Copyright and Citation Considerations for This Thesis/Dissertation

Creative Commons

- Attribution — You must give appropriate credit, provide a link to the license, and indicate if changes were made. You may do so in any reasonable manner, but not in any way that suggests the licensor endorses you or your use.

- NonCommercial — You may not use the material for commercial purposes.

- ShareAlike — If you remix, transform, or build upon the material, you must distribute your contributions under the same license as the original.

How to cite this thesis

DISSOLUTION OF MINERALS FROM GEOPHAGIC CLAYS FROM SELECTED AREAS AND THEIR PROBIOTICS BEHAVIOURS.

BY
OKEREAFOR GODWIN UCHENNA
Submitted in Fulfilment of the Requirements for the Degree of
MAGISTER TECHNOLOGIAE:
EXTRACTION METALLURGY
In the
Faculty of Engineering and the Built Environment
University of Johannesburg
Supervised by
Professor Antoine F Mulaba-Bafubiandi
Dr. Vuyo Mavumengwana
January, 2017
EXECUTIVE SUMMARY

Clay and its associated minerals are crucial materials with various applications ranging from industrial, health to traditional activities. Since time immemorial, the application of clays for traditional and health purposes have remained evident amongst Africans and rapidly gaining grounds in the world at large. Indigenous knowledge has constantly supported geophagia – the deliberate consumption of earthy material for several benefits such as mineral supplement to the body and treatment of diseases. In Southern Africa, geophagia is becoming a common practice irrespective of the social class, age and religion of the geophagists. Few reports on the characterisation of geophagic clays are available but none had considered probiotics and volatile fatty acids. Characterisation plays a vital role in the study of geophagic clays, as it provides useful information about the safety of its application by humans. The quality of such earthy material had been solely based on characterisation, which stirs issues of safety and environmental health. It was therefore imperative to consider other studies beside characterisation of geophagic clays to better appreciate the effects upon consumption by humans.

Probiotics are known to be beneficial as they help maintain a healthy gut in humans. The effect of clays on these microorganisms and the possible impacts on humans is necessary. This current research study considered the application of Atomic absorption spectroscopy (AAS) together with Infrared fluorescence (XRF) and Infrared diffraction (XRD) as well as a front-line chromatographic and mass spectrometric analytical technique, i.e. two dimensional gas chromatography coupled with a time-of-flight mass spectrometry (GC×GC-TOFMS) for investigating the production of volatile fatty acids (VFAs) by the probiotics upon interaction with geophagic clays.

The first phase of the study considered the physicochemical characterisation of geophagic clays sold in informal South African markets using 2g of the various samples moistened with 100mL of 0.5M, 1M and 2M HCl acid. The subsequent analysis of the filterate on AAS revealed the different clay samples containing several heavy metals such as copper (Cu), lead (Pb), zinc (Zn), iron (Fe), cobalt (Co), manganese (Mn), chromium (Cr). These heavy metals have been reported to have deleterious effects on humans when above the minimum permissible level. Further analysis using infrared fluorescence indicated average values of major elements such as SiO$_2$ (54.02%), Al$_2$O$_3$...
(35.45%), Fe$_2$O$_3$ (6.73%), K$_2$O (2.76%), MgO (1.16%) with MnO, Na$_2$O and P$_2$O$_5$ falling below 0.5%. Although the potential medicinal application of these clayey soils is supported by their kaolinite contents, titanium was a major heavy metal identified from the samples which may result in heavy metal toxicity. In a subsequent experiment, an attempt was made to isolate microorganisms (using nutrient agar media) from the different pulverised geophagic clay samples collected from informal markets to ascertain the potential pathogenic exposure of individuals who consume these materials. Over and above this, studies were conducted to ascertain the effect of these clay materials on *Lactobacillus fermentum* and to evaluate the impact they have on the production of VFA’s by these probiotics. Considering that these clays are consumed as is when purchased from the open market, the results revealed that the clays had opportunistic microorganisms that are likely to infect immunocompromised individuals such as children, unborn babies (exposed through pregnant women), HIV infected persons and the elderly. These pathogens cause damage to their host via invasion, colonisation and growth, and use several strategies to establish virulence; (the relative ability of a pathogen to cause disease in the host). Some of the identified pathogenic microorganisms included *Achromobacter* specie, *Acinetobacter* specie, *Actinobacillus* specie, *Agrobacterium* specie, *Enterobacter* specie and *Pseudomonas* specie.

In conclusion, our findings validate various indigenous knowledge on the application of geophagic clays for health purposes such as nutrient supplements and treatment of certain diseases such as diarrhea. The geology of the area where clay is found affects or determines the physiological and mineralogical contents of the clay. The handling of clay exposes it to harmful pathogenic microbes. The ingestion of clay affects the production of VFAs such as Ethyl chloride, Methyl formate, Propanoic acid, 2-oxo-Butane, 2-methyl- by probiotics (*Lactobacillus fermentum*) that are likely to be found in the large intestines.

As much as people claim that ingestion of clay has no negative side effects on humans, over a long period of time negative health effects do arise due to the pathogenic microorganisms present, accumulation of heavy metals in the body and the alteration of the production of VFAs by probiotics in the body.

**Key words:** Geophagic clay, GC×GC-TOFMS, heavy metals, probiotics, volatile fatty acids.
DECLARATION

By submitting this dissertation, I the undersigned, hereby declare that the work contained herein completely belongs to me and as such, I am the sole author thereof. In the event that there is a reproduction and publication in parts or whole thereof by the University of Johannesburg, I will not infringe upon any third party rights and I have not at any time previously, in its entirety or in part, submitted this dissertation for obtaining any qualification.

Signature: ..................................................

Date: ............./.........../......................
DEDICATION

I dedicate this work to the Almighty God, for His grace. Also to my wife and family for their love and support.
ACKNOWLEDGEMENTS

My profound gratitude firstly goes to God Almighty who has continued to inspire me despite the various bottle-necks during the period of this study.

Special acknowledgement is also given to my supervisor, Professor Antoine Mulaba-Bafubiandi who saw the potentials in me. Thank you for the opportunity to study within your research group. To my amiable co-supervisor Dr. Vuyo, words alone cannot describe my heartfelt gratitude for the roles you played throughout my study.

I say merci to Dr. Clarence Yah for introducing me to the University of Johannesburg and setting the pace. Dr. & Mrs. Obinna Ozumba, many thanks for your words of encouragement. Prof. Gabriel Adegoke, Dr. Derek Ndinteh, Dr. Eugenie Kayitesi and Dr. Obinna Nwiyi I am most grateful for your kind words.

My darling wife – Nkemdinma, thank you for always being by me especially at moments when it seems the world was on my shoulders. My beloved father – Mr Daniel Uchewuakor Okereafor and siblings – Samuel, Christian, Ugonna, Ngozi, and Bright, I appreciate your total confidence in me. To my in-laws, Chief (Sir) & Lady Luke Dike, thank you for all your prayers. Mr. Davidson Kotegbe, Mr. Jeffrey Idemudia, Mr. Akintunde Olusola and Mr. Timothy Oludare, words alone can not express how much I appreciate your support.

Pastor & Deac. Franly Nkulu, Pastor Alfred Enitayo, Jude Von Benecke, Eric Kyngombe, Imani and Akila Nkulu, thank you for sowing the seed of determination in me.

This study would not have materialised without the huge support of colleagues - Dr. Biswas and Edward Malenga of the Mineral Processing and Technology Research Centre of the Department of Metallurgy, University of Johannesburg. To Mr. Alastair Campbell, I say a big thank you for allowing me use the Biotechnology laboratories. Special thanks goes to the following: Nomsa Baloyi, Phindile Hlekane, Fanoro Mokesioluwa, Vitalis Aguba, Adeline Megne, Tendani Sebola, Bongeka Mbambo, Paul Nwaiwu, Tsakani Muohshwana, Nomfundo, Adebo Oluwafemi, Olusola Ayeleru, Chukwu Daniel, Babatope Adebiyi, Ifeoluwa Oluto, Sharon Pelo, Amarachi Ugorji,
Sefater Gbashi and Evonia Nchabeleng, for their supports and kind words. You all rocked my world.

I would also like to say a special word of gratitude to Dr. Njobeh for his financial support and encouragement. To my mentors, Dr Khanyi and Dr Mathe, I appreciate you support.

Special appreciation to my friend Bubele Vuba for his motivation and endless encouragement.

