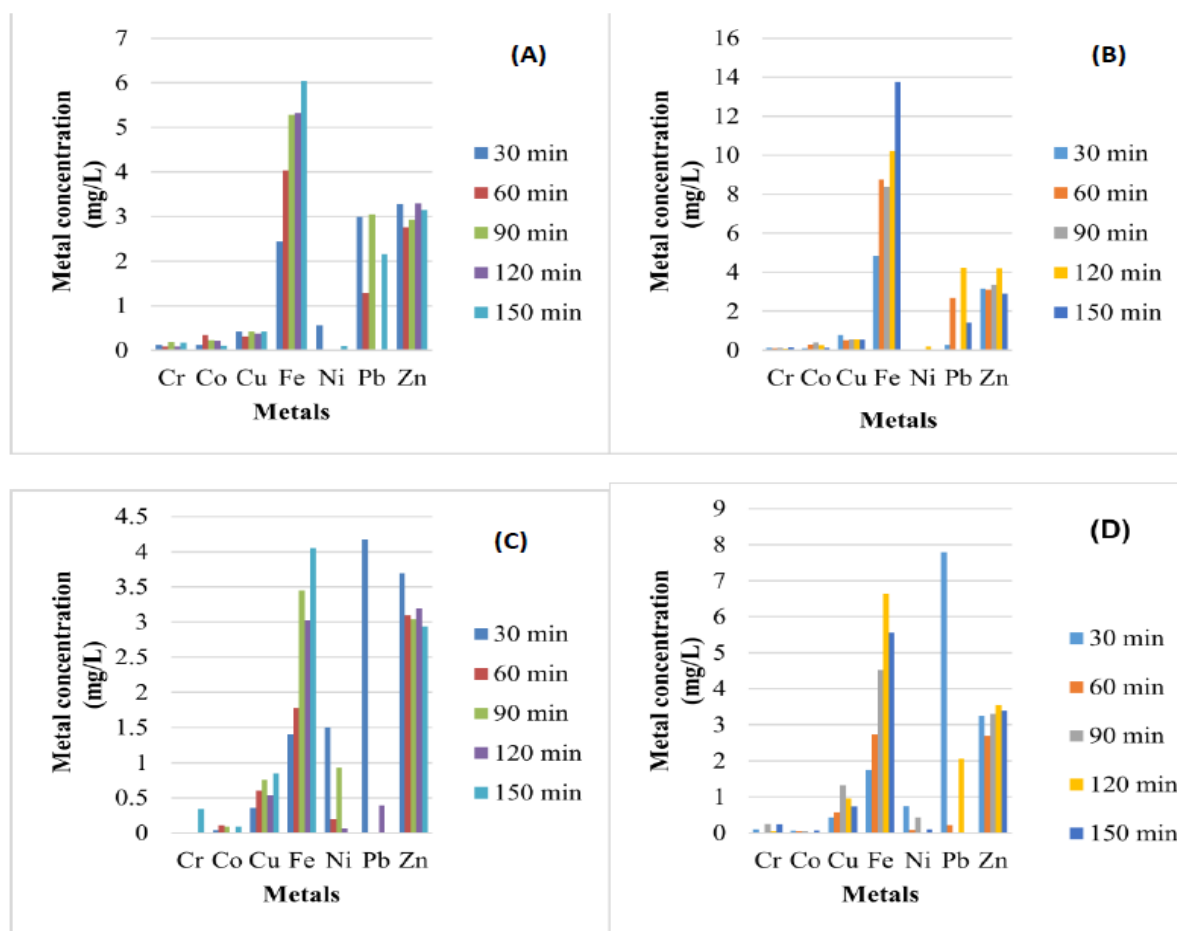


**Figure 3:** Amount of metal (mg/L) leached from the geophagic clays of Pheramindi: (A) Market sample S/L of 1/100; (B) Market sample S/L of 2/100; (C) Source sample S/L of 1/100; (D) Source sample S/L of 2/100.

When compared to the adequate daily intake of trace elements in the human body, the amounts of elements released from all the geophagic clays samples were relatively high (Figure 1a -Figure3d). The Pb content that was leached from the Pheramindi samples was significantly higher (Figure 3a) than 20  $\mu\text{g/L}$  set as the standard limit for body function. The Cu content leached from all geophagic clays samples was in the range of 0.15 mg/L-0.85 mg/L, less than the standard limit of 1000  $\mu\text{g/L}$  required for body function (Figure 1a - Figure3b). The amount of Zn leached from all the geophagic clays was below 3.81 mg/L which is within the standard limit required for body function, set as 5.0 mg/L.

The average concentration of leached Ni was in the range of 0.06 - 1.5 mg/L, but only 0.025 – 0.03 mg/kg /day is required for body functions. The average Co content in these geophagic clays was in the range of 0.001 – 0.60 mg/L, while the body requirement for this element is 0.002 – 0.1 mg/kg daily. Lead (Pb), Ni, Co and Fe were released in

concentrations exceeding the value required for the proper functioning of the human body. The mobility of the metals was mostly dependent on their initial concentration in the clay as well as the mineralogical composition of the clay.

#### *Micro-organisms and potential health risks.*

Micro-organisms are found everywhere in our body, soil, water and plants. Bacteria can be either beneficial or harmful to people depending on their classification. Soil is an example of an extreme environment that contains large numbers of varied and specific bacteria. In this study, the bacterial community structure and species in the metagenomics of six samples were analysed. The results (Table 4) only show the selected microorganisms with potential effects to the geophagic individuals. The microorganisms that were found on the geophagic clays were introduced in the source and markets by either run-off water, human activities or occurred naturally. Most of the geophagists consume the clay without any pretreatment for disinfection, therefore exposing themselves to pathogenic microorganisms. In our recent study, it was demonstrated that the load of microorganisms in the clay can be significantly reduced by simply exposing the clay to sun light [28]. The risk of infection can at least be minimized through the use of sustainable pretreatment method such as solar disinfection.

**Table 4:** Microorganisms occurring in geophagic clays from Ikageng, Phelandavha and Pheramindi samples and the potential health risks

| Sampling area | Source                            |                                                                                                              | Market                            |                                                                                                                                        |
|---------------|-----------------------------------|--------------------------------------------------------------------------------------------------------------|-----------------------------------|----------------------------------------------------------------------------------------------------------------------------------------|
|               | Microorganisms                    | Potential disease                                                                                            | Microorganisms                    | Potential disease                                                                                                                      |
| Ikageng       | <i>Pseudomonas aeruginosa</i>     | Urinary tract infection, Pneumonia, Septic shock, Gastrointestinal infection, Skin and soft tissue infection | <i>Mycobacterium sp</i>           | Tuberculosis                                                                                                                           |
|               | <i>Spiroplasma sp</i>             | Creutzfeldt-Jakob (CJD)                                                                                      | <i>Tsukamurella sp</i>            | Pneumonia                                                                                                                              |
|               | <i>Pseudonocardia yunnanensis</i> | Brain and Renal inflammatory disease, Chronic kidney disease                                                 | <i>Pseudonocardia yunnanensis</i> | Brain and renal inflammatory disease, chronic kidney disease                                                                           |
|               | <i>Propionibacterium acnes</i>    | Acne, Chronic blepharitis, Endophthalmitis                                                                   | <i>Klebsiella oxytoca</i>         | Colitis and sepsis                                                                                                                     |
|               |                                   |                                                                                                              | <i>Pseudomonas aeruginosa</i>     | Urinary tract infection, Pneumonia, Septic shock, Gastrointestinal infection and Skin and soft tissue infection.                       |
| Phelandavha   | <i>Neisseria meningitidis</i>     | Sepsis, Meningitis, Meningococcal disease                                                                    | <i>Shigella sonnei</i>            | Diarrhea, Dysentery                                                                                                                    |
|               | <i>Synechococcus sp</i>           | Cyanotoxins                                                                                                  | <i>Escherichia coli</i>           | Gastroenteritis, urinary infection, neonatal meningitis                                                                                |
|               | <i>Streptococcus pneumoniae</i>   | Pneumonia                                                                                                    | <i>Pseudomonas sp</i>             | Nosocomial infection, Urinary tract infection, Pneumonia, Septic shock, Gastrointestinal infection and Skin and soft tissue infection. |

|                |                                 |                                                                                                             |                                 |                                                                                                                                        |
|----------------|---------------------------------|-------------------------------------------------------------------------------------------------------------|---------------------------------|----------------------------------------------------------------------------------------------------------------------------------------|
| Pherami<br>ndi | <i>Propionibacterium acnes</i>  | Acens, Chronic blepharitis, Endophthamitis                                                                  | <i>Propionibacterium acnes</i>  | Acens, Chronic blepharitis, Endophthamitis                                                                                             |
|                | <i>Pseudomonas aeruginosa</i>   | Urinary tract infection, Pneumoni, Septic shock, Gastrointestinal infection, Skin and soft tissue infection | <i>Spiroplasma sp</i>           | Creutzfeldt-Jakob (CJD)                                                                                                                |
|                | <i>Alpha proteobacterium</i>    | Bacteraemia                                                                                                 | <i>Klebsiella oxytoca</i>       | Colitis and sepsis                                                                                                                     |
|                | <i>Spiroplasma sp</i>           | Creutzfeldt-Jakob (CJD)                                                                                     | <i>Mycobacterium avium</i>      | Lungs infection                                                                                                                        |
|                | <i>Streptococcus mitis</i>      | Endocarditis                                                                                                | <i>Peptoniphilus sp</i>         | Bloodstream infection                                                                                                                  |
|                | <i>Streptococcus agalactiae</i> | Neonatal sepsis, Postpartum infection                                                                       | <i>Streptococcus salivarius</i> | Endocarditis                                                                                                                           |
|                | <i>Propionibacterium acnes</i>  | Acens, Chronic blepharitis, Endophthamitis                                                                  | <i>citrobacter amalonaticus</i> | Neonatal meningitis, Gastroenteritis                                                                                                   |
|                | <i>Spiroplasma sp</i>           | Creutzfeldt-Jakob (CJD)                                                                                     | <i>Lactobacillus jensenii</i>   | Urinary tract infection, Pneumonia, Septic shock, Gastrointestinal infection and Skin and soft tissue infection.                       |
|                |                                 |                                                                                                             | <i>Leptotrichia sp</i>          | Endocarditis, Oral diseases                                                                                                            |
|                |                                 |                                                                                                             | <i>Streptococcus mitis</i>      | Endocarditis                                                                                                                           |
|                |                                 |                                                                                                             | <i>Propionibacterium acnes</i>  | Acens, Chronic blepharitis, Endophthamitis                                                                                             |
|                |                                 |                                                                                                             | <i>Pseudomonas sp</i>           | Nosocomial infection, Urinary tract infection, Pneumonia, Septic shock, Gastrointestinal infection and Skin and soft tissue infection. |

### Susceptibility of Microorganisms

Excessive concentration of heavy metals can be toxic to all organisms. When the soil is ingested and enters into the stomach which has the pH of 2, there is possible reaction with clay minerals and micronutrients contained in soil ingested [23, 29]. The acidic stomach causes a decrease in the pH of ingested clay (Table 3). The bacterial activity decreased over specified time (Figure 4, 5). Figure 4 shows that the number of bacteria in the clay decreases with the increased released of metals into solution; however it is likely that the decrease of the number of bacteria is a result of the combined inhibitory effect of metal and low pH. It has been reported that the physiochemical parameters such as pH, and metal ion concentration are correlated with microbial community composition [30]. The low pH in stomach increases the solubility of metals and other cations in the gastrointestinal tract [31; 32]. The concentration of metal was low at the initial time (25.3 mg at 30 min) and at the end of exposure the concentration of metals was 34.39 mg (Figure 4). The bacterial activity decreased over specified time ranged from 71.67 CFU\ml -38.33 CFU\ml (Figure 4, 5). Microorganisms that inhabit in metal-contaminated environment are able to develop many various types of resistance mechanisms in order to survive heavy metals, as results not all the bacteria were inhibited [31, 33].