Finally, special appreciation to Zandile Funde who took out time to peruse the entire dissertation before the submission of the first draft.
ARTICLES PUBLISHED OR WRITTEN FOR PUBLICATION

Journal Papers


* Okereafor GU, Mulaba-Bafubiandi AF, Biswas S, Mavumengwana V, *Clay minerals and clayey soil as possible microorganism repository*, Transactions of the Royal Society of South Africa, Accepted TTRS-2016-0046. R2

Conference Papers

# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>TITLE PAGE</td>
<td>1</td>
</tr>
<tr>
<td>EXECUTIVE SUMMARY</td>
<td>2</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>4</td>
</tr>
<tr>
<td>DEDICATION</td>
<td>5</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>6</td>
</tr>
<tr>
<td>ARTICLES PUBLISHED OR WRITTEN FOR PUBLICATION</td>
<td>8</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>9</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>14</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>15</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>16</td>
</tr>
<tr>
<td>LIST OF SYMBOLS AND UNITS</td>
<td>18</td>
</tr>
<tr>
<td>DISSERTATION OUTLINE</td>
<td>19</td>
</tr>
<tr>
<td>CHAPTER ONE</td>
<td>21</td>
</tr>
<tr>
<td>1.0 General Introduction</td>
<td>21</td>
</tr>
<tr>
<td>1.1 Background</td>
<td>21</td>
</tr>
<tr>
<td>1.2 Rationale</td>
<td>21</td>
</tr>
<tr>
<td>1.3 Hypothesis</td>
<td>24</td>
</tr>
<tr>
<td>1.4 Research Aim(s) and Objectives</td>
<td>24</td>
</tr>
<tr>
<td>1.5 Scope of the Study</td>
<td>24</td>
</tr>
<tr>
<td>1.6 Study site</td>
<td>25</td>
</tr>
<tr>
<td>References</td>
<td>26</td>
</tr>
<tr>
<td>CHAPTER TWO</td>
<td>28</td>
</tr>
<tr>
<td>2.0 LITERATURE REVIEW</td>
<td>28</td>
</tr>
<tr>
<td>Abstract</td>
<td>28</td>
</tr>
<tr>
<td>2.1 Introduction</td>
<td>29</td>
</tr>
<tr>
<td>2.2 Clayey Soils: Origin and mode of Formation</td>
<td>31</td>
</tr>
<tr>
<td>2.3 Physical and chemical properties of clays</td>
<td>32</td>
</tr>
<tr>
<td>2.4 Clay minerals and their classification</td>
<td>33</td>
</tr>
<tr>
<td>2.5 Properties of clay minerals</td>
<td>35</td>
</tr>
</tbody>
</table>
CHAPTER THREE ................................................................................................................................. 67

3.0 Mineralogical Profile of Geophagic Clayey Soils Sold in Selected South African Informal Markets.......................................................................................................................... 67

Abstract ................................................................................................................................................. 67

3.1 Introduction ...................................................................................................................................... 68

3.2 Experimental methods ....................................................................................................................... 69

3.2.1 Materials ........................................................................................................................................ 69

3.2.2 Digestion of clay samples ................................................................................................................. 70

3.2.3 Loss of ignition (LOI) ....................................................................................................................... 70

3.2.4 Determination of oxides using X-ray fluorescence (XRF) ................................................................. 70

3.2.5 Determination of the mineral phases using X-ray diffractometer (XRD) ........................................ 70

3.2.6 Determination of trace Elements Using Atomic Absorption Spectrophotometer (AAS) .................. 71

3.3 Results .............................................................................................................................................. 71

3.3.1 Physico-chemical characterization of geophagic clays ................................................................... 71

3.4 Discussion ....................................................................................................................................... 74

3.4.1 Colour ........................................................................................................................................... 74

3.4.2 Texture ........................................................................................................................................... 75

3.4.3 pH.................................................................................................................................................... 76

3.4.4 Electrical conductivity (EC) ............................................................................................................. 77

3.4.5 Loss of ignition (LOI) ....................................................................................................................... 78

3.4.6 Physico-chemical characterization ................................................................................................. 78

3.4.7 Mineral phases ............................................................................................................................... 80

3.5 Conclusion ...................................................................................................................................... 85

3.6 Acknowledgment ............................................................................................................................... 86

References .............................................................................................................................................. 86

CHAPTER FOUR..................................................................................................................................... 91

4.0 THE EFFECTS OF AN ACIDIC ENVIRONMENT ON SELECTED GEOPHAGIC CLAYEY SAMPLES .......................................................................................................................... 91

Abstract ................................................................................................................................................. 91

4.1 Introduction ...................................................................................................................................... 92

4.2 Methods ......................................................................................................................................... 94
4.2.1 Clay soil samples ........................................................................................................94
4.2.2 Hydrochloric acid extraction of minerals .................................................................94
4.2.3 Mineral Analysis .........................................................................................................94
4.2.4 Determination of oxides using X-ray fluorescence (XRF) .........................................95
4.2.5 Loss of Ignition ...........................................................................................................95
4.3 Results ..........................................................................................................................95
4.4 Discussion .......................................................................................................................97
4.5 Conclusions ....................................................................................................................100
References ...........................................................................................................................101

CHAPTER FIVE ..................................................................................................................105
5.0 CLAY MINERALS AND CLAYEY SOILS AS POSSIBLE MICROORGANISMS REPOSITORY .........................................................................................................................105

Abstract ..............................................................................................................................105
5.1 Introduction .....................................................................................................................106
5.2 Materials and methods .................................................................................................108
  5.2.1 Clay samples and study area ......................................................................................108
  5.2.2 Soil analysis ...............................................................................................................108
  5.2.3 Digestion of clay samples ..........................................................................................108
  5.2.4 Loss of ignition (LOI) ...............................................................................................109
  5.2.5 Determination of oxides using XRF .........................................................................109
  5.2.6 Determination of the mineral phases using X-ray diffractometer (XRD) ...............109
  5.2.7 Determination of trace elements using atomic absorption spectrophotometer (AAS) .................................................................................................................................109
  5.2.8 Culturing of microorganisms from soil sample .......................................................110
  5.2.9 Isolation of Bacterial and Fungal Isolates ...............................................................110
  5.2.10 Morphological and Biochemical Characterization of the Bacteria Isolates ............110
5.3 Results and discussion ..................................................................................................110
  5.3.1 Physical characterisation .........................................................................................110
  5.3.2 Chemical Characterisation (XRF) ............................................................................111
  5.3.3 Mineralogical characterisation (XRD) .......................................................................113
  5.3.4 Identification of bacterial isolates ............................................................................114
5.3.5 Pathogenicity and toxicity of identified microorganisms .................. 116
5.4 Conclusion ...................................................................................... 117
References .......................................................................................... 118
CHAPTER SIX ....................................................................................... 121
6.0 GC×GC-TOFMS ANALYSIS OF VOLATILE FATTY ACIDS PRODUCED BY Lactobacillus fermentum WHEN IN CONTACT WITH GEOPHAGIC CLAY .......... 121
Abstract ............................................................................................ 121
6.1 Introduction .................................................................................... 122
6.2 Materials and methods .................................................................. 123
  6.2.1 Sample Collection ..................................................................... 123
  6.2.2 Sample Preparation .................................................................. 123
  6.2.3 Instrumental Conditions .......................................................... 124
6.3 Results ........................................................................................... 125
6.4 Discussions ................................................................................... 129
6.5 Conclusion ..................................................................................... 131
References .......................................................................................... 132
CHAPTER SEVEN ................................................................................ 135
7.0 GENERAL DISCUSSION AND CONCLUSION ........................................ 135
7.1 General discussion .......................................................................... 135
7.2 Recommendations .......................................................................... 136
References .......................................................................................... 137
APPENDIX ......................................................................................... 138
LIST OF FIGURES

Figure 2.1: Classification of Silicates ................................................................. 36
Figure 3.1 X-ray diffractogram of geophagic soil (GSA1) indicating mineral peaks .......... 83
Figure 3.2 X-ray diffractogram of geophagic soil (GSA2) indicating mineral peaks .......... 83
Figure 3.3 X-ray diffractogram of geophagic soil (GSA3) indicating mineral peaks .......... 84
Figure 3.4 X-ray diffractogram of geophagic soil (GSA4) indicating mineral peaks .......... 84
Figure 3.5. X-ray diffractogram of geophagic soil (GNG1) indicating mineral peak .......... 84
Figure 3.6. X-ray diffractogram of geophagic soil (GNG2) indicating mineral peaks......... 85
Figure 5.1: X-ray diffractogram of representative clayey soil indicating mineral peaks ....... 114
LIST OF TABLES

Table 2.1 Differences between Clay and Clay mineral ................................................................. 36
Table 2.2 Clay minerals associated with geophagic, cosmetic/medicinal and pottery applications ........................................................................................................................................ 56
Table 3.1: Physical characterization of geophagic clay samples .................................................... 72
Table 3.2: Colour and macroscopic description of 6 geophagic samples from informal markets.72
Table 3.3: Chemical composition in oxide state of geophagic clayey soils obtained from open markets .................................................................................................................................... 73
Table 3.4: Chemical composition in elemental state of geophagic clayey soils............................ 74
Table 3.5: Trace element composition of geophagic clayey soils .................................................. 74
Table 3.6: Mineral phases ............................................................................................................. 81
Table 4.1: Chemical composition of solid clay samples .................................................................. 96
Table 4.2: Trace element contents of clayey soil before treatment with hydrochloric acid ....... 97
Table 4.3: Acid extractable mineral contents of clayey soil ingested by geophagists in Johannesburg ........................................................................................................................................ 97
Table 5.1. Physical characterization of geophagic clayey samples ............................................. 111
Table 5.2. Major oxides analysis of clayey soil samples ................................................................. 112
Table 5.3. Trace Element composition of clayey soil samples ....................................................... 112
Table 5.4. Mineralogical composition of clayey soil samples ....................................................... 113
Table 5.5: Bacteria associated with the clay samples .................................................................... 115
Table 5.6. Diversity of bacterial phylum identified from the clay samples .................................... 115
Table 5.7: Microbial classes composition detected in the clay samples ..................................... 116
Table 5.8 Pathogenicity and toxicity of identified microorganisms .............................................. 116
Table 6.1: Results showing the effects of geophagic clay on Lactobacillus fermentum probiotic. ........................................................................................................................................... 126
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAS</td>
<td>Atomic absorption spectrophotometer</td>
</tr>
<tr>
<td>AD</td>
<td>After death</td>
</tr>
<tr>
<td>Al</td>
<td>Aluminium</td>
</tr>
<tr>
<td>As</td>
<td>Arsenic</td>
</tr>
<tr>
<td>ATCC</td>
<td>American type culture collection</td>
</tr>
<tr>
<td>BC</td>
<td>Before Christ</td>
</tr>
<tr>
<td>Ca</td>
<td>Calcium</td>
</tr>
<tr>
<td>Ca⁺</td>
<td>Calcium ion</td>
</tr>
<tr>
<td>CAPD</td>
<td>Central auditory processing disorder</td>
</tr>
<tr>
<td>Cd</td>
<td>Cadmium</td>
</tr>
<tr>
<td>CEC</td>
<td>Cation exchange capacity</td>
</tr>
<tr>
<td>Cu</td>
<td>Copper</td>
</tr>
<tr>
<td>EC</td>
<td>Electrical conductivity</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agricultural Organisation</td>
</tr>
<tr>
<td>Fe</td>
<td>Iron</td>
</tr>
<tr>
<td>GC</td>
<td>Gas chromatography</td>
</tr>
<tr>
<td>GC×GC-TOFMS</td>
<td>Two Dimensional Gas chromatography coupled to time of flight mass spectrometry</td>
</tr>
<tr>
<td>GIT</td>
<td>Gastrointestinal tract</td>
</tr>
<tr>
<td>H</td>
<td>Hydrogen</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>Hg</td>
<td>Mercury</td>
</tr>
<tr>
<td>HIV/AIDS</td>
<td>Human Immunodeficiency Virus/ Acquired Immunodeficiency Syndrome</td>
</tr>
<tr>
<td>H₂O</td>
<td>Water</td>
</tr>
<tr>
<td>K⁺</td>
<td>Potassium ion</td>
</tr>
<tr>
<td>LOI</td>
<td>Loss of ignition</td>
</tr>
<tr>
<td>Mg</td>
<td>Magnesium</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
</tr>
<tr>
<td>Na</td>
<td>Sodium</td>
</tr>
<tr>
<td>Na⁺</td>
<td>Sodium ion</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>O</td>
<td>Oxygen</td>
</tr>
<tr>
<td>OH</td>
<td>Hydroxyl</td>
</tr>
<tr>
<td>OM</td>
<td>Organic matter</td>
</tr>
<tr>
<td>Pb</td>
<td>Lead</td>
</tr>
<tr>
<td>RDA</td>
<td>Recommended daily allowance</td>
</tr>
<tr>
<td>Sb</td>
<td>Antimony</td>
</tr>
<tr>
<td>Se</td>
<td>Selenium</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscope</td>
</tr>
<tr>
<td>Si</td>
<td>Silicon</td>
</tr>
<tr>
<td>TO</td>
<td>1:1</td>
</tr>
<tr>
<td>TOT</td>
<td>2:1</td>
</tr>
<tr>
<td>USDA</td>
<td>United States Department of Agriculture</td>
</tr>
<tr>
<td>UTI</td>
<td>Urinary tract infection</td>
</tr>
<tr>
<td>VFAs</td>
<td>Volatile fatty acids</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
<tr>
<td>WRC</td>
<td>Water retention capacity</td>
</tr>
<tr>
<td>XRD</td>
<td>X-ray powder diffraction</td>
</tr>
<tr>
<td>XRF</td>
<td>X-ray fluorescence</td>
</tr>
<tr>
<td>Zn</td>
<td>Zinc</td>
</tr>
</tbody>
</table>
# LIST OF SYMBOLS AND UNITS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Abbreviation</th>
<th>Unit/Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Å</td>
<td>Angstrom</td>
<td></td>
</tr>
<tr>
<td>α</td>
<td>Alpha</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>Percentage</td>
<td></td>
</tr>
<tr>
<td>±</td>
<td>Plus or minus</td>
<td></td>
</tr>
<tr>
<td>&lt;</td>
<td>Less than or equal to</td>
<td></td>
</tr>
<tr>
<td>cm</td>
<td>Centimeter</td>
<td></td>
</tr>
<tr>
<td>°C</td>
<td>Degree Celsius</td>
<td></td>
</tr>
<tr>
<td>°F</td>
<td>Degree Fahrenheit</td>
<td></td>
</tr>
<tr>
<td>μg</td>
<td>Microgram</td>
<td></td>
</tr>
<tr>
<td>μg/mL</td>
<td>Microgram per milliliter</td>
<td></td>
</tr>
<tr>
<td>μm</td>
<td>Micrometer</td>
<td></td>
</tr>
<tr>
<td>μs/cm</td>
<td>Microsecond per centimeter</td>
<td></td>
</tr>
<tr>
<td>g</td>
<td>Gram(s)</td>
<td></td>
</tr>
<tr>
<td>kV</td>
<td>Kilovolts</td>
<td></td>
</tr>
<tr>
<td>nm</td>
<td>Nanometer</td>
<td></td>
</tr>
<tr>
<td>meq/g</td>
<td>Milliequivalent per gram</td>
<td></td>
</tr>
<tr>
<td>mg/L</td>
<td>Milligram per litre</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>Mole</td>
<td></td>
</tr>
<tr>
<td>Min</td>
<td>Minute(s)</td>
<td></td>
</tr>
<tr>
<td>ml</td>
<td>Microliter</td>
<td></td>
</tr>
<tr>
<td>mA</td>
<td>Milliampere</td>
<td></td>
</tr>
<tr>
<td>mL</td>
<td>Millilitre</td>
<td></td>
</tr>
<tr>
<td>mm</td>
<td>Millimeter</td>
<td></td>
</tr>
<tr>
<td>ppm</td>
<td>Parts per million</td>
<td></td>
</tr>
<tr>
<td>Sec</td>
<td>Second(s)</td>
<td></td>
</tr>
<tr>
<td>v/v</td>
<td>Volume by volume</td>
<td></td>
</tr>
</tbody>
</table>
DISSERTATION OUTLINE

This dissertation covers studies on mineralogical profile of selected geophagic clayey soils, microorganisms associated with these clays and the effects of the clays on probiotics using Lactobacillus fermentum as analyzed on a Two-Dimensional Gas Chromatography coupled with Time-of-Flight Mass Spectrometry (GCxGC-TOFMS). A brief outline of the chapters presented in this dissertation is provided below.

Chapter One: General introduction
This chapter provides a general overview of the research study, providing relevant background information as well as describing the problem under investigation. The chapter also highlights the assumptions (hypothesis), aim and objectives of the study.

Chapter Two: Literature review
This chapter presents an in-depth evaluation of the research focus. It provides a description of the applications of clayey soils ranging from the deliberate consumption of earthy materials by humans and animals, to their usage in cosmetics and medicine as well as pottery applications. This aspect of the dissertation titled ‘Clayey soil as used in geophagic, medicinal and traditional pottery applications: A review.’ has been submitted for publication in the journal - *International Journal of Environmental Science and Technology* JEST-D-15-01205.

Chapter Three: Mineralogical profile of geophagic clayey soils sold in selected South African Informal markets.
Chapter Three describes the physico-chemical characterization of some of the earthy materials ingested by humans particularly pregnant women using techniques such as X-ray fluorescence (XRF), X-ray powder diffraction (XRD) and Atomic absorption spectroscopy (AAS). The work described in this chapter won the session best paper at the International Conference on advances in science, engineering, technology and natural resources (ICASETNR-16) November 24-25, Parys, South Africa.
Chapter Four: The effects of an acidic environment on selected geophagic clayey samples.
Chapter Four describes the simulation of the human stomach using 0.1M hydrochloric acid to ascertain the amount of extractable minerals that the body can absorb upon consumption of 20g of clay as compared to the recommended dietary allowance by the World Health Organisation 2005. This chapter is presented in the format suitable for submission to the *International Journal of Environmental Health Research* ISSN: 0960-3123 for publication.

Chapter Five: Clay minerals and clayey soils as a possible repository for microorganisms.
Chapter Five focused on a critical investigation into the presence of microorganisms within different clayey soil samples. Also discussed were the possible types of microbial flora associated with each type of clayey soil as well as their functions within the human body. This section of the dissertation titled ‘Clay minerals and clayey soils as possible microorganisms repository’ was accepted for a poster presentation at the 35th International Geological Conference, Cape Town, South Africa and has been submitted for publication in the journal - *Transactions of the Royal Society of South Africa*, TTRS-2016-0046.

Chapter Six: GC-MS Analysis of volatile fatty acids produced by probiotic: *Lactobacillus fermentum* when in interaction with geophagic clay.
Considering that often times, geophagic clay eaten by humans ends up in the large intestines due to some of the clay not being absorbed by the body, this chapter highlights some of the possible effect of the clay on probiotics using GCxGC-TOFMS.

Chapter Seven: General discussion and conclusion
This chapter reiterates the research focus (problem statement and aim) of this dissertation as well as presents an overall discussion of the issues addressed in Chapters Three, Four, Five and Six and reaches a final judgment. Recommendations and prospects for future studies are also provided.
CHAPTER ONE

1.0 General Introduction
1.1 Background
The practice of geophagy, which is the habit of eating earth including clay and other types of soil (Halsted, 1968) is extending from Africa to other parts of the world and is commonly observed in pregnant and breast-feeding women. This practice is deeply rooted and traceable to some cultural beliefs. To some sets of people, the act of eating clay is considered normal despite the conflicting views of the beneficial and harmful implications of the consumption of clay/soil. Physicians consider geophagy as a form of pica, an abnormal and harmful practice associated with elemental deficiencies, parasites and intestinal irritation. Some soils whose ingestion is associated with deleterious effects have been discovered to contain high amounts of smectite (phyllosilicate mineral species). However, many geophagic based soils not specifically associated with such effects, which do contain smectite, also contain larger amounts of kaolinite or halloysite (Mahaney et al., 2000a; Szajewska et al., 2006).