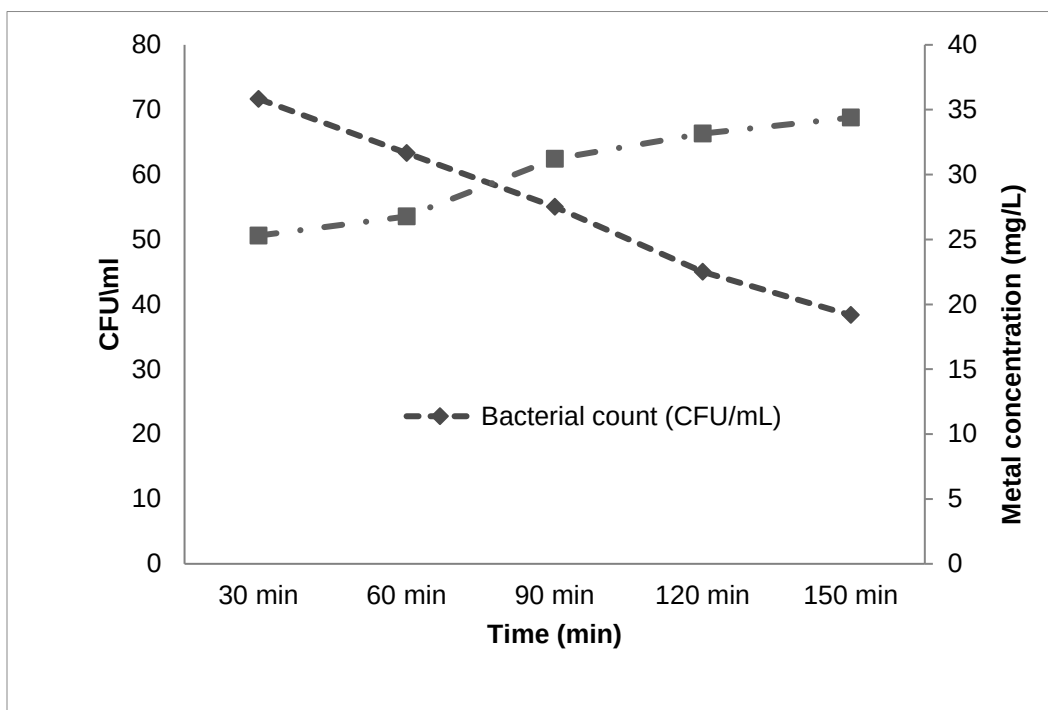


**Figure 4:** The metals concentration released and the microorganism that survived

Study has reported that heavy metals (Pb, Cd, Hg, Ag, and Cr) are toxic at low concentration and have no known beneficial effects on bacterial cells [33]. Clay particles can also bind to metals cations and microbes and reduce the toxicity of certain metals towards microorganisms; metals such as Zn enter the crystal lattice and become unavailable to organism [34]. Clay minerals such as kaolinite and montmorillonite protect certain bacteria from inhibitory effect of metals [34]. The acidity of the environment increases solubility of metals and decreases the concentration of microorganisms (Table 3, Figure 5). The identification of microorganisms through sequencing of 16S rDNA showed that the more resistant species (still alive after two and half hours) included *Bacillus subtilis*, *Paenibacillus*, *Bacillus cereus*, *Bacillus thuringiensis*, *Bacillus humi* and *Arthrobacter arilaitensis* which are all Gram-positive bacteria. Renal failure can be caused by *Paenibacillus sp.* The microbial concentration decreased significantly within the first 30 min while only a slight decrease was observed between 30 and 150 min (Figure 5). The acidity directly affects both metal release and bacterial activity. The low availability of organic carbon is extremely challenging and hostile environment affect the growth of microorganisms. Bacteria needs organic carbon to survive, this can be converted to energy and biomolecules required for cell structure [32].

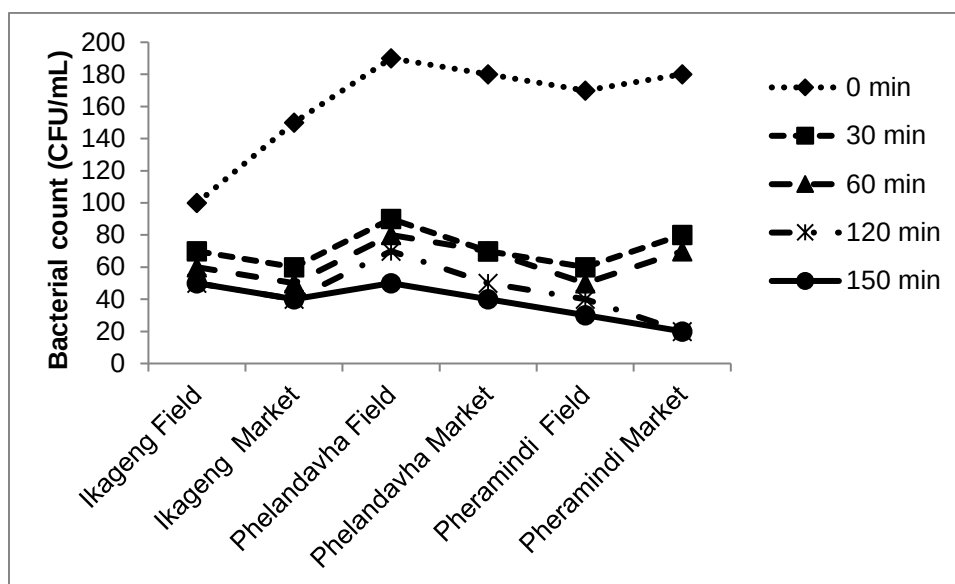


Figure 5: The behavior of microorganism in the geophagic clay subjected to a simulated gastric fluid

## Conclusion

The aim of this study was to determine the susceptibility of microorganisms to conditions similar to the gastrointestinal tract during ingestion of clays. Our findings show that low pH promotes the release of metals from the clays and the combination of acidity as well as increased concentration of metals contributes to the considerable reduction of the load of microorganisms in the clay. However, it was also found that microorganisms are not totally inhibited under such conditions; Gram-positive bacteria were found to resist after longer exposure and will therefore be likely to reach the intestine of geophagists alive. It therefore ensues that despite the adverse conditions in the estomach some microorganisms are likely to persist in the digestion system and cause disease. It is advisable for consumers to consider the pretreatment of clay prior to ingestion; affordable and sustainable technique such solar disinfection was found to be effective for the treatment of clay [28].

## Acknowledgements

The authors are grateful to the sponsor from the North-West University and the National Research Foundation (NRF) in South Africa. Any opinion, findings and conclusions or recommendations expressed in this material are those of the authors and therefore the NRF does not accept any liability in regard thereto. The authors appreciate the contribution of Mr N. Lemmer, and Mr G. Van Rensburg from the North-West University; Mr E. Malenga and Ms N. Baloyi from the University of Johannesburg in South Africa.

## References

- [1]. Johnson EB. (1990). Pica. In: Walker HK, Hall WD, Hurst JW, (editors).
- [2]. Ekosse, G-I.E., de Jager, L. and Ngole, V (2010). Traditional mining and mineralogy of geophagic clays from Limpopo and Free State provinces, South Africa. *African Journal of Biotechnology*. 9(47): 8058 – 8067.
- [3]. Woywodt A, Kiss A (2002). Geophagia: The history of earth-eating. *Journal of the Royal Society of Medicine*. 95: 143 – 146. 32: 2129 – 2135.
- [4]. Young SL, Wilson JM, Hillier S, Delbos E, Ali SM, Stoltzfus RJ. (2010). Differences and Commonalities in Physical, Chemical and Mineralogical Properties of Zanzibari Geophagic Soils. *Journal of Chemical Ecology*. 36: 129 – 140.
- [5]. Brand CE, de Jager L. Ekosse, G-I.E (2009). Possible Health Effects Associated With Human Geophagic Practice: An Overview. *Medical Technology SA*. 23(1): 11 - 13.
- [6]. Walker ARP, Walker BF, Sookaria FI, Canaan RJ. (1997). Pica. *Journal of health*. 997(117), 280-284.
- [7]. Reilly C, Henry J (2000). Why do humans consume soil? *Nutrition Bulletin* 25: 141–144.
- [8]. Hunter JM (1973). Geophagy in Africa and in the United States: a culture-nutrition hypothesis, *Geographical Review* 63, 170–195.
- [9]. Bisi-Johnson MA, Obi CL, Ekosse G-I.E (2010). Microbiological and health related perspectives of geophagia: An overview. *African Journal of Biotechnology*. 9(19): 5784 – 5791.
- [10]. Kawai K, Saathoff E, Antelman G, Msamanga G, Fawzi WW (2009). Geophagy (Soil-eating) in Relation to Anemia and Helminth Infection among HIV–Infected Pregnant Women in Tanzania. *The American Journal of Tropical Medicine and Hygiene*. 80(1): 36 – 43.
- [11]. Halsted JA (1968). Geophagia in Man: Its Nature and Nutritional Effects. *The American Journal of Clinical Nutrition*. 21(12): 1384 - 1391.
- [12]. Morman S.A, Plumlee S.G, Smith D.B. (2009). Application of *in vitro* extraction studies also involve using the simulated human gastric fluid to evaluate element bio accessibility in soils from a transect across the United States and Canada. *Applied geovhistry* 24:1454-14.63.

- [13]. Fosso-Kankeu E, Mulaba-Bafubiandi AF. 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.
- [14]. Kutalek R, Wewalka G, Gundacker C, Nuier H, Wilson J, Haluza D, Huhulescu S, Hillier S, Jager M, Prinz A (2010). Geophagy and potential health implication: Geohelminths, microbes and heavy metals. Transaction of the royal society of tropic medicine and hygiene.104:787-795.
- [15]. Callahan GN (2003). Eating Dirt. Emerging Infect. Dis. 9(8): 1016-1021.
- [16]. Nchito M, Geissler PW, Mubila L, Friis H, Olsen A (2004). Effects of iron and multi-micronutrient supplementation on geophagy: a two-by-two factorial study among Zambian schoolchildren in Lusaka. Transactions of the Royal Society of Tropical Medicine and Hygiene. 98(4): 218 – 227
- [17]. Kawai K, Saathoff E, Antelman G, Msamanga G, Fawzi WW (2009). Geophagy (Soil-eating) in Relation to Anemia and Helminth Infection among HIV–Infected Pregnant Women in Tanzania. The American Journal of Tropical Medicine and Hygiene. 80(1): 36 – 43.
- [18]. Luoba, A.I., Geissler, P.W., Estambale, B., Ouma, J.H., Magnussen, P., Alusala, D., Ayah, R., Mwaniki, D. and Friis, H. (2004). Geophagy among pregnant and lactating women in Bodo District, western Kenya. Transactions of the Royal Society of Tropical Medicine and Hygiene. 98(12): 734 - 741.
- [19]. Luoba, A.I., Geissler, P.W., Estambale, B., Ouma, J.H., Alusala, P., Ayah, R., Mwaniki, D., Magnussen, P. and Friis, H. (2005). Earth-eating and reinfection with intestinal helminths among pregnant and lactating women in western Kenya. Tropical Medicine and International Health. 10(3): 220 – 227.
- [20]. Hoorman JJ, Islam R (2010). Understanding soil microbes and nutrients recycling. Agricultural and natural resource. The Ohio State University. SAG-16-10.
- [21]. Zhu H, Hart CA, Sales D, Roberts NB. (2006). Bacterial killing in gastric juice – effect of pH and pepsin on *Escherichia coli* and *Helicobacter pylori*. Journal of Medical Microbiology (55) 1265–1270.