Based on the rich history attached to geophagy, adequate consideration must be given to aspects such as the evolution of the landscape, the soil systems, the soil microorganisms and the effects of animals within an area; in the choice of a sample site for geophagy soils. Several studies have proven some claims about the increased food efficiency attached to clay consumption (Habold et al., 2009a). Some of the adverse effects associated to geophagy include iron deficiencies (Hooda et al., 2002), anaemia (Geissler et al., 1998a, Kawai et al., 2009b) and geohelminth infections (Saathoff et al., 2002; Luoba et al., 2005). Traces of heavy metal such as lead are found in geophagic clays which affect the kidneys and brain (Kutalek et al., 2010). An association between deleterious effects in the renal and brain during pregnancy and early childhood and exposure to heavy metals has been established by some studies, as such, it is pertinent to scientifically establish the mineralogical and potential pathogenic microorganisms of the various clayey soils (Tchounwou et al., 2012).

1.2 Rationale
The existence of human life, plants and other organisms revolves around clayey soils. Clayey soils are naturally occurring earthy material comprising of clays and clay minerals that have continued
to be mined from time immemorial (Staubach, 2013a). Over and above the fact that these soils constitute a part of the geological structure of the earth, in recent times, they remain one of the major materials used in the manufacturing and environmental industries, which make them an essential in life.

As far back as 69 – 30 B.C, Cleopatra, Queen of Egypt, used mud from the Dead Sea for cosmetic purposes (Newton, 1991, Robertson, 1996, Reinbacher, 1999). During the era of Ancient Greek, mud materials were used as antiseptic cataplasms to cure skin afflictions and; snake bites. Clay minerals are also heavily utilised in normally ceremonial and religious events by a number of societies (Popoola et al., 2013). The use of clays in modern civilisation goes as far as ingesting them for therapeutic purposes. The first mention of the deliberate consumption of clayey soils by humans, for therapeutic and religious purposes, was made by Aristotle around 384 – 322 B.C. During his travels, Marco Polo also described the use of pink earth by Muslim pilgrims in the cure of fevers. This is still in vogue in several parts of the world (Mahaney et al., 2000a).

Clayey soils are mostly consumed by pregnant women while others apply them topically on the skin for various ailments whereas potters use them for pot making (Mahaney et al., 2000a). To a large extent they are used in the production of feedstuffs, beverages, paper, rubber, plastics, artificial leather, protective coatings for interior and exterior use, pharmaceutics, cosmetics, paints, pencils, pastels, porcelain, electro-porcelain, other fine ceramics, coarse ceramics and sialon ceramics; in the foundries, various branches of the chemical, petroleum and cement industries, agriculture and forestry; in the preparation of agrochemicals and special fertilizers, lubricating oils and gels, lightweight ceramics and effective sorbents; in the manufacture of mineral wool, in briquette and pelletizing processes; as ingredients in grinding and polishing pastes, in the insulations of dumping sites of various kinds of waste (including toxic and radioactive waste), in thermally, electrically, acoustically and chemically resistant insulations, and in filters for the treatment of industrial, agricultural and similar outflows (Konta, 1995).

Geologists and earth scientists apply clay minerals for various tasks such as: (1) indicators of the environment during weathering, allothi- and authigenesis in the sediments and in the study of the source areas of the detrital supply; (2) as pH indicators and indicators of processes in micro- and
mega-environments, and of changes in the course of diagenesis and metamorphosis. Mineralogical, petrological, geological and geochemical investigations directed to clay minerals serve as one of the correlation methods, in the recognition of processes in the petroleum-bearing sediments, coal-bearing formations, and origin of riverine, lacustrine, marine and oceanic sediments, in the climatic, geodynamic, paleogeographical, stratigraphic and weathering rate interpretations (Adebisi et al., 2013).

Over the years, extensive studies have been carried out on clayey soils in geophagy, pottery and cosmetics by the layman in isolation but there are no significant unifying reports focusing on clayey soils as in all these aspects. The similarities as well as differences in both the microbial and toxic heavy metal content of various clayey soils also need to be established with respect to them being in their natural and processed states.

It is important therefore to critically study the different clay materials on account of their physicochemical properties such as, pH, electrical conductivity, bulk density, porosity density, soil texture and colour to determine possible heavy metal pollutants and microbes. Also, the influence of these properties and identified heavy metals on the availability of microbes (Young & Crawford, 2004).

Clayey soils are ubiquitous and remain essential materials in life. Due of their abundance in nature and varieties coupled with the high specific surface area, optimum rheological features and absorptive capacity, clay minerals such as Kaolinite, Talc, Montmorillonite, Saponite, Hectorite and Palygorskite are widely used as active ingredients and ideal excipients in the production of many pharmaceutical and cosmetic products (Khurana et al., 2015).

Nevertheless, not all clays possess the same benefits, while some are useful in pottery and construction; others are beneficial in medical and agricultural fields. Over the years and in present times, locally and internationally, the issue of geophagy has continued based on the healing potential of some earthy materials. However, recent studies revealed a high level of contamination by geohelminths and toxic heavy metals in most clayey soils. The continued consumption of geophagic materials at high dosages may pose a threat in a human’s health particularly in
pregnancy (Ekosse & Jumbam, 2010). Potters involved in the application of clay materials are to some degree exposed to toxic heavy metals (Dart, 2004). With the controversies raging over the applications of clay, it is imminent to investigate further into the components of these clays to scientifically substantiate their similarities in mineralogy, toxic heavy metals and microbial contamination. Knowledge obtained will assist in the recommendation of clay for specific applications with little or no deleterious effects.

This project focuses therefore on studying the combined geophagic and medicinal applications using clayey soils in South Africa. It also focuses on looking at the importance of microorganisms within these soils.

1.3 Hypothesis
It is generally hypothesised that the different clayey soil types including those mostly used in geophagic applications amongst women within the Gauteng province of South Africa due to the mode of collection, transportation and exposure to airborne contaminants at informal market venues possess harmful microorganisms as well as contain toxic heavy metals.

1.4 Research Aim(s) and Objectives
This study is aimed at investigating the quality of clayey soils as used in geophagia and their microbiological implications on probiotics.

To accomplish the aims stated above, the objectives of the intended study are:

- The investigation of the physicochemical and mineralogy of the different clayey soils.
- Investigation of toxic heavy metal (lead, mercury and cadmium) content of clayey soil.
- Isolation/Identification of in situ bacteria and fungi on each clay type.
- To determine the production of VFAs when in contact with probiotics.

1.5 Scope of the Study
The different clayey soils sourced from selected informal markets were incorporated into this study which included investigations on their mineralogical composition. Investigations on the prevalence of pathogenic bacteria were conducted by selecting the most prevalent environmental human and faecal contaminants. Different bacteria were cultured from the different geophagic clay
samples and their known pathogenicity and toxicity were reported. The effect(s) of ingested geophagic clay on volatile fatty acids produced by probiotics in the large intestine was investigated using *Lactobacillus fermentum* as a case study.

1.6 Study site
The study was carried out using clayey soils purchased from selected informal markets within South Africa; this included 6 known markets and 15 vendors selling either cosmetic or geophagic soils. Markets considered included Boulders Taxi Rank (in Midrand), MTN Taxi Rank (in Johannesburg Central), Community market (Hillbrow), Nigerian Village (Doornfontein) and West African Market (Yeoville).
References


CHAPTER TWO

2.0 LITERATURE REVIEW

Abstract
This review discusses various applications of clayey soils ranging from the deliberate consumption of earthy materials by humans and animals, to their usage in cosmetics and medicine as well as pottery applications. Geophagic clayey soils are naturally occurring nanomaterials that exist in several forms such as kaolin. Geophagia, the deliberate ingestion of soil by humans and animals has both beneficial and detrimental health implications that are dependent on the clay’s physico-chemical, mineralogical and geochemical content. Clay minerals had remained indispensable in human activities following their applications in many traditional and scientific applications. The application of clay minerals in different aspects of human activities is supported by certain properties such as structure and chemical composition, nature of exchangeable ion and particle size. A critical look into the contributions of clay minerals in pharmaceutical products, cosmetic products, pottery, and spa and beauty therapy is discussed. Also considered, are certain deleterious effects associated with the applications of clay minerals in human and animal health. It is crucial to acknowledge the various studies done so far on clays; however, new frontiers will continue to emerge on the application of clayey soils.

Keywords: Aesthetic medicine - cosmetic –geophagia -mud –pelotherapy.

Submitted for publication as: Okereafor, G. U; Mulaba-Bafubiandi, A.F; and Mavumengwana, V. (2016). Clayey soil as used in geophagic, medicinal and traditional pottery applications: A review. International Journal of Environmental Science and Technology.
2.1 Introduction

Clays and clay minerals, either as such or after modification, are recognised as the materials of the 21st century because of their abundance, cost effectiveness and because they are environmentally friendly (Maisanaba et al., 2015). Phyllosilicates are essential minerals used by environmental and manufacturing industries, thus constituting a vital material in life. The role of clay is crucial in industrial processes, in agricultural applications, in engineering and construction applications, in environmental remediation, in geology, and in many other diverse applications (Murray, 2007). Clay minerals constitute part of the geological structure of the earth (Mahaney et al., 2000b). Based on their abundance in nature and properties such as high specific surface area, optimum rheological features and absorptive capacity, they are widely used as active ingredients and ideal excipients in pharmaceutical and cosmetic products (Carretero, et al., 2006, Lopez-Galindo, et al., 2007, Carretero & Pozo, 2009, Gomes, et al., 2013). The use of clay minerals for medicinal purposes is almost as old as mankind itself. Since prehistoric times, clay minerals have been used for curative purposes. There are indications that *Homo erectus* and *Homo neanderthalensis* used ochres mixed with water and different types of muds in order to cure wounds, soothe irritations, as well as cleansing the skin (Carretero, 2002). Some of the well-known early reports of using ochre describe the early inhabitants of Australia where they used the clay minerals to burial ceremonies and rock paintings including their use in Mesopotamia and Ancient Egypt (McGrath and Phillips, 2008; Carretero, 2002). Mud materials during the era of Ancient Greek were used as antiseptic cataplasms to cure skin afflictions, snake bites and cicatrizes (Williams, 2009).

In modern civilisation the use of clay dates back to the period when it was used for therapeutic purposes. The first mention of the deliberate consumption of earth/clay by humans for therapeutic and religious purposes was done by Aristotle (384 – 322 B.C). Marco Polo described the use of pink earth by Muslim pilgrims in the cure of fevers which is still done in several parts of the world (Mahaney, et al., 2000, Carretero, 2002). Clay minerals are largely indispensable, while some are useful in pottery and construction; others are beneficial in medical and agricultural fields. Over the years and in present times, locally and internationally, the issue of geophagy had continued based on the healing potentials in some earthy materials. Recent studies however revealed a high level of contamination by geohelminths, other microbes and toxic heavy metals, and that continued consumption of geophagic materials at high dosages
may pose a threat to a human’s health particularly during pregnancy. Due to the different attributes of clay in various disciplines, a specific definition for the term clay had been somewhat impossible.

The word clay is derived from the German word “Kleben”, which means ‘to stick to’. There is no distinctive definition for the term. However, it may refer to materials having a particle size of less than 2 micrometers (25,400 micrometers = 1 inch) and to the group of minerals that has similar chemical compositions and common crystal structural characteristics (Velde, 1995). Clays are natural occurring materials that are composed primarily of fine-grained minerals, which are generally plastic at appropriate water contents and harden when dried or fired (Heckroodt, 1991, Guggenheim & Martin, 1995). Clay is the finest fraction of a sediment or soil in the classification of clay, silt and sand. The term ‘pure’ has often been used to describe the cleanliness of a clay deposit. Exposure to pollutants and faecal contamination is responsible for the preference of subsurface deep deposit of clays to surface clays.

The colour of clay is dependent on the components of the soil type and ranges from white to dull gray or brown to a deep orange-red (Guggenheim, 1995). The particle size of clay minerals ranges from 10's of angstroms to millimeters. (An angstrom (Å) is a unit of measurement at the scale of atoms.) Thus, clays may be composed of mixtures of finer grained clay minerals and clay-sized crystals of other minerals such as quartz, carbonate, and metal oxides. Differences in size and mineralogy help to distinguish clay from other fine-grained soils. Silts and clays are different based on particle size as the former tend to have larger particle sizes. Based on particle size, geologists and soil scientists distinguish silt from clay at a particle size of 2µm with clay being finer than silts. On the other hand, sedimentologists consider 4 - 5µm while Colloid chemists use 1µm. Based on the plasticity soil properties, geotechnical engineers e.g. Atterberg could distinguish silts from clay.

Associated phases in clay may be minerals such as quartz, calcite, dolomite, feldspar, oxides, hydroxides, organic phases, etc. or non-crystalline phases, such as colloidal silica, iron hydroxide gels, organic gels (Guggenheim, 1995). All clays are natural products of volcanic ash that has weathered over the years with exceptions of some that have been processed or tampered with by human activities.
Clay is a valuable mineral due to its abundance, plasticity and durability; it has an ideal formula $\text{Al}_2\text{O}_3\text{2SiO}_2\text{2H}_2\text{O}$. Plasticity in clay refers to the ability of a clay material to be molded to any shape, and it is greatly influenced by the chemical composition and aggregate particle size of the material (Guggenheim, 1995); durability describes the clays ability to resist weathering action, chemical attack and abrasion. The plasticity of clay could be related to the particle’s flatness, 2-dimensions and electrical charge. The easiest way to determine clay’s plasticity is using the “coil” test where a sample of clay is taken, rolled into a coil, and tested for its deformability. The presence of water acts like a lubricant and creates attraction between the particles which makes them slide.

### 2.2 Clayey Soils: Origin and mode of Formation

The discussion of clay and its minerals as raw materials cannot be better understood without the consideration of their geologic origins. In nature, clay is an extremely common and abundant material (Gillott, 2012b). To a large extent, it is a nuisance to the farmer, miner, and road construction worker or engineer. It is an important part of the earthy material which makes up the surface of the planet. The geologic weathering of the earth surface which is a continuous process results in the formation of clay (Christie et al., 2014). Clays and their associated minerals occur in nature under certain geologic settings. The depositional environments of most clay comprise of the soil horizons, continental and marine sediments, geothermal fields, volcanic deposits, and weathering rock formations. Most clay minerals are formed when rocks come into contact with water, air, or steam.

Feldspar remains the most common mineral whose weathering largely accounts for clay formations. It is important to mention that only five minerals account for over 90 percent of the bulk of the earth's surface. Hundreds of different minerals have been identified in nature, but most of these are relatively rare, and many might almost be considered freaks of nature.

Basically, two categories of clay deposits exist in respect to environment of deposition (Siddiqui and Ahmed, 2005): primary and secondary. Primary clays are residual deposits commonly formed *in situ* by surface weathering of the original rock. They are formed from the mechanical weathering of rock or shale: a dark fine-grained sedimentary rock composed of layers of compressed clay, silt, or mud (Uddin, 2008). They are often less plastic and their formation involves the chemical
decomposition of rock bodies such as granite, containing silica and alumina; solution of rocks such as limestone containing clayey impurities and the disintegration of shale (Uddin, 2008). Secondary clays refer to those that have been carried away or transported by wind and volcanic ash, rains, rivers and streams, and in time, deposited great distances from the original bedrock. As such, due to the distance travelled, clays are considered “plastic” (Geissler et al., 1998b).

2.3 Physical and chemical properties of clays
The resultant characteristics of clays and clay minerals are largely dependent on their chemical composition, size, and layered structure. The properties of clays that support their various applications include plasticity, shrinkage upon firing and air drying, degree of fineness of grain particles, cohesion, hardness and ability of the surface to take decoration (Attah, 2008). There is a great affinity for water by clay minerals with some swelling and probably doubling in thickness when wet. A host of clay minerals have the potential of absorbing electrically charged atoms and molecules from a solution and releasing the ions later when conditions are varied.

Water molecules are strongly attracted to clay mineral surfaces. The introduction of clay particles to water comprise of slurry which is as a result of the clay distributing itself evenly throughout the water. This property of clay–carrier is adopted by the paint industry to disperse pigment (colour) evenly throughout paint. A mixture of a lot of clay and a little water results in a mud that can be shaped and dried to form a relatively rigid solid. This property is exploited by potters and the ceramics industry to produce plates, cups, bowls and pipes. Environmental industries use both these properties to produce homogeneous liners for containment of waste.

The process by which certain clay minerals swell when they come in contact with water is revocable by changing some environmental factors. Hydration and dehydration can vary the thickness of a single clay particle by almost 100 percent (for example, a 10Å-thick clay mineral can expand to 19.5Å in water (Velde, 1995). Buildings constructed with swelling clays may suffer structural damage due to seasonal swelling of the clay portion of the soil.

The ability of clay minerals to exchange ions is dependent on their surfaces. Ions are either attracted to the surface of a clay particle or taken up within the structure of the minerals (Karpinski
The property of clay minerals that causes ions in solution to be fixed on clay surfaces or within internal sites applies to all types of ions, including organic molecules like pesticides. As such, clays can be regarded as important agent for conveying and extensively dispersing toxins from one area to another (Ziaie Moayed & Allahyari, 2012).

2.4 Clay minerals and their classification
Clay minerals differ from clays in a variety of ways. Often times, scholars misuse the words: clay and clay minerals believing that they have similar meaning. Unlike clays, clay minerals can be natural or synthetic; they contain both phyllosilicate and non-phyllosilicate minerals and have no specific criterion for size. They are basically the minerals which are used for therapeutic purposes in pharmaceutical formulations as active principles or excipients, in spas and aesthetic medicine (Carretero, 2002).

Clay minerals are formed as a result of processes such as: crystallisation from solution, comparable to anthogenesis or neoformation; substitution by clay minerals; weathering of silicate minerals and rocks (not clay minerals); weathering of other clay minerals; diagenesis, reconstitution, and ion exchange; and hydrothermal alteration of minerals and rocks (Meunier, 2005).

In this review five categories of clay minerals will be discussed; these include kaolinite; montmorillonite/smectite; illite; chlorite and sepiolite. Granite has its major constituents as quartz, mica and feldspar. The quartz is usually resistant to chemical alteration and can only be obtained as mineral grains of quartz. On the contrary, micas and feldspars are susceptible to alteration and thus disintegrate to form clay minerals. The alteration of micas and feldspars results in the carrying away of some elements in solution as ions (Na⁺, Ca⁺ and K⁺) which end up with clay that are rich in aluminum and silicon. The layers of the main groups of clay minerals reveals kaolinite as having fairly weak bonds while a much stronger bonding exist in illite and montmorillonite which could be attributed to the presence of positively charged metal ions (Ekosse and Mulaba-Bafubian, 2008). Potassium is the metal ion found in illite while calcium and sodium are the metal ions in montmorillonite. It is important however to state that the formation of kaolinite is as a result of the breakdown of potassium feldspar; micas when weathered forms illite, while ferromagnesian minerals break down to form Montmorillonite (Islam et al., 2002).
Kaolinite holds the chemical composition $\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$ and is an aluminum silicate material with a low “shrink-swell” capacity. It is a soft, white mineral but is often colored orange or red by iron oxide found in the soil. Kaolinite is found in major parts of the world and is the most common clay in the Mountain and Piedmont regions of North Carolina. It is the most prominent mineral found in kaolin, or porcelain, pottery (Ekosse & Mulaba-Bafubiandi, 2003).