- [22]. Baath E. (1989). Effects of heavy metals in soil on microbial processes and populations (a review). *Water Air Soil Pollut.* 47:335–379.
- [23]. Ngole VM. (2015). Chemical characteristics of geophagic soils. Human and enzootic geophagia: Ingested soils and practice. Lambert Academic Publishing. 57-70.
- [24]. Otto CC, Haydel SE (2013). Microbial clays: composition, activity, mechanisms of action and therapeutic applications. pp 1169-1180.
- [25]. Juhasz AL, Weber J, Naidu R, Gancarz D, Rofe A, Todor D, Smith E.(2010). Determination of cadmium relative bioavailability in contaminated soils and its prediction using in vitro methodologies. *Environmental Science and Technology.* 44:5240–5247. [PubMed: 20527788].
- [26]. Sauvé S, Hendershot W, Allen HE. (2000).Solid-solution partitioning of metals in contaminated soils: Dependence on pH, total metal burden, and organic matter. *Environmental Science and Technology.* 34:1125–1131.
- [27]. Lar UA, Agene JI, Umar AI. (2015). Geophagic clay materials from Nigeria: a potential source of heavy metals and human health implication in mostly women and children who practice it. *Environ Geochem Health*, 37:363-375.
- [28]. Fosso-Kankeu E, Netshitanini TL, Abia ALK, Ubomba-Jaswa E, Waanders FB. (2015). Application of solar treatment for the disinfection of geophagic clays from markets and mining sites. *African Journal Biotechnology.*14 (50); 3313-3324.
- [29]. Oomen AG, Sips AJ, Groten AM, Dick JP, Sijm TJM, Tolls THM. (2000). Mobilization of PCBs and lindane from soils during in vitro digestion and their distribution among bile salt micelles and proteins of human digestive fluids and the soil. *Environment science technology.* 34:297-303.
- [30]. Johnson DB, Hallberg KB. (2003).The microbiology of acidic mine waters. *Res Microbial.* 154:466-473.
- [31]. Dopson M, Baker-Austin C, Koppineedi PR, Bond PL. (2003). Growth in sulfidic mineral environments: metal resistance mechanism in acidophilic micro-organisms. *Microbiology* 149:1959-1970.

- [32]. Young SL, Wilson JM, Miller D, Hillier S. (2008). Toward a Comprehensive Approach to the Collection and Analysis of Pica Substance, with Emphasis on Geophagic Materials. PLoS ONE. 3(9): 1 - 11.
- [33]. Nies DH. (2004). Metals and their compounds in the environment. Part II. In: Anke K, Ihnat M, Stoeppler M (eds) The elements: essential and toxic effects on microorganism. Wiley-VCH, Weinheim, Germany.
- [34]. Gadd GM, Griffiths AJ. (1978). Micro-organisms and heavy metal toxicity. Microbial ecology. 4:303-317.

# Chapter 5

**The effect of sunlight on the inactivation of *Escherichia coli*, *Salmonella enterica* and naturally occurring microorganisms in edible geophagic clay**

The article is in preparation to be submitted to the journal of Science of the Total Environment.

## 5.1 Introduction

This paper describes the effect of sunlight on the microorganisms in edible geophagic clays. Microorganisms that are used in the study include *Escherichia coli*, *Salmonella enterica* and naturally occurring microorganisms. The aim of the study was to investigate the ability of sunlight to inactivate microorganisms that are detrimental to human health. Naturally occurring microorganisms are all microorganisms that occur naturally on the soils. These organisms can either be on the surface of clay samples or inside the layers of the clay. *E. coli* and *S. enterica* were inoculated into the geophagic clays and exposed to sunlight. Two experimental set-ups were employed: 1) direct exposure to sunlight and 2) dark conditions.

The results of the study showed total inactivation of microorganisms in clay samples that were exposed to direct sunlight and no possible regrowth was observed. The study entailed the inactivation of *Escherichia coli*, *Salmonella enterica* and naturally occurring microorganisms exposed directly to sunlight on sunny days. During cloudy days, incomplete inactivation of microorganisms in all the geophagic clays was observed as regrowth occurred. Complete inactivation of microorganisms in geophagic clay exposed to direct sunlight was achieved after 3 h and 5 h, without any observed regrowth. The complete inactivation of naturally occurring microorganisms was achieved after 2 h of sunlight exposure. Soil temperatures of both experimental set-ups showed an increase, especially in clays exposed to direct sunlight, which caused a synergistic effect for the inactivation of microorganisms in geophagic clays. This study confirms that significant inactivation of microorganisms in geophagic clays can be achieved, using sunlight exposure which is an environmentally friendly, sustainable and affordable method.

The article is intended for submission to the journal of Science of the Total Environment.

# The effect of sunlight on the inactivation of, *Escherichia coli*, *Salmonella enterica* and naturally occurring microorganisms in edible geophagic clay

## Abstract

Geophagic clay materials in open markets are placed in different set-ups: in open space directly exposed to the sun; loose in bags; packed in plastics bags and under shade. The effect of sunlight on the inactivation of *Escherichia coli*, *Salmonella enterica* and naturally occurring organisms in edible geophagic clay was studied for the first time. Geophagists prefer different soils, including ground soil, riverbank soil, mountain soil and termite mound soil, but commonly high preference is for soil sold in open markets. Experiments were set up in two different set up clay samples were 1) clay exposed directly to sunlight and 2) clay exposed to dark conditions. These samples were inoculated with  $10^9$  CFU ml<sup>-1</sup> concentrations of *Escherichia coli* and *Salmonella enterica* and allowed to stand for 5 h. The clays sold in markets and mined clays were collected and exposed on the same day to the different experimental conditions to determine the effectiveness of sunlight to disinfect naturally occurring microorganisms. In winter and summer days, complete inactivation of *Escherichia coli* and *Salmonella enterica* (0 CFU/100 ml) occurred within 5 h and no regrowth of *E. coli* and *Salmonella enterica* occurred within 24 h and 48 h following sunlight exposure. Under dark and cloudy conditions prolonged exposure was needed and incomplete inactivation was achieved and regrowth of *Escherichia coli* and *Salmonella enterica* was observed. The naturally occurring microorganisms in open marketed and mined clays were completely inactivated within 2 h after exposure to sunlight. This study confirms that sunlight is effective in ridding the clay of microorganisms. After disinfection, the geophagic clays in the open market must be handled carefully to avoid recontamination.

**Keywords:** Sunlight inactivation; regrowth; *Escherichia coli*; *Salmonella enterica* ; naturally occurring organisms; clay temperature; geophagic clays; solar radiation.

## 1. Introduction

Microorganisms are found everywhere in the environment, e.g. in water, soil, food and plants. Microorganisms can be classified as harmful or beneficial. They can either be introduced or occur naturally. There are different methods used to get rid of microorganisms in water, and solar disinfection (SODIS) is the easiest water treatment method used to improve the microbiological quality of drinking water by inactivating waterborne pathogens (Conroy *et al.*, 1996; McGuigan *et al.*, 1998; Conroy *et al.*, 2001; Mani, 2006; Gómez-Cousoa *et al.*, 2012; McGuigan *et al.*, 2012). Inactivation of pathogens is achieved using a synergy between optical and thermal inactivation at temperatures above 45°C (Wegelin *et al.*, 1994; McGuigan *et al.*, 2012).

More than 45 million people use SODIS daily, mostly in developing countries (Conroy *et al.*, 1996; McGuigan *et al.*, 1998; Conroy *et al.*, 2001; Mani, 2006; Rainey & Harding, 2005). The SODIS method is simple in its application, cheap and environmentally sustainable (SODIS, 2002; McGuigan *et al.*, 2012). The method involves filling transparent containers with contaminated water and then exposing the containers to direct sunlight for at least 6 hours after which the water is safe to drink (McGuigan *et al.*, 2012).

Microorganism inactivation by SODIS treatment is the result of two synergistic effects: 1) optical inactivation-effect by UVA between 320 nm-400 nm and visible irradiance between 400 nm -490 nm; and 2) thermal inactivation by increased water temperature, which must reach at least 45°C (Wegelin *et al.*, 1994; Sommer *et al.*, 1997; McGuigan *et al.*, 1998). Exposure of microorganisms to mild heat and UVA light, simultaneously cause the microorganisms to be inactivated fast. The UV radiation damages the DNA of microorganisms and mild heat (45-50°C) is assumed to denature essential cellular enzymes (Wegelin *et al.*, 1994; Ubomba-Jaswa *et al.*, 2008). High inactivation of bacteria under sunlight is attributed to solar UVA which damages cells (Ubomba-Jaswa *et al.*, 2010).

Geophagia is the deliberate consumption of earthy materials, including clay and soil, by both humans and animals (Abrahams, 2002; Ekosse *et al.*, 2010) and is more common among pregnant women and children (Brand *et al.*, 2009). Geophagia practice is believed to be practiced for various reasons, such as cultural, medicinal, psychological, traditional and nutritional needs, and mineral deficiency (Ekosse *et al.*, 2010; Grace & Adams, 2012). Pregnant women consume clay because they believe it reduces nausea and morning

sickness during pregnancy (Reilly & Henry, 2000; Kawai *et al.*, 2009; Bisi-Johnson *et al.*, 2010). Lar *et al.* (2015) reported the average amount of 20 g of clay ingested by pregnant women on a daily basis.

Clay may contain undesirable compounds such as bacteria, parasites, heavy metals, human/ animal faeces and helminths (Callahan, 2003; Ekosse & Jumbam, 2010). Microorganisms in clay from open markets can be from either natural environment contamination or introduced through the handling of the clay by vendors and consumers. Ingestion of clay holds dangers for human health such as microbiological infections, heavy metal poisoning, dental abrasion, helminthiasis, intestinal obstruction and anemia (Geissler *et al.*, 1998; Kawai *et al.*, 2009). Microbial contamination can be prevented by taking specific precautions. Mined geophagic clays are consumed directly before undergoing processing such as pounding, sieving, slurring or grinding (Ekosse *et al.*, 2010).

Soil pasteurization is used to kill lethal pathogens in contaminated soil and it involves sterilizing soil by artificial heating or heating the soil by covering it with transparent polyethylene mulching while the soil is kept wet (Baker & Cook, 1974; Mahrer, 1979). Fosso-Kankeu *et al.* (2015) recently demonstrated that microorganisms in geophagic clay could be inactivated after exposure to UVA light for 8 hours in a photocatalytic chamber. However, the effects of sunlight on organisms in geophagic clays have still not been evaluated.

This study aims to evaluate the effect of sunlight on the disinfection of geophagic clay through inactivation of microorganisms including *Escherichia coli*, *Salmonella enterica* and naturally occurring microorganisms. Experiments were carried out in summer (October to December 2015) and in winter (June and July 2016) on cloudy and sunny days. It is of interest to develop a low cost, simple and environmentally sustainable method to disinfect microorganisms in geophagic clay. The vendors lack the knowledge about reduction and disinfection of microorganisms in edible geophagic clays, but with this simple method they can be educated. In the open markets, some of the clays are placed in direct sunlight for drying because it has been collected wet.

In the SODIS treatment, *Escherichia coli* inactivation is evaluated because it is a main bacterial indicator and more sensitive to the effect of solar irradiation and temperature compared to other waterborne human pathogens. Berney *et al.* (2006) suggested the use

of *Salmonella spp.*, for it is an extremely pathogenic microorganism and reported to be more resistant to SODIS treatment (Smith *et al.*, 2000; Rincon & Pulgarin, 2003; Winfield & Groisman, 2003; Sciacca *et al.*, 2011).

## **2. Materials and methodology**

### **2.1. Sample collection**

Clay samples were obtained in markets around Gauteng and North-West Province and mined from geophagic clay mining sites. Clay samples were packed into sterile plastic bags, loose and picked from the heap. Sample collection took place during different seasons: in summer (2015) and winter (2016). The samples were kept in a refrigerator at 4°C until analyzed.

### **2.2. Bacterial growth**

Laboratory strains of *Escherichia coli* and *Salmonella enterica* were obtained from frozen stocks (Liquid Stock Culture) and 100 µl was inoculated on the plate and incubated at 37°C for 15 to 18 h (Ubomba-Jaswa *et al.*, 2008). A single colony was then inoculated into 250 ml of sterile Luria broth and was incubated at 37°C for 18 h on a rotary shaker to obtain a stationary phase culture. A volume of 500 µl of stock concentration was inoculated into clays in order to obtain a  $10^9$  CFU ml<sup>-1</sup> starting concentration of bacteria for each experiment (Ubomba-Jaswa *et al.*, 2008).

### **2.3. Bacterial enumeration techniques**

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 dilution procedure was carried out by diluting 100 µl of the sample into a test tube containing 900 µl of 0.1 M (1X) PBS. Enumeration of bacteria exposed to sunlight was conducted through the standard plate count method after a series of 10 fold dilutions. To ensure that the starting concentration was indeed  $10^9$  CFU ml<sup>-1</sup> a sample of clay was taken before clays had been exposed to sunlight (Ubomba-Jaswa *et al.*, 2008).

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 37°C for 18 h and counted the following day. Regrowth counts of bacteria were determined for *Salmonella enterica* and *Escherichia coli* experiment in winter by leaving the last samples at room temperature (22°C) for 24 h and 48 h. The plate count method (Membrane Lactose Glucuronide agar plates and Nutrient agar plates) as described above was then used to determine the number of remaining bacteria in the clay. The inactivation was determined by a log reduction in the growth of microorganisms and was plotted with the standard error of the mean for each time point. Expected levels of inactivation were dependent upon the observed weather conditions, the ambient temperature, and the clay temperatures of the test clays (Ubomba-Jaswa *et al.*, 2008).

#### **2.4. Sunlight inactivation of *Escherichia coli*, *Salmonella enterica* and naturally microorganism**

Inactivation of *Escherichia coli*, *Salmonella enterica* and naturally microorganisms using sunlight was carried out in the metal zinc aluminium painted roof of the CSIR Environmental Building No 33, in full sunshine. The clay samples were autoclaved before inoculating with *Escherichia coli* and *Salmonella enterica*. Samples were collected on the experimental day and exposed the same day to remove any naturally occurring microorganisms in the open marketed and mined clay samples. **Figure 1** shows the setups of geophagic clay material in open markets.

To effectively explore the effect of the different settings in the open markets, two experimental set up were conducted: 1) clay exposed directly to sunlight and 2) clay exposed in dark conditions. The experimental configuration for clay exposed directly to sunlight included clay samples exposed to sunlight, covered by polyethylene terephthalate (PET) bottles. Clay samples exposed to sunlight while covered with PET bottles wrapped in paper towels to maintain dark condition. The clay temperature was taken at predetermined times and the climatic conditions were monitored. The clay samples were irradiated with sunlight for periods of 5 hours. Samples were taken every hour until the end of the experiment. The last clay samples were stored in the dark at room temperature and sampled at 24 h and 48 h to monitor regrowth (Ubomba-Jaswa *et al.*, 2008).

SODIS of *Escherichia coli* using sunlight was carried out as the control assay. Polyethylene terephthalate (PET) bottles of 2 litres 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 prepared in triplicate, placed on the roof of the building and exposed to full sunshine. During the experiment the water temperature was monitored. The solution was irradiated for 6 h. Samples were taken after 15, 30, 45, 60, 90, 120, 180, 240, 300, and 360 minutes of exposure to sunlight. Serial dilutions were performed in PBS, incubated overnight, and counted using the plating techniques described previously (Ubomba-Jaswa *et al.*, 2008).



**Figure 1:** Geophagic clay materials sold in the open market in South Africa. Photograph taken by Thembuluwo (2015).

### 3. Results and discussion

#### 3.1. Effect of different set-up on the inactivation of *Escherichia coli*, *Salmonella enterica* and naturally occurring microorganisms.

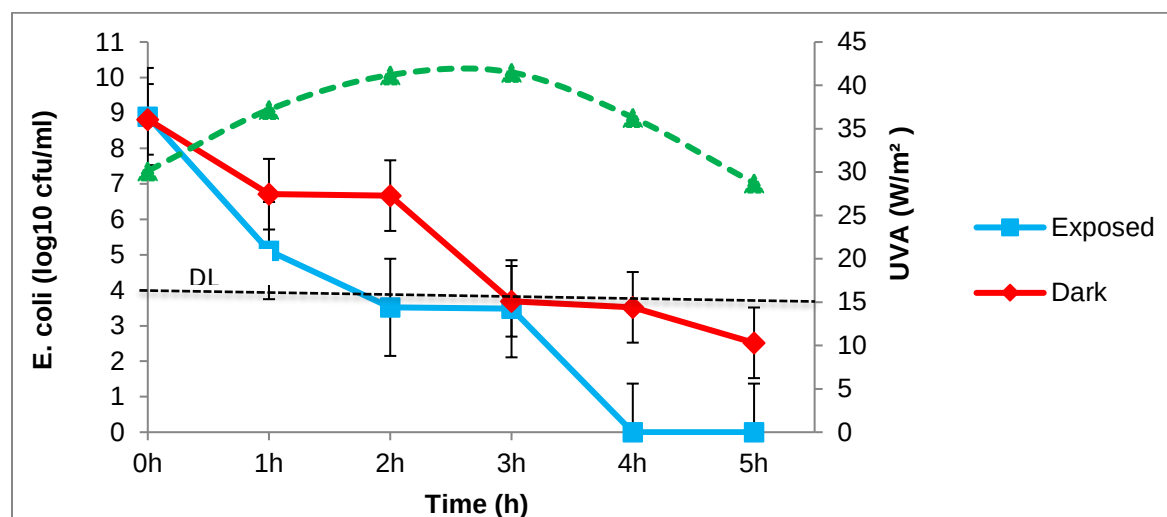
As shown in Figures 2a, 3, 4, 5a and 6a clays exposed directly to sunlight were completely inactivated within 5 h of sunlight exposure. Inactivation of *Salmonella enterica* after 1 h of direct exposure to sunlight decreased the *Salmonella enterica* population by a factor of  $10^4$  see Figure 3a while exposure of clay in dark conditions resulted to the decrease of *Salmonella enterica* number by a factor of  $10^2$  after 1 hour. As seen in Figure 6a complete inactivation of *Salmonella enterica* was achieved within 3 h, with a high level of solar irradiance at the start of the exposure ( $17.5 \text{ Wm}^{-2}$ ), an ambient temperature of  $23^\circ\text{C}$  and a clay temperature of  $19.2^\circ\text{C}$ . Figure 4 shows the inactivation of naturally occurring microorganisms in market clay sample was complete within 1 h with a high level of solar irradiance of  $39.50 \text{ Wm}^{-2}$ , and an ambient temperature of  $40^\circ\text{C}$ . Based on the results, the inactivation of *Escherichia coli* and *Salmonella enterica* occurred after 4 hours of exposure, from 10:00 to 14:00.

According to Figure 5a the inactivation of *Escherichia coli* in dark conditions was achieved with a high level of solar irradiance of  $21.6 \text{ Wm}^{-2}$  and ambient temperature of  $20^\circ\text{C}$  resulting in a clay temperature of  $22.2^\circ\text{C}$  after 2 h of sunlight exposure. However, solar irradiance increased the clay temperature, but the *Escherichia coli* population remained constant sees Figure 5a. Clays exposed in dark conditions were blocked from UV rays because of the paper towel shields. Decrease of the *Salmonella enterica* population is observed in Figure 3a by a factor of  $10^7$  after 5 h sunlight exposure. The UV rays can penetrate a blocking object, but insufficient UV radiation reaches the clay to cause inactivation (Herman & Celarier, 1997). The inactivation of bacteria was slower with changes in solar irradiance (Ubomba-Jaswa *et al.*, 2010).

The strongest sunlight occurs from 10:00 to 14:00 (Rokjo, 2003). Clays exposed directly to sunlight achieved complete inactivation because of the synergistic effect of UV radiation and increase in temperature. Wegelin *et al.* (1994) reported that high levels of solar irradiance combined with an increase in water temperature have a strong synergistic effect. The inactivation rate of these two experiments achieved different results regarding the level of disinfection achieved. Ubomba-Jaswa *et al.* (2010) reported that useful inactivation can

still occur, even in greatly reduced amounts of UVA. The synergy between the optical and the thermal inactivation mechanisms causes the rate of microorganism inactivation to increase (Wegelin *et al.*, 1994; McGuigan *et al.*, 2012).

(a)



(b)

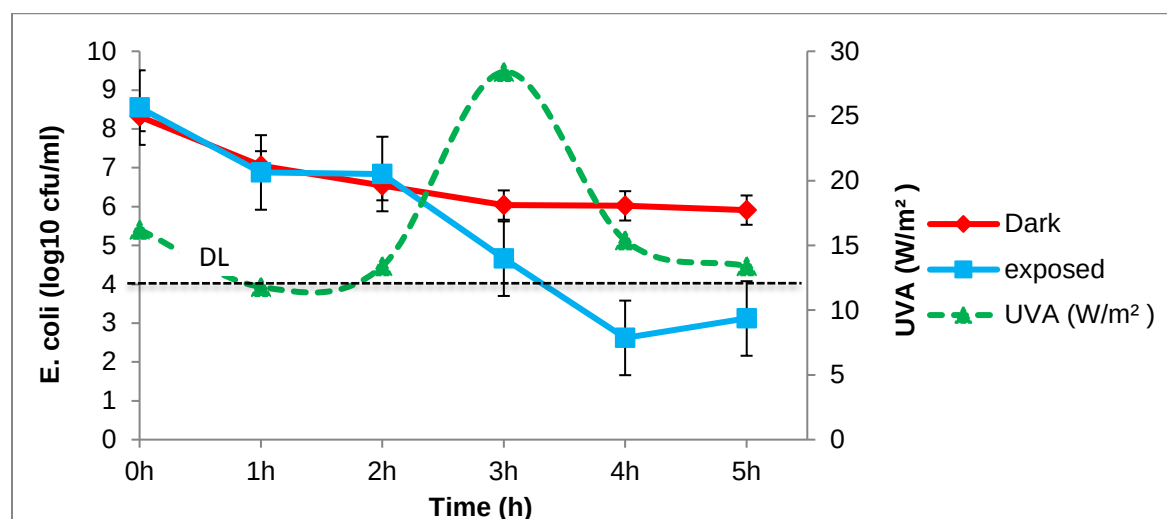
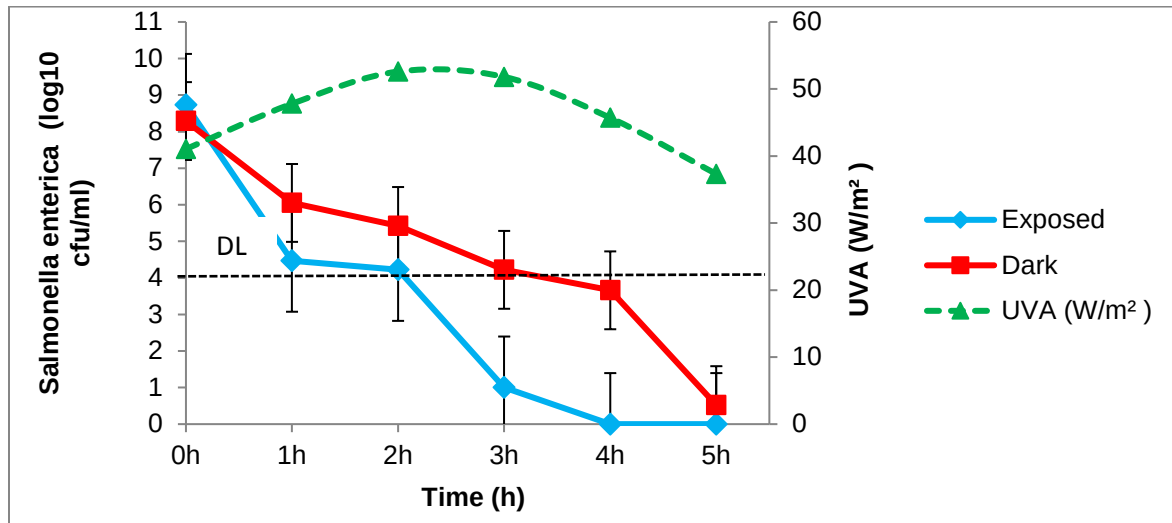


**Figure 2:** Inactivation of *Escherichia coli* in the geophagic clay exposed directly to sunlight and in dark conditions. Summer season experiment (Figure a) on a sunny day and cloudy day (Figure b). Error bars indicate standard error of triplicate measurements.