Illite is a name for a group of mica-like clay minerals proposed by R.E. Grim, R.H. Bray, and W.F. Bradley in 1937 to honour the US state of Illinois, a source of illite and a state that had supported research on clay (Grim, 1968; Bates & Jackson, 1987). Illite minerals show considerably no expanding lattice characteristics and are characterized by intense 1.0-nm 001 and 0.33-nm 003 peaks that are not modified by glycerol or ethylene glycol solvation, potassium saturation, or heating to 550 °C (Fanning et al., 1989). Previous studies indicated that illite includes both trioctahedral (biotite) and dioctahedral (muscovite) types of crystallization (Grim, 1968). It has an absence of swelling due to its tightly interlaced molecular spacing. It is a common mineral found in sediments, soils, and metamorphic rock. The distinct properties of illite are derived from its molecular structure. Its colour is determined by its iron content and may be white, yellow, lilac, or reddish brown. It is also an important impurity in limestone, affecting the properties and thus the value of the stone for construction and other purposes (Carr et al., 1994). Illite reacts with both inorganic and organic ions and has a cation exchange capacity of 10–40 meq/100 g, a value intermediate between those of montmorillonite and kaolinite (Grim, 1968). By heating, the ion exchange capacity of illite is reduced.

Chlorite is a group of silicate minerals with similar properties primarily found in weakly metamorphosed rock and form from the alteration of clay-rich sedimentary rocks and from alteration of pyroxenes, amphiboles and micas (Yoneda and Maeda, 2008). The general formula of chlorite is $(\text{Fe, Mg, Al})_6(\text{Si, Al})_4\text{O}_{10}(\text{OH})_8$. The structure permits extensive isomorphic substitution which enables it to have a wide range of chemical composition. Chlorite crystallises in the monoclinic symmetry system and exhibit a basal cleavage on account of their stacked structure. It is most commonly found in hydrothermal ore deposits and has a soft, flexible consistency. Often times, chlorite is referred to as inclusions in or coatings on quartz, calcite, topaz and other minerals.
Sepiolite, formerly known as Meerschaum (sea froth), is a non-swelling, lightweight, porous clay with a large specific surface area. The individual particles of sepiolite unlike other clays have a needle-like morphology as well as a high surface area and porosity, which account for its outstanding sorption capacity and colloidal properties that make it a valuable material for a wide range of applications. Sepiolite is a very uncommon clay mineral; however, considerable deposits are found in places such as New England, Arizona, and California in the United States. Chemically, sepiolite is a hydrated magnesium silicate with the ideal formula $\text{Si}_{12}\text{Mg}_{8}\text{O}_{30}(\text{OH})_{4}(\text{OH}_2)_4\cdot8\text{H}_2\text{O}$ (Murray & Zhou, 2006). Sepiolite based clays are stable in high salt environments and are typically found as dull, white colored deposits. The high surface area and porosity of sepiolite account for the remarkable adsorptive and absorptive properties of this clay. As non-swelling clay, its granules do not disintegrate even when saturated with liquids.

Smectite as a clay mineral is the weathering product of mafic silicates (figure 2.1). It is a 2:1 clay consisting of an octahedral sheet sandwiched between two tetrahedral sheets (Murray, 1999). It has a variable net negative charge, which is balanced by Na, Ca, Mg and, or, H adsorbed externally on interlamellar surfaces. The structure, chemical composition, exchangeable ion type and small crystal size of smectite clays are responsible for several unique properties, including a large chemically active surface area, a high cation exchange capacity, interlamellar surfaces having unusual hydration characteristics, and sometimes the ability to modify strongly the flow behaviour of liquids. Smectite clay minerals are unique in their ability to interact with liquids and their finite crystal shape. The composition $(\text{Na, Ca})_{0.33}(\text{Al, Mg})_2(\text{Si}_4\text{O}_{10})\cdot(\text{OH})_2\cdot\text{nH}_2\text{O}$ causes a high plasticity allowing Smectite clays to absorb and swell easily (Vasu, 2011). These can be found as white, pale green, pink and yellow deposits.

### 2.5 Properties of clay minerals
Clay differs from clay minerals based on their different properties (Table 2.1). Some of the characteristic features of clay minerals are stated thus: (i) They have a layer structure that has one dimension in the nanometer range; the thickness of the $1:1$ (TO) layer is about 0.7 nm, while that of the $2:1$ (TOT) layer is about 1 nm, (ii) they are anisotropic in nature due to the different crystallographic orientations of the layers or particles, (iii) they have varying types of surfaces: external basal (planar) and edge surfaces as well as internal (interlayer) surfaces (Annabi-Bergaya
et al., 1979), (iv) both the external and internal, surfaces are easily changed by adsorption, ion exchange, or grafting (v) they are easily molded into desired shapes without rupturing when stress is applied, and retain the shapes after the stress is removed (vi) they harden on drying or firing.

Table 2.1: Differences between Clay and Clay mineral.

<table>
<thead>
<tr>
<th>Clay</th>
<th>Clay mineral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural</td>
<td>Natural and synthetic</td>
</tr>
<tr>
<td>Fine-grained</td>
<td>No size criterion</td>
</tr>
<tr>
<td>Phyllosilicates as principal constituents</td>
<td>May include non-phyllosilicates</td>
</tr>
<tr>
<td>Plastic (with some exceptions like flint clays)</td>
<td>Plastic</td>
</tr>
<tr>
<td>Hardens on drying or firing</td>
<td>Hardens on drying or firing</td>
</tr>
</tbody>
</table>

Figure 2.1: Classification of Silicates as adapted from (World Health Organisation, 2005).

2.6 Geophagic clayey soils
Geophagy has seldom been viewed with distain from a distance whereas it is strongly linked to healing beliefs in some cultures (World Health Organization, 2005). The word geophagia or geophagy was coined from two Greek words, geo – (earth) and phag – (eat) (Ferrell Jr, 2008).
Geophagia amongst humans has been linked to nutritional, psychological, cultural and medical (Abrahams, 2002), social taste (Danford et al., 1982), spiritual, religious, ritual (Geissler et al., 1999) and physiological demands (Vermeer, 1979, Harvey, et al., 2000, Callahan, 2003). It is comparatively practiced in virtually all parts of the world (Oliver, 1997, Mogongoa, et al., 2011) irrespective of the people’s diverse cultures, ages, races and socio-economic group (Hunter, 1984).

Many colloquial expressions and scientific terms are used for edible clays, including beidelliticmontmorillonite, chalk, clay dirt, white dirt, clay tablets, colloidal minerals, and white mud (Hunter, 1993). Geophagic soils are largely known to contain clay minerals – secondary minerals that result from the chemical disintegration of feldspars and micas. They are dominantly alumino silicates comprising of tetrahedron and octahedron sheet that make up a unit cell. The unit cell is characterised by layering yielding 1:1 and 2:1 classes of clay comprising Kaolinite – Serpentine and Smectites group respectively (Reinbacher, 1999). Some organic pollutants in the form of pesticides could be observed in geophagic clayey soils.

The practice of geophagy, habit of eating earth including clay and other types of soil (Kikouama, et al., 2002, George & Ndip, 2011) has been practiced for several years in different ethnic, religious, and social groups of the world (Ekosse et al., 2010a). Geophagy, usually associated with a craving, is prevalent among pregnant women and children (Abrahams et al., 2013), women of all ages, educational level and women who are breast-feeding. To some sets of people this act is considered normal despite the conflicting views of the beneficial and harmful implications of the consumption. Some of the reasons to support the practice of geophagia include nutrient supplementation, detoxification, and alleviation of gastrointestinal disorders such as diarrhea, craving and relief from morning sickness (Young, 2011, Diko, 2013). Clay minerals such as kaolinite and plagioclase may adsorb organic toxins, and often times support their excretion. These minerals have been found to change gut pH making it more conducive for the growth of symbiotic bacteria. Edible clays are sold in a variety of ways such as clay tablets, discs, and as rough blocks that may be raw or smoked (Ekosse & Anyangwe, 2012).

Physicians consider geophagy as a form of pica, an abnormal and harmful practice associated with iron deficiency anaemia (Geissler, et al., 1998, Kawai, et al., 2009, Mogongoa, et al., 2011),
hypokalemia (Vermeer & Ferrell Jr, 1985), enamel damage, erosion of the mucosal surface of the stomach, perforation of the colon (Bisi-Johnson et al., 2010), parasitic infections resulting from transmission of *Ascaris lumbricoides*, and other major toxigenic bacteria, causative agents of gas gangrene, tetanus and botulism and geohelminth infections (Saathoff, *et al*., 2002, Luoba, *et al*., 2005). Traces of heavy metal contents such as lead are found in geophagic clays which affect the kidneys and brain (Barker, 2005, Ruth *et al*., 2010). Exposure to these heavy metals during pregnancy and early childhood has deleterious effects in the renal and brain development of children. Most geophagic earthy materials have low bioavailability of micronutrients which jeopardises their function as dietary sources. Some adsorb and bind nutrients, thus limiting their availability to the body (Bonglaisin *et al*., 2011). Many clay minerals are very rich in total iron (Fe) compared to normal food, but it does not necessarily mean that the Fe is bioavailable (Young, 2011).

The resultant positive and negative effects of geophagia are dependent on the physico-chemistry, mineralogy and geochemistry of the consumed soils which are principally affected by: pedogenetic development of the soils, type, amount and degree of crystallinity of clay minerals together with the non-clay minerals (Ngole & Ekosse, 2012, Detellier & Schoonheydt, 2014). Some soils whose ingestion is associated with deleterious effects have been discovered to contain high amounts of smectite, however, many geophagy soils not specifically associated with such effects, (which do contain smectite) also contain larger amounts of kaolinite or halloysite (William, *et al*., 2000, Szajewska, *et al*., 2006).