(a)



(b)

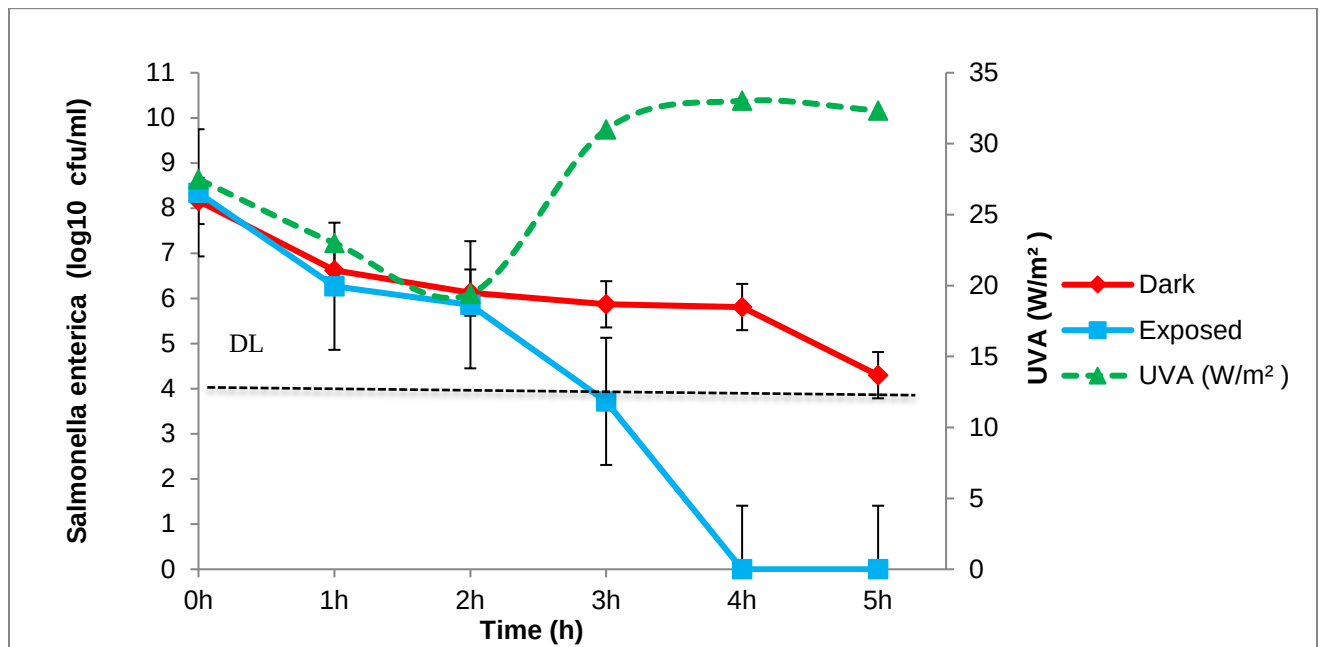


**Figure 3:** Inactivation of *Salmonella enterica* in geophagic clay exposed directly to sunlight and dark conditions. Summer season experiment (Figure a) on a sunny day and cloudy day (Figure b). Error bars indicate standard error of triplicate measurements.

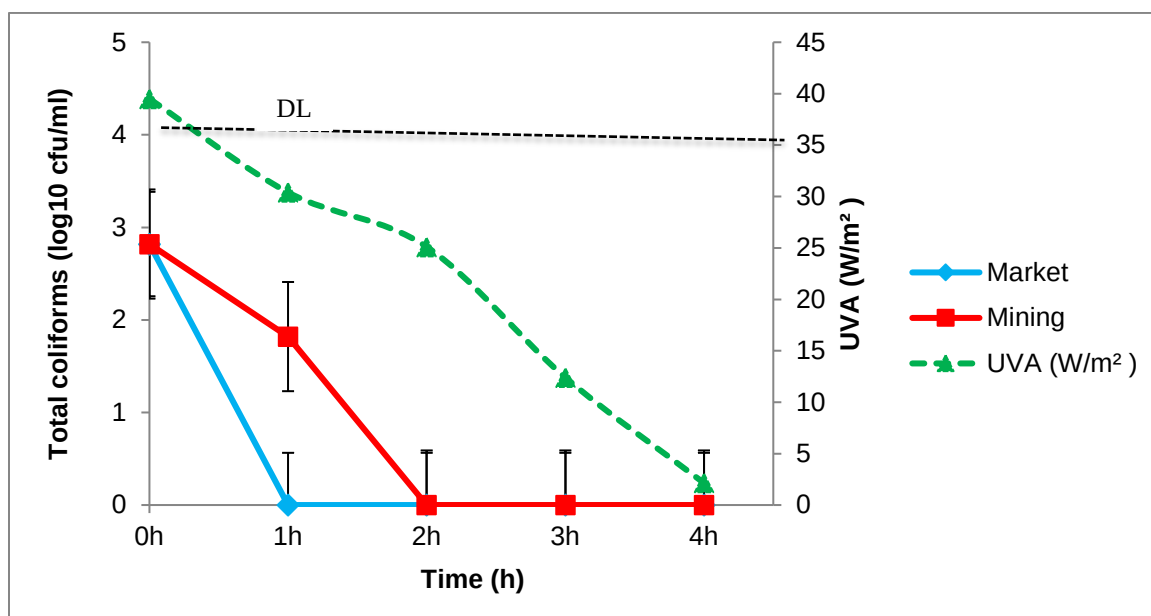
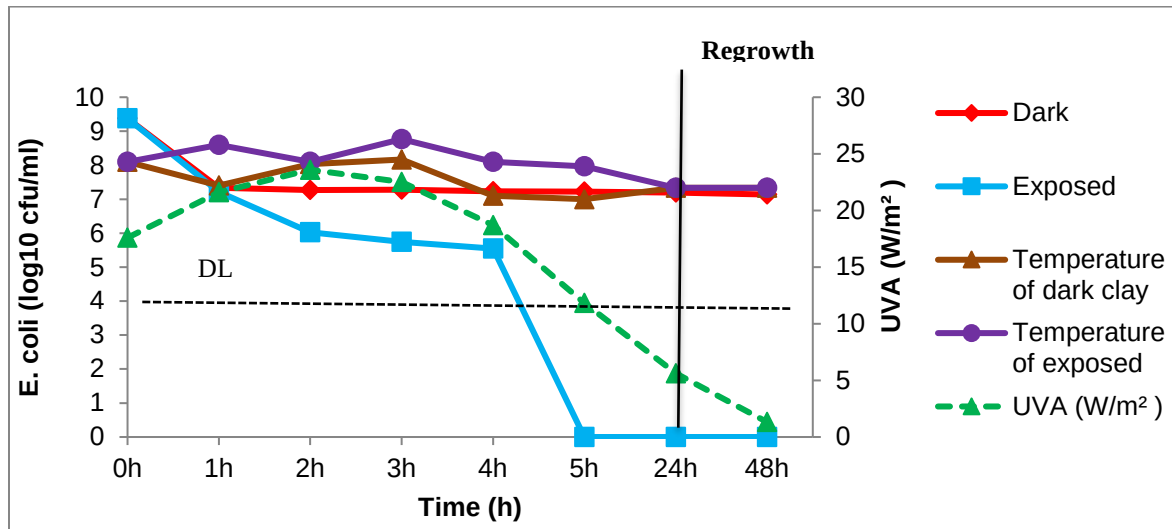


**Figure 4:** Inactivation of naturally occurring organisms of marketed clays and mined clays. Error bars indicate standard error of triplicate measurements.

(a)



(b)

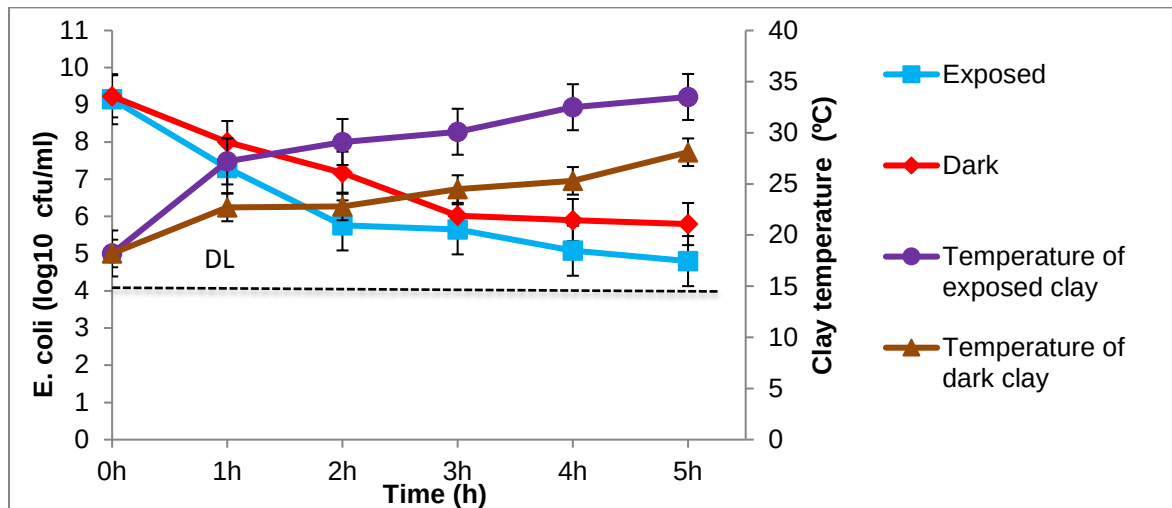
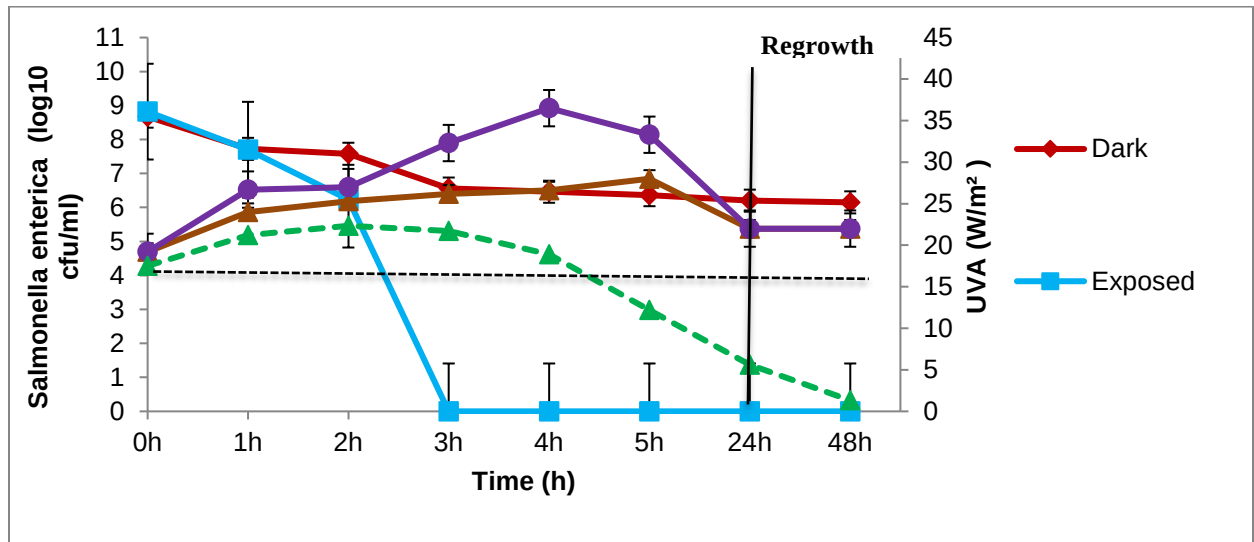


**Figure 5:** Inactivation of *Escherichia coli* in geophagic clay exposed directly to sunlight and in dark conditions. Winter season experiment (Figure a) on a sunny day and cloudy day (Figure b). Error bars indicate standard error of triplicate measurements.