Based on the rich history attached to geophagy, adequate consideration must be paid with respect to the evolution of the landscape, the soil systems, the soil microorganisms and the effects of animals within an area; in the choice of a sample site for geophagy soils (Seim *et al*., 2013). Researches had proven some claims about the increased food efficiency attached to clay consumption (Mahaney *et al*., 2000b).

Geophagic clayey soils are majorly composed of kaolin of which kaolinite is the dominant type of mineral (Habold *et al*., 2009b). Palygorskite and montmorillonite (usually from Bentonite) are other clay minerals associated with geophagic clayey soils (Wilson, 2003a). Most geophagic
clayey soils - with particular reference to those consumed by gorillas - contain quartz, atatite, analbite, sanidine, amphibole, limonite, ilmenite, magnetite and halloysite (Mahaney, et al., 1993, Ekosse & Anyangwe, 2012). As far back as 1600 B.C., geophagic clays were employed as vital minerals for treating ailments such as diarrhea, dysentery, tapeworm, hookworm, wounds, and abscesses (Ekosse & Anyangwe, 2012). The 20th century witnessed the use of kaolin for the treatment of cholera (Nunn, 2002). Apparently, it is obvious that there are numerous clays consumed on account of mineralogy and geochemical composition (Schoenenberger, 1911).

2.7 Characteristics of geophagic clays
Geophagic materials are usually referred to as “soils”, while a host of others are referred to “saprolites” on the grounds that they look like decomposed and disaggregated rock, like those often found at the base of soil profiles developed by in situ weathering. It is important to understand some of the physico-chemical properties of geophagic clayey soils which help in understanding the reasons behind their many applications.

2.7.1 Colour
This remains one of the major parameter used in determining the suitability of soil for ingestion (Ferrell Jr, 2008). It provides information as to the palatability of a geophagic clayey soil. A scientific way of determining the colour of soil is the use of a Munsell colour chart. The Munsell colour chart describes colors in order of their hue (actual color), value (degree of lightness), and chroma (strength of color). For example, 7.5R 7/2 describes a reddish color (7.5R), of light value (7) and weak chroma (2).

When dealing with clays, colour can be quite diagnostic of the predominant form of iron oxide. Thus, clays having predominantly hematite (Fe₂O₃) as the main iron oxide mineral are often a deep red (5YR), whereas those having goethite [FeO(OH)] as the main iron oxide mineral are yellowish brown (2.5YR–7.5YR) (Ekosse & Anyangwe, 2012). In addition, clays containing lepidocrocite, another form of iron oxyhydroxide, are a distinctive orange color (7.5YR). Previous studies particularly in Africa reveal most geophagic clayey soils as possessing colours such as white, yellow, light grey, light yellow, brown, reddish tint, dark greyish brown etc. (Sera et al., 2008).
On account of colour, several geophagic practitioners prefer basically the white and dark greyish brown clayey soils. The white, grayish or khaki colouration could be linked to the presence of kaolin, smectite, and calcite. A reddish or yellowish appearance depicts the presence of hematite and goethite (Ekosse & Anyangwe, 2012). It is a wide spread belief amongst geophagic individuals that reddish clayey soils are rich in Fe and could be used to supplement Fe in humans. Nevertheless, the reddish tint is not necessarily an indicator of the quantities or availability of iron (Fontes, et al., 2005, Ngole, et al., 2010). Generally, iron in heam form (Fe$^{2+}$) gets absorbed easily by the human body compared to the non heam form (Fe$^{3+}$), with optimal uptake taking place in the duodenum (Ekosse et al., 2010b).

2.7.2 Texture
Most studies reveal geophagic clayey soils to be texturally dominated by silty clay with an appreciable level of sand. The presence of sand poses some health issues due to the fact that contain mainly quartz (SiO$_2$) which is a very hard mineral with a measurement of seven (7) on the Mohr hardness scale. The main inorganic component of the human tooth, dental enamel; is predominantly made up of hydroxylapatite (Ca$_5$(PO$_4$)$_3$(OH)) – a Calcium Phosphate mineral with a hardness of five (5) on the Mohr hardness scale. Imperatively, the sand in most geophagic clayey soils may result in the damage of the dental enamel via cracking, grinding, breakage and splitting during mastication. Previous studies indicated that medium sized sand (250 - 500µm) created severe dental damage in hominid species (Bain et al., 2011a).

2.7.3 Particle size
Generally, Soils are made of a mixture of inorganic mineral materials and organic material (also known as humus). The particle size fractions of the inorganic part of most soils have been classified according to a number of systems. The two most common are the United States Department of Agriculture (USDA) and the international Union of Soil Sciences. The USDA scheme ranks particle sizes from medium to very coarse sand (0.25–2 mm in diameter), very fine to fine sand (0.05–0.25 mm), silt (0.002–0.05 mm) and clay (<0.002 mm). In the International scheme the medium to coarse sand fraction is (0.2–2 mm), fine sand (0.02–0.20 mm), silt (0.002–0.02 mm) and clay (<0.002 mm) (King et al., 1999a).
The relative proportion of each of these fractions is crucial to the characterization of geophagic clays, as it provides relevant information to both their nutritional and protective hypotheses. By physically using an ultrasonic probe and deionised water, clayey soils are disaggregated into their primary size fractions. Granulometric properties of soils describe their particle size characteristics and play an important role in several soil reactions. Particle size distribution (PSD) of soils could provide clues to possible health threats that the habit of geophagia may present to an individual. The ingestion of coarse textured soils results in severe dental damage in hominid species (Sera et al., 2008). Most geophagic materials comprise of <20% clay content (King et al., 1999b).

2.7.4 pH
Geophagic clayey soils have a pH ranging between 5 and 7 which is similar to that of human saliva (5 and 8) with no noticeable chemical reactions while in the mouth (Ngole-Jeme & Ekosse, 2015b). The pH of most geophagic clayey soils has a significant impact on their taste. Previous studies revealed that most acidic soils have a sour taste (Omen et al., 2000). The use of soil to control the secretion of saliva during pregnancy is as a result of the taste of the soil which could be linked to the soil pH and dissolved salts content (Abrahams & Parsons, 1997b). Due to the acidity (pH = 2) of the gastric juice in the stomach, certain chemical reactions involving clay minerals and organic matter contained in geophagic clays may occur (Ibeanu et al., 1997a). Although, due to the residence time of approximately 2 hours of ingested material in the stomach, no significant reaction is noticed. The release of cations, which may have been absorbed on the exchange sites of ingested soil by the intestines, is as a result of the low pH. Interestingly, with a pH of about 8 in the duodenal and intestinal section of most gastrointestinal tract (GIT), some clay sized particles undergo chemical reactions but the silt and sand-sized particles dominated mostly by silica may not be altered (Omen et al., 2000). Most of the unaltered particles that go through the GI are lodged in the diverticula of the sigmoid colon. The silica rich particles that are abrasive in nature results in rupturing of the colon; a condition referred to as perforation of the sigmoid colon (Omen et al., 2000). The solubility of Fe and other cations in the GIT tends to increase with a decrease in pH.

2.7.5 Electrical conductivity
Electrical conductivity (EC) of geophagic clays is a property that is determined by standardised measures of soil conductance (resistance-1) by the distance and cross sectional area through which a current travels (Jahanshahee et al., 2004a). The amount of dissolved salts in clays and soils can
be indicated using electrical conductivity (Ekosse, 2000, Ngole, et al., 2006). The electrical conductivity of a geophagic material is dependent on the presence of charged ions that it contains. Often times, electrical conductivity increases with the number of ions in solution but does not necessary exhibit a non-linear relationship. This is because at higher concentrations, the mobility of ions is impeded. The mobility relies on the ionic size and charge of the clay particles.

Generally, the presence of fine sediments affects the electrical conductivity of clays. There is usually an increase in electrical conductivity when suspended sediments from geophagic clay solution are filtered. This increase could be attributed to desorption of ions held on sediment surfaces. A low Electrical Conductivity is indicative of a low amount of dissolved salts within the clay sample.

2.7.6 Organic matter content and water retention capacity

Soil organic matter tends to increase as the clay content increases. Organic matter in soils is a source of Nitrogen from which soil microbes could synthesise their proteins and deoxyribonucleic acid (DNA). Geophagic clayey soils with a high level of organic matter will potentially promote the harbouring of more bacteria some of which may be pathogenic. This result in the risk of bacterial infection of *Escherichia coli*, *Enterobacter histolytica*, *Salmonella typhi* and helminth infection such as those caused by *Ascaris lumbricoides*, *Trichuris trichiura* and *Strongyloides stercoralis*.

Water retention refers to the ability of soils to process and hold large amounts of water (Hartsock *et al.*, 2000) and it varies in geophagic clayey soils. A higher water retention capacity is observed in geophagic clayey soils with high clay content in contrast to those with high sand content (Brady & Weil, 1999). The Water retention capacity (WRC) observed in most geophagic clayey soils such as kaolin encourages their wide application in medicinal and pharmaceutical sciences. They are applied in the preparation of formulations used in the treatment of diarrhea and other GIT related ailments (Brady & Weil, 1999). Due to the high clay content of most geophagic clayey soils, there is the tendency to have numerous micro pores in which bacteria some of which may be pathogenic, could be lodged. Survival rate of the pathogens in such soils would considerably be low because of the low amount of nutrients as indicated by the low levels of organic matter (OM). Soils with a
considerable water retention capacity help in the absorption of water from the GIT that is infected with diarrhea causing pathogens.