(a)



(b)

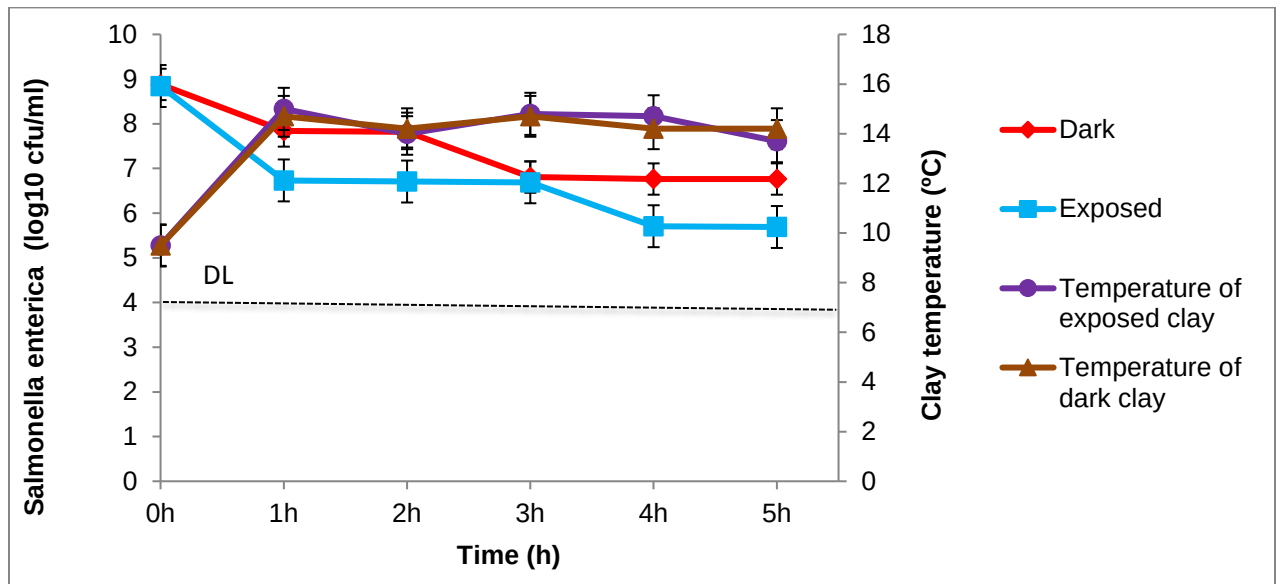


**Figure 6:** Inactivation of *Salmonella enterica* in the geophagic clay exposed directly to sunlight and dark conditions. Winter season experiment (Figure a) on a sunny day and cloudy day (Figure b). Error bars indicate standard error of triplicate measurements.

### 3.2. Synergy effect (optical and thermal effect) in inactivation

The clay temperature was monitored during the exposure to sunlight during winter only. During winter the ambient temperature was very low (in the range of 23°C) with solar irradiance of 10.76 Wm<sup>-2</sup>.) At an ambient temperature of 23°C the clay temperature reached 33°C during 5 h of sunlight exposure. The ambient temperature and the solar irradiation (UV dose) that the soil absorbs control the soil's internal temperature. Soil temperature involves the heat that the soil absorbs and the flow of heat (Comerford, 2013).

When the soil has adequate internal energy it can kill organisms that live in the soil (Comerford, 2013), including plants roots and microorganisms. Results obtained from inactivation of *Escherichia coli* on a cloudy day showed that inactivation was incomplete for both exposed directly to sunlight and in dark conditions see Figure 5b. As seen in Figures 5a and Figure 6a an increase in the ambient temperature and solar irradiance resulted in an increase in inactivation of microorganisms. When the clay temperature reached 20°C with UV irradiance of 21.73 Wm<sup>-2</sup>, complete inactivation of *E. coli* and *Salmonella enterica* was observed. At high ambient temperatures and high UV dose, the internal energy of the soil is high, causing effective disinfection of microorganisms that may be present in the clay. However, at later hours of the day, the ambient temperature and UV dose decrease, which results in a decrease in internal energy of the clay.

When clays are exposed directly to sunlight, the clays loose water. Fosso-Kankeu *et al.* (2015) observed low moisture content in the geophagic clays exposed to simulated UVA light for 4 weeks. Increased temperature associated with high levels of solar irradiance caused a decrease of the water content of soils and clays (Dunn *et al.*, 1985; Fosso-Kankeu *et al.*, 2015). A decrease in ambient temperature and UV irradiance caused a decrease in clay temperature see Figure 6b which resulted in incomplete inactivation of *Salmonella enterica*. Clay temperature and UV irradiance play significant roles in the disinfection of microorganisms present in clay.

When the clay temperature reached levels below 20°C incomplete inactivation was observed see Figure 6b. The clay temperature reached 27°C and the complete inactivation was achieved with UV irradiance of 22.34 Wm<sup>-2</sup>. Similar synergistic effect occurs during SODIS treatment when the water temperature is higher than 45°C (Wegelin *et al.*, 1994; McGuigan *et al.*, 1998; Navntoft *et al.*, 2008). In contrast, the study of *Escherichia coli*

SODIS showed a greater reduction of *Escherichia coli* at water temperature  $\geq 45^{\circ}\text{C}$  (Ubomba-Jaswa *et al.*, 2008).

### **3.3. Seasonal variation in inactivation**

Experiments were conducted from June 2015 to July 2016, under sunny and cloudy day, for 5 hours. As seen in Figure 5a, during the winter months of June and July, solar irradiance levels were low and resulted in complete inactivation of *Escherichia coli* after 5 h of sunlight exposure, with low levels of solar irradiance at the start of the exposure ( $17.6 \text{ Wm}^{-2}$ ). In October and November solar irradiance levels were high enough to result in complete inactivation of bacteria. With high levels of solar irradiance of  $30.12 \text{ Wm}^{-2}$  at the start of the exposure, complete inactivation of *Escherichia coli* was achieved after 4 h of sunlight exposure see Figure 2a. However, on cloudy days in summer, solar irradiance levels were low ( $15 \text{ Wm}^{-2}$ ) at the start of exposure and increased and decreased during exposure, possibly due to cloud conditions see Figure 2b. This resulted in incomplete *Escherichia coli* inactivation since the solar irradiance was not sufficient for the disinfection of clay. Ubomba-Jaswa *et al.* (2010) reported that the presence of clouds decreases the accumulated UVA dose because of diminished UVA irradiance. On a cloudy day, achieving complete inactivation is difficult, especially under low ambient temperature and high wind speed conditions, but UVA is still available to penetrate the clays for inactivation (Saitoh & El-Gethany, 2000; Ubomba-Jaswa *et al.*, 2010). Climatic conditions varied from summer to winter, with ambient temperatures in winter ranging between  $8^{\circ}\text{C}$  and  $23^{\circ}\text{C}$  during the experiments, and between  $11^{\circ}\text{C}$  and  $40^{\circ}\text{C}$  in summer, corresponding to variations in a temperate climate. Clay temperatures reached a maximum of  $36.5^{\circ}\text{C}$  in winter. In summer the clay temperature was not monitored.

### **3.4. Effect of sunlight of *Escherichia coli*, *Salmonella enterica* and naturally occurring microorganism in geophagic clays.**

As seen in Figure 1a, 3 h sunlight exposure was sufficient to decrease the *Escherichia coli* population by a factor of  $10^5$  and the inactivation of *Salmonella enterica* after 3 h sunlight exposure was sufficient to decrease the *Salmonella enterica* population by a factor of  $10^7$  see Figure 3a. However, complete inactivation of *Escherichia coli* was achieved after 4 h see Figure 2a and 5 h see Figure 5a, respectively, without any observed regrowth. The complete inactivation of *Salmonella enterica* exposed directly to sunlight was achieved after

4 h see Figure 3a and b and 3 h see Figure 6a, respectively. After 2 hours of sunlight exposure, the naturally occurring microorganisms from mined clay were completely inactivated, whereas those in the markets clay samples were completely inactivated after 1 hour see Figure 4. High UV irradiance value was observed within 2 hours of the experiment and ranged from 39.5- 30.4 W/m<sup>2</sup> and with a maximum ambient temperature of 40°C. During the winter season the naturally occurring microorganisms were not observed in open marketed clays and mined clays; however, naturally occurring microorganisms in clays were observed in the summer season. Both mined and open marketed samples were contaminated with microorganisms see Figure 4.

The load of naturally occurring microorganisms in mined clays was higher than what was observed in the marketed clays. After geophagic clays are collected from mining sites, they are exposed to sunlight on the market (Fosso-Kankeu *et al.*, 2015). This could explain the inactivation of naturally occurring microorganisms in marketed clays. Henry and Cring (2013) proposed that marketed soil is prepared in a cleaner way; however, in this study microorganisms were found in such clays.

The occurrence of microorganisms in the marketed clays results from unhygienic handling by vendors (Fosso-Kankeu *et al.*, 2015), customers and surrounding regions. Microbial contamination can occur during post-processing handling (unhygienic handling or unsanitary conditions where clay is processed and distributed) (Tano-Debrah & Bruce – Baiden, 2010). Davies and Evison (1991) reported the effectiveness of solar disinfection, which resulted in a 10 times reduction of *Escherichia coli* in 10 h of exposure to sunlight and with a factor of 10<sup>4</sup> inactivation of *Salmonella typhimurium* in 4 h of exposure. Berney *et al.* (2006) suggest that the inactivation mechanism can be different depending of the enteric pathogen. In SODIS treatment *Escherichia coli* was found to be more resistant to the effect of solar radiation and temperature. However, Berney *et al.* (2006) disagree, suggesting that *Salmonella Typhimurium* is more resistant to SODIS treatment.

### **3.5. Regrowth of *Salmonella enterica* and *Escherichia coli***

It is suggested that, after solar disinfection, complete disinfected water should be used within 24 h to avoid the possibility of regrowth (McGuigan *et al.*, 2012). The clay samples exposed for 5 hours were left in the laboratory at the room temperature of 22°C for 1-2 days. The results obtained from regrowth of *Escherichia coli* and *Salmonella enterica* with different initial microorganism concentrations can be seen in Figures 6a and 7a. Bacterial

regrowth can be influenced by storage temperature, water, nutrient and level of disinfection achieved (McGuigan *et al.*, 2012; Giannakis *et al.*, 2014; Vivar & Fuentes, 2016). After inactivation of *Escherichia coli* and *Salmonella enterica* by sunlight, no regrowth was observed.

These results are in agreement with a recent study of regrowth of *Escherichia coli*, which reported no *Escherichia coli* regrowth in the water that had been completely disinfected (Vivar & Fuentes, 2016). Studies reported no regrowth of *Escherichia coli* on completely disinfected water after 2-3 days of storage (Wegelin *et al.*, 1994; Sommer *et al.*, 1997; Oates *et al.*, 2003; Navntoft *et al.*, 2008; Ubomba-Jaswa *et al.*, 2010). The damage on the cells cannot be repaired and recover after the sunlight exposure (Berney *et al.*, 2006; Ubomba-Jaswa *et al.*, 2008). For the water to be considered safe to drink, it must reach a level of 0 CFU/ 100 mL, according to the World Health Organisation Guidelines for Drinking-Water (WHO, 2011). Regrowth of *Escherichia coli* and *Salmonella enterica* was observed in clays exposed in the dark and those incompletely disinfected clays.

The regrowth could be due to the level of disinfection achieved in clays. Fernandez *et al.* (2005) observed the regrowth of *Escherichia coli* K12 in water treated with SODIS after 24 hours at room temperature, and concluded that the reason for regrowth was the presence of viable bacteria in water that was not completely disinfected after sunlight exposure. A residual bacterial concentration of  $10^7$ - $10^6$  CFU/mL regrowth was observed. Regrowth of *Salmonella enterica* and *Escherichia coli* clays are similar for the clays exposed in dark conditions, but significantly different to clays exposed directly to sunlight. The *Escherichia coli* and *Salmonella enterica* inactivation had a low level of regrowth and no bacterial reactivation. Regrowth of bacteria could occur because bacteria are viable if conditions allow the cells to be repaired (McGuigan *et al.*, 1999; Kehoe *et al.*, 2001; Ubomba-Jaswa *et al.*, 2008).

#### **4. Conclusion**

This study confirms that significant inactivation can be achieved using sunlight. Treated clay exposed directly to sunlight achieved complete inactivation (0 CFU/100 mL) of *E. coli*, *Salmonella enterica* and naturally occurring organisms in both the summer and winter days. On cloudy days, 5 h sunlight exposure was sufficient to decrease the bacterial population by  $10^6$ . During sunny day bacteria were completely inactivated within 5 h. Experimental set of clays exposed in dark conditions blocked sunlight rays to reach clays resulting to

incomplete inactivation of *E. coli* and *Salmonella enterica* and regrowth of *Escherichia coli* and *Salmonella enterica* occurred. No regrowth of *Escherichia coli* and *Salmonella enterica* in completely disinfected clays occurred with 24 h and 48 h following sunlight inactivation.

The UV radiation damaged cells of the microorganism (Ubomba-Jaswa *et al.*, 2008) and no regrowth occurred. Ultraviolet (UV) rays and ambient temperature caused an increase of internal energy of clay to reach complete inactivation of bacteria. UV rays reach the earth even on cloudy days, but the strength of UV rays can change seasonally (Wegelin *et al.*, 1994; Herman & Celarier, 1997; Ubomba-Jaswa *et al.*, 2010). The open marketed clay was completely disinfected under 1 h of sunlight exposure because geophagic clay materials in open markets had already been exposed to sunlight before collection. However, recontamination could be avoided in the open markets by placing clay materials in plastics bags.

Completely disinfected treated clays with values of 0CFU/100ML according to World Health Organization drinking-water guidelines could be safely consumed after sunlight exposure as it does not pose any health risk associated with no regrowth of microorganism (Vivar & Fuentes, 2016). Therefore, prolonged exposure is needed for geophagic clays under dark conditions not to pose risks to human health. This study contributes to confirm that using sunlight is safe and freely available for the inactivation of bacteria if applied accurately so that complete inactivation is achieved (0 CFU/100 mL).

## References

- Abrahams, P.W. 2002. Soils: Their implications to human health. *Science of Total Environment*, 291:1-32.
- Baker, K.F. & Cook, J.R. 1974. Biological Control of Plant Pathogens. 1st ed. San Francisco, Freeman.
- Berney, M., Weilenmann, H. & Egli, T. 2006. Flow-cytometric study of vital cellular functions in *Escherichia coli* during solar disinfection (SODIS). *Microbiology*, 152:1719-1729.
- Bisi-Johnson, M.A., Obi, C.L. & Ekosse, G-I.E. 2010. Microbiological and health-related perspectives of geophagia: An overview. *African Journal of Biotechnology*, 9(19): 5784 – 5791.

Brand, C.E., de Jager, L. & Ekosse, G-I.E. 2009. Possible Health Effects Associated With Human Geophagic Practice: An Overview. *Medical Technology SA*, 23(1): 11 - 13.

Callahan, G.N. 2003. Eating Dirt. *Emerging Infect Dis*, 9(8): 1016-1021.

Comerford, N. 2013. Soil science: Soil temperature. <https://soilsmatter.wordpress.com/2013/12/31/155/> Date of access: 25 May 2015.

Conroy, R.M., Elmore-Meegan, M., Joyce, T., McGuigan, K.G. & Barnes, J. 1996. Solar disinfection of drinking water and diarrhoea in Maasai children: a controlled field trial. *Lancet*, 348: 1695–1697.

Conroy, R.M., Meegan, M.E., Joyce, T., McGuigan, K.G. & Barnes, J. 2001. Solar disinfection of drinking water protects against cholera in children under 6 years of age. *Archives of disease in childhood*, 85: 293–295.

Davies, C., & Evison, L. 1991. Sunlight and the survival of enteric bacteria in natural waters. *Journal of Applied Bacteriology*, 70: 265-274.

Dunn, P.H., Barro, S.C. & Poth, M. 1985. Soil moisture affects the survival of microorganisms in heated chapparal soil. *Soil Biology and Biochemistry*, 17(2):143-148.

Ekosse, G.I. & Jumbam, N.D. 2010. Geophagic clays: Their mineralogy, chemistry and possible human health effects. *African Journal of Biotechnology*, 9(40): 6755 – 6767.

Ekosse, G-I.E., de Jager, L. & Ngole, V. 2010. Traditional mining and mineralogy of geophagic clays from Limpopo and Free State provinces, South Africa. *African Journal of Biotechnology*, 9(47): 8058 – 8067.

Fernandez, P., Blanco, J., Sichel, C. & Malato, S. 2005. Water disinfection by solar photocatalysis using compound parabolic collectors. *Catalysis Today*, 101(3): 345–352.

Fosso-Kankeu, E., Netshitanin, T.L., Abia, A.L.K., Ubomba-Jaswa, E. & Waanders F.B. 2015. Application of solar treatment for the disinfection of geophagic clays from markets and mining sites. *African Journal of Technology*, 14(50): 3313-3324.

Geissler, P.W., Mwaniki, D., Thiong'o, F. & Friis, H. 1998. Geophagy as a risk factor for geohelminth infections: a longitudinal study of Kenyan primary school children. *Transactions Royal Society Tropical Medicine & Hygiene*, 92: 7-11.

Giannakis, S., Gamo, A. I.M., Darakas, E., Escalas-Canellas, A. & Pulgarin, C. 2014. Monitoring the post – irradiation *E. coli* survival patterns in environmental water matrices: implications in handling solar disinfected water. *Chemical Engineering journal*, 253:366–376.

Gómez-Cousoa, H., Fontán-Sainza, M., Fernández-Ibáñezb, P. & Ares-Mazása, E. 2012. Speeding up the solar water disinfection process (SODIS) against *Cryptosporidium parvum* by using 2.5 l static solar reactors fitted with compound parabolic concentrators (CPCs). *Acta Tropica*, 124:235–242.

Grace, G. & Adams, A. 2012. Physiological effects of geophagy (soil eating) with reference to iron nutritional status in pregnant women: a study in selected antenatal clinics in KSD municipal area of the Eastern Cape, South Africa. (*In* 2012 3rd International Conference on Biology, Environment and Chemistry: IPCBEE 46(21). Singapore: IACSIT Press. p. 96-98).

Herman, J.R. & Celarier, E.A.L. 1997. Earth surface reflectivity climatology at 340nm to 380nm from TOMS Data. *Journal of Geophysical Research*, 102: 28003-28011.

Henry, J.M. & Cring, D. 2013. Geophagy: an anthropological perspective E.C. (*In*: Brevik, L.C. Burgess., eds. *Soils and Human Health*. USA: CRC Press Taylor & Francis Group. p. 179–198).

Kawai, K., Saathoff, E., Antelman, G., Msamanga, G. & Fawzi, W.W. 2009. Geophagy (Soil-eating) in Relation to Anemia and Helminth Infection among HIV–Infected Pregnant Women in Tanzania. *The American Journal of Tropical Medicine and Hygiene*, 80(1): 36 – 43.

Kehoe, S.C., Joyce, T.M., Ibrahim, P., Gillespie, J.B., Shahar, R.A. & McGuigan, K.G. 2001. Effect of agitation, turbidity, aluminum foil reflectors and container volume on the inactivation efficiency of batch process solar disinfectors. *Water Res*, 35:1061–1065.

Keogh, M.B., Castro-Alferez, M., Polo-Lopez, M.I., Fernandez Calderero, I., Al-Eryani, Y.A., Joseph-Titus, C., Sawant, B., Dhodapkar, R., Mathur, C., McGuigan, K.G. & Fernández-Ibañez, P. 2015. Capability of 19-litre polycarbonate plastic water cooler containers for efficient solar water disinfection (SODIS): field case studies in India, Bahrain and Spain. *Solar Energy*, 116: 1– 11.

Lar, U.A., Agene, J.I. & Umar, A.I. 2015. Geophagic clay materials from Nigeria: a potential source of heavy metals and human health implication in mostly women and children who practice it. *Environmental Geochemical Health*, 37: 363-375.

Mahrer, Y. 1979. Predictions of soil temperature of a soil mulched with transparent polyethylene. *Journal of Applied Meteorology*, 18: 1263-1267.

Mani, S.K. 2006. Development and evaluation of small-scale systems for solar disinfection of contaminated drinking water in India. Newcastle-upon-Tyne, UK: Northumbria University. (Thesis-PhD).

McGuigan, K.G., Joyce, T.M. & Conroy, R.M. 1999. Solar disinfection: use of sunlight to decontaminate drinking water in developing countries. *Journal of Medical Microbiological*, 48: 785-787.

McGuigan, K.G., Joyce, T.M., Conroy, R.M., Gillespie, J.B. & Elmore-Meegan, M. 1998. Solar disinfection of drinking water contained in transparent plastic bottles: characterizing the bacterial inactivation process. *Journal Applied Microbiology*, 84: 1138–1148.

McGuigan, K.G., Conroy, R.M., 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. *Journal of hazardous materials*, 235-236, 29-46

Navntoft, C., Ubomba-Jaswa, E., McGuigan, K.G. & Fernández-Ibañez, P. 2008. Effectiveness of solar disinfection using batch reactors with non-imaging aluminium

reflectors under real conditions: natural well water and solar light. *Journal Photochemical and Photobiology B: Biology*, 93 (3): 155–161.

Oates, P., Shanaman, P. & Polz, M. 2003. Solar disinfection (SODIS): simulation of solar radiation for global assessment for point of use water treatment in haiti'. *Water Res*, 37: 47–54.

Rainey, R.C. & Harding, A.K. 2005. Drinking water quality and solar disinfection: effectiveness in peri-urban households in Nepal. *Journal of Water Health*, 3:239–248.

Reilly, C. & Henry, J. 2000. Why do humans consume soil? *Nutrition Bulletin*, 25: 141–144.

Rincon, A.G. & Pulgarin, C. 2003. Photocatalytical inactivation of *E. coli*: effect of (continuous-intermittent) light intensity and of (suspended fixed) TiO<sub>2</sub> concentration. *Applied Catalysis. B – Environment*, 44: 263–284.

Rokjo, C. 2003. Solar disinfection of drinking water. Potchefstroom: North-West University. (Thesis: Master).

Saitoh, T.S. & El-Ghetany, H. H. 2000. Exposure time as a disinfecting index in a solar water disinfecting system. *IECEC 35th Intersociety*, 2:1162–1166

Sciacca, F., Rengifo-Herrera, J.A., Wethe, J. & Pulgarin, C. 2011. Solar disinfection of wild *Salmonella sp.* in natural water with an 18 L CPC photoreactor: Detrimental effect of non-sterile storage of treated water. *Solar energy*, 85:1399-1408.

(SODIS) Solar water disinfection. 2002. A guide for the application of SODIS. *SANDEC report*, 6(02):1-88.

Sommer, B., Marinõ, A., Solarte, Y., Salas, M.L., Dierolf, C. & Valiente, C. 1997. SODIS – an emerging water treatment process. *Journal Water Supply Res Technol.-Aqua*, 46 (3): 127–137.

Smith, R.J., Kehoe, S.C., McGuigan, K.G & Barer, M.R. 2000. Effect of simulated solar disinfection of water on infectivity of *Salmonella typhimurium*. *Letter Applied Microbiology*, 31: 284–288.

Tano-Debrah, K. & Bruce-Baiden, G. 2010. Microbiological characterization of dry white clay, a pica element in Ghana. *Nature Science Report and Opinion*, 2(6): 77-81.

Ubomba-Jaswa, E., Boyle, M. A. R. & McGuigan, K. G. 2008. Inactivation of enteropathogenic *E. coli* by solar disinfection (SODIS) under simulated sunlight conditions. *Journal of Physics: Conference Series*, 101: 1-4.–595.

Ubomba-Jaswa, E., Fernánde z-Ibañ ez, P., Navntoft, C., Polo-Lo´pez, M. & McGuigan, K.G. 2010. Investigating the microbial inactivation efficiency of a 25L batch solar disinfection (SODIS) reactor enhanced with a compound parabolic collector (CPC) for household use. *Journal Chemistry Technology Biotechnology*, 85: 1028–1033.

Vivar, M. & Fuentes, M. 2016. Using solar disinfected water: On the bacterial regrowth over 1-week of water usage including direct intake after sun exposure and long-term dark storage. *Solar energy*, 131:138-14.

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. *Journal Water SRT-Aqua*, 43: 154-169.

WHO (World Health Organization). 2011. Guidelines for Drinking Water Quality. 4th Ed. World Health Organization.

Winfield, M.D. & Groisman, E.A. 2003. Role of Nonhost environments in the lifestyles of *Salmonella* and *Escherichia coli*. *Applied Environmental Microbiology*, 69: 3687–3694.

# Chapter 6 Conclusion and recommendations

## 6.1 Introduction

This Chapter provides the conclusion and recommendations on the research study. General conclusions are presented, based on the outcome of the experimental study. This study provided valuable insights into the use of solar treatment and sunlight to lower the microbial contaminants in geophagic clays of the Gauteng and North-West provinces of South Africa. The main findings of this study are reported here, followed by recommendations that can possibly improve the scientific value of the study in the future.

## 6.2 General conclusion

Geophagic practice has a high prevalence in South Africa. Different clays are preferred according to colour, texture, smell and source. Geophagic clay materials collected from Pretoria and Potchefstroom, South Africa, in open markets and original sources, showed that it contained different levels of mineral nutrients including, calcium, potassium, zinc, phosphorus, magnesium, iron, and copper.

The chemical composition of geophagic clay materials showed that geophagic clays from the source contained almost similar contents of major oxides than open market geophagic clay materials (Ikageng source contained 55.62 mg/kg SiO<sub>2</sub>, 26.07 Al<sub>2</sub>O<sub>3</sub>, 0.41 MgO, and 12.6 FeO while Ikageng market clays contained 55.37 mg/kg SiO<sub>2</sub>, 25.55 Al<sub>2</sub>O<sub>3</sub>, 0.39 MgO and 13.45 FeO). Geophagic clay materials are known to provide an important source of essential elements (minerals or trace elements).

Phelandavha and Pheramindi clay samples contained low levels of Zn and other oxides including MgO, CaO, and NaO. Pheramindi clay samples contained amounts of Pb presenting a risk of lead toxicity because even a low level of lead can be harmful to humans. In addition, depending on the type of clay eaten and the source obtained from, people who practice geophagia may generally be exposed to different levels of chemical elements.

The geophagic clays samples were reported to contain kaolinite, quartz, muscovite, and bentonite. The dominance of quartz in all geophagic clays corresponds to a high amount of SiO<sub>2</sub> and Al oxides. Kaolinite mineral was found in all the samples except in Ikageng

samples. The results of XRF are in agreement with the presence of kaolinite because of a high  $\text{SiO}_2/\text{Al}_2\text{O}_3$ , and less alkali, as revealed by the chemical results. The mineralogical composition of the clays appears similar and therefore suggests the same source as the market samples. The results of mineralogical analyses, with ratios of  $\text{SiO}_2/\text{Al}_2\text{O}_3$  ranging from 1.53 to 3.33 signified the presence of siliceous sands resulting in high quartz content.

Large amounts of nutrients were released from the clay over time (Fe of 13.8 mg/kg). These results suggest that significant amounts of mineral nutrients are bioavailable after consumption of the clay, contributing to mineral nutrition such as zinc, iron and calcium. The clay minerals can potentially increase absorption of bioavailable nutrients, particularly Pb, Co, Ca, and Fe, and geophagia can adversely affect human health. When the clay is ingested, there is a release of metals, nutrients and microorganisms and in this study large amounts of metals were found to be released in the acidic environment. Decrease of bacterial activity was observed with increase of metal concentration at low pH. The acidity of the gastrointestinal tract inhibits the growth of bacteria and promotes metal release. However, the geophagic clay materials have to be processed to reduce microorganisms in the clays and the source of clay need to be examined for the presence of metals such as lead (Pb).

Clays exposed in dark conditions with low clay temperature were incompletely disinfected. No regrowth of bacteria occurred within 24 h and 48 h after the complete disinfection of clays exposed directly to sunlight. However, regrowth of microorganisms in clay kept in dark conditions occurred within 24 h and 48 h. The naturally occurring microorganisms showed a far higher sensitivity to sunlight than *Escherichia coli* and *Salmonella enterica*. Geophagic clays that had been exposed directly to sunlight were completely disinfected (values of 0 CFU/mL), and are therefore considered safe for consumption according to WHO drinking water guideline with respect to microbial quality. It poses no health risk associated with potential regrowth of *Escherichia coli* and *Salmonella enterica*. This study confirms that significant inactivation of microorganisms in geophagic clays can be achieved using sunlight exposure, which is an environmentally friendly, sustainable and affordable. It was expected that sunlight can disinfect microorganism in geophagic clays. The study proved that when geophagic clays are exposed for at least 7 hours, complete inactivation of microorganism can be achieved

To conclude the study the purpose of the study was to identify the contaminants in geophagic clays and to reduce the amount of microorganisms in the clays using solar treatment and sunlight. Moreover, these identified microorganisms were destroyed by simulated UV-A lights in a chamber and low amount of water contents caused death to the microorganism because of lack of nutrients. Furthermore, naturally occurring microorganism, *Escherichia coli*, and *Salmonella enterica* were disinfected in geophagic clays when exposed directly to sunlight and no regrowth was observed. Minerals and trace of metals were identified in the geophagic clays and high levels of metals leached from geophagic clays using simulated gastric fluid pose a health risk to human health.

### **6.3 Recommendations**

From this study the following recommendations can be made for future studies.

- There is a need to educate the vendors about the need to process and store the clay sold in the market.
- Health education is required to raise awareness of the health risks of geophagia (microbiological infection, gastro-intestinal problems, heavy metal poisoning, and dental damage) and to improve the microbiological quality of clay by pre-treatment before consumption (heating, burning, cooking, and baking).
- Regarding the mining sites of geophagic clays the vendors should be advised to collect the clays from locations far from industrial areas, dumping sites and contaminated areas.
- Future investigation should also focus on other treatments that vendors use and compare them with solar treatment and sunlight treatments.
- An assessment of the microbiological contents of geophagic clays sold around South Africa should be made in future. Not all soil is safe to ingest.
- Further studies are required to investigate the effect of sunlight on geophagic clays conducted according to the different sets the vendors uses in markets

## Appendix A



**Figure 1.1:** Geophagic clay materials exposed directly to sunlight at the open markets at Gauteng local markets. Pheramindi and Phelandavha markets samples. Photograph taken by Thembuluwo (2015)



**Figure 1.2:** Geophagic clay materials mined in large quantities from the respective sources, ready to be sold to others vendors at Gauteng local markets. Photograph taken by Thembuluwo (2015).



**Figure 1.3:** Geophagic clay materials packed in the plastics bags ready to be sold to the geophagists at Gauteng local markets. Photograph taken by Thembuluwo (2015)



**Figure 1.4:** Geophagic clay mining sites at Pretoria. Photograph taken by Thembuluwo (2015).



**Figure 1.5:** Geophagic soil mining sites at Potchefstroom. Photograph taken by Thembuluwo (2015).

## Appendix B

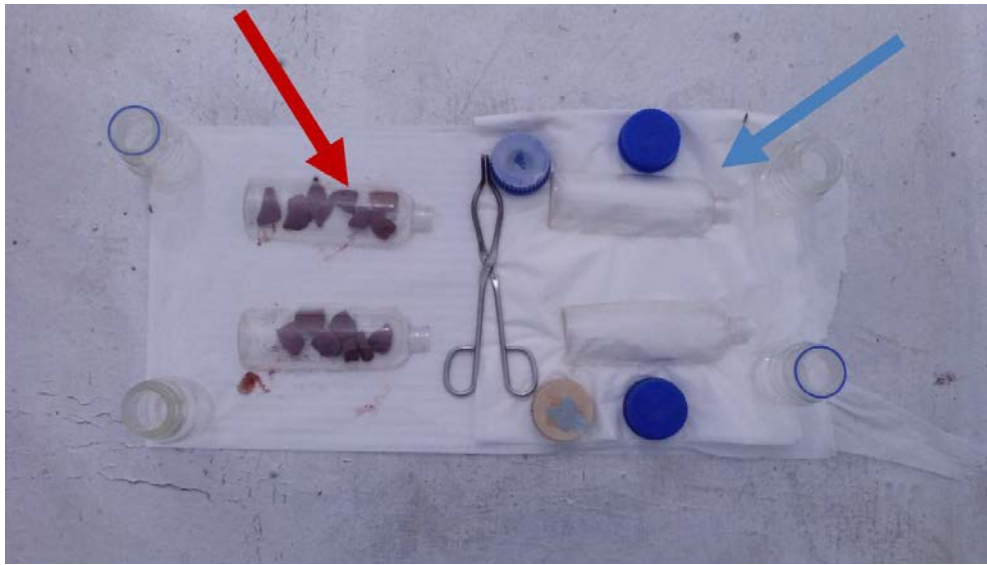


**Figure 2.1:** The experimental set up of inactivation of *Escherichia coli*, *Salmonella enterica* and naturally microorganism in geophagic clays exposed directly to sun and in dark conditions. Red arrow represents experimental set up of geophagic clays exposed directly to sunlight and blue arrow is experimental set up of geophagic clays exposed under dark conditions.

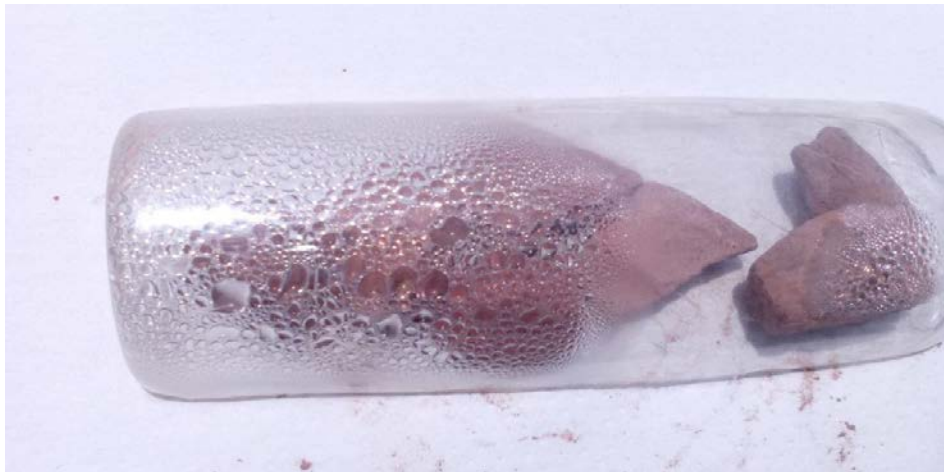


**Figure 2.2:** Moisture reaction in geophagic clays exposed directly to sunlight.