2.7.7 Cation exchange capacity
Cation exchange is the phenomenon that describes the chemical modification of clays for the production of more organophilic clays (Pavlidou & Papaspyrides, 2008, Paul & Robenson, 2008). Quaternary ammonium salts are one of the widely used modifiers. The interlayer surfaces of expandable clays have ions and molecules bound to them. Cation exchange of clays is best carried out by immersing the fractions of clay in a concentrated (1M) solution containing ideal cations or ions with the best fit in ionic radius and charge for the interlayer exchange sites (Murdoch, 1985). Geophagic clayey soils that are used as supplements for any nutrient possess such nutrient in sufficient state in the available form. Most antibacterial agents are linked to ions that are presumably in the exchangeable sites of a particular clay fraction. The surface energy of a clay mineral often times is affected by cation exchange which also affects the pH of clay surface. The manufacture of inorganic antibacterial materials had been linked to clays high cation exchange capacity (Moore & Reynolds, 1997). A high cation exchange capacity in geophagic clays helps in the absorption of diarrhea causing enterotoxins (Marini et al., 2007).

2.8 Pharmaceutical formulations - Clay minerals as major constituents
The application of clay minerals in pharmaceutical formulations dates back to decades ago and has been well documented based on previous studies (Bolger, 1995, Lopez Galindo & Viseras, 2000, Carretero, 2002, Cerri, et al., 2004, Love, 2004, Carretero, et al., 2006, Carretero & Pozo, 2009). The medicinal uses of clays were well recorded in antiquity by the Greek philosopher Aristotle (4th century BC), Pliny the Elder and Dioscorides (1st century AD), and in the writings of Galen of Pergamon (2nd century AD) (Rateau et al., 1982). Due to economic circumstances, most of the minerals used in the pharmaceutical industry are synthetic analogues. However, in the application of clay minerals, there is a great exception on the account of the difficulty and cost implications in having them synthesised. Certain physical and physic-chemical properties of clay minerals such as particle size fractionation, thermal treatment and acid activation support their usage in pharmaceutical products (Williams & Hillier, 2014). Adsorption capacity of clay minerals coupled with their ability to release microelements is important properties which enable the binding of noxious compounds and their subsequent expulsion from the body (Carretero & Pozo, 2009). Clay
minerals, when used as active ingredients, have therapeutic traits while as excipients are more or less inert minerals that determine the consistency, form, and volume of the pharmaceutical preparations (Tenorio Arvide et al., 2008).

Excipient clay minerals have an organoleptic property that causes them to induce liberation of the active ingredient or facilitate the elaboration and conservation of the pharmaceutical preparation (Carretero & Pozo, 2009). Smectite, kaolinite, talc and palygorskite are some of the clay minerals used in pharmaceutical formulations either as active principles or excipients (Carretero & Pozo, 2009). Their usage centers on certain characteristics such as a high specific area, adsorption capacity (towards ions or molecules), cation exchange capacity, rheological properties, chemical inertness and low toxicity for patients, thixotropy and colloidal properties, swelling capacity, dispersivity, plasticity and low cost (Carretero & Pozo, 2009). There is an on-going research into possible application of sepiolite in pharmaceutical formulations as active principles or excipient (Viseras et al., 2007). The beneficial effects of clay minerals on human health could be: as active ingredients that are administered either orally (by ingestion) or topically (by application on the skin) and excipient in pharmaceutical products. The medicinal use of clays has evolved from the common consumption in “first grade” to pharmaceutical uses in “first aid,” and recently, to antibacterial applications and as vehicles for drug delivery and cancer treatment.

2.9 Clay minerals as active principles in pharmaceutical formations
Clay minerals, depending on the therapeutic need for which they are required can be administered orally or topically to patients. The therapeutic ingestion of clay minerals in the form of pills, dispersions, powders and emulsions can be observed in gastrointestinal protection, osmotic oral laxatives and antidiarrheals.

2.9.1 Gastrointestinal protectors
Antimicrobial clays – clays that kill human pathogens on contact – have been identified from hydrothermal clay deposits (Lopez Galindo & Viseras, 2000). In these clays, the role of bactericidal action is as a result of redox-sensitive transition metals. Recently, a lacustrine deposit from the Colombian Amazon basin containing kaolin (kaolinite, 30%; halloysite, 16%), along with smectite (31%), quartz (16%), and muscovite (7%) has been shown to be antibacterial against *E.*
coli and Bacillus subtilis (Morrison et al., 2013). The clay lowers the capability of Gram-negative and Gram-positive bacteria tested by 3-5 orders of magnitude (Williams & Hillier, 2014).

Palygorskite and kaolinite are the major clay minerals used for the purpose of gastrointestinal protection which could be supported by their high specific area and sorption capacity (Williams & Hillier, 2014). Other minerals in this category include sepiolite and smectites. They carry out their protective function by attaching themselves to the gastric and intestinal mucous membranes thereby absorbing toxins, bacteria and viruses (Cygan & Tazaki, 2014). Kaolin administered orally may interfere with the digestive tract by coating the epithelial cells lining the small intestine villi and mixing with and modifying the physical and chemical properties of the gastrointestinal mucus layer. This effect is as a result of the morphology of kaolin (platy, tubular, or spherical) coupled with the increased viscosity and stability of the gastric mucus alongside the decreased degradation of glycoproteins in the mucus (Droy-Lefaix & Tateo, 2006). The surface area of kaolin crystallites being greater than that of the villi will necessitate the exchangeable ions of the clays to acting as a source or a sink for both nutrients and toxic compounds (Droy-Lefaix & Tateo, 2006).

The issue of eliminating enzymes and other vital nutritive elements is one of the challenges associated with these minerals which have a deleterious effect on prolong use by humans. Clay minerals are administered to patients in the form of suspensions, pills or powders and are discharged from the body as faecal matter as they cannot be absorbed by the intestinal tract and not readily soluble in aqueous media. Smectites from previous studies exhibited a high level of adsorption and a high specific area but are not applied as gastrointestinal protectors on account of the stomach’s hydrochloric acid (pH 2), a condition that destroys them (Williams & Hillier, 2014). However, the use of smectites as gastrointestinal protector requires a stomach buffer such as Na-Beidellite.

2.9.2 Clay minerals as osmotic oral laxatives
The application of clay minerals as orally administered laxatives is usually to support defecation which they do via a process of osmosis or by irritating the colon-rectum. A typical clay mineral that is used for laxative effect is smectite. It functions by the process of osmosis as the therapeutic effect is triggered by the interlayered Na\(^+\) that spread through the stomach’s fluids up to the bowel,
resulting in a rise in the osmotic pressure of the gastric tracts. A balance in the osmotic pressure is maintained by the movement of water from the blood plasma through the walls of the bowel. This action propels an increase in the volume of the bowel’s contents thereby stimulating a propulsive motor activity of the smooth intestinal muscle which gives rise in liquid faeces (Carretero, 2002). The laxatives are generally administered as suspensions and are eliminated together with faeces when the trigger defecation. About 15% to 20% of the Na+ is absorbed while crossing the intestinal wall into the blood plasma and are later eliminated by the kidneys or by perspiration.

2.9.3 Clay minerals as antidiarrheal
Diarrhea is seldom associated with an increase in the fluidity of faeces as well as their rate of evacuation (Carretero, 2002). This acute pathological situation could be linked to food poisoning, bacterial infections, allergic states and defective intestinal absorption. Tackling these root causes had been the best way of treating diarrhea. Often times, formulations used in treating diarrhea combat the effects by reducing the amount of liquid that reaches the colon-rectum from the small bowel by either reducing the speed of passage through the bowel thus supporting the absorption of water and electrolytes or by direct absorption of water by the active principle itself. Kaolin and palygorskite are the widely used clay minerals in diarrhoeics as they help in absorbing both excess water in faeces and gases in the digestive tract (Carretero, 2002). Alternatively, Calcium smectites are applied as laxatives due to the astringent action of Ca\(^{2+}\) ion which forms a non-soluble hydrated phosphate that gives rise to pulverulent faeces that are hard to evacuate, resulting in constipation. The clay minerals are administered orally either as powders or pills and are excreted through the faeces. In the event that the diarrhoeics is calcium smectites, part of the Ca\(^{2+}\) gets absorbed in the bowel and a fraction of the absorbed Ca\(^{2+}\) are eliminated by the kidney which ends up in faeces.

2.10 Topical application of clay minerals
Some clay minerals constitute pharmaceutical formulations that are applied to the exterior part of the body for the purpose of dermatological protectors, cosmetics or anti-inflammatory.

2.10.1 Clay minerals as dermatological protectors
Generally, powders, creams and ointment that protect the skin against external agents and materials exuded by the skin itself are referred to as Dermatological protectors. Kaolinite, talc and smectites
are some of the clay minerals used as dermatological protectors, on account of their high absorbent capacity (Carretero, 2002). Kaolinite is effective as hemostatic wound dressings as demonstrated during the Iran and Afghanistan wars on the grounds that with little or no damage to body tissues can clot blood from traumatic injury (Carretero, 2002). Unlike kaolinite, when dry zeolite is hydrated, it results in just enough heat (~100°C) to cauterize blood vessels (Williams & Hillier, 2014), but unfortunately it causes severe damage to vascular tissue, requiring post-trauma grafting. Smectites also had proven problematic by resulting in severe endothelial injury, massive thrombosis, and occlusion of the injured blood vessels when used on wounds (Baker et al., 2007). Following modern understanding of the mineralogy of kaolin, the claims of hazardous effects from nanoparticles of kaolinite on human tissues had been resolved. The surface charge of kaolinite, which is negative at the pH of blood and human plasma, is positively correlated with its blood-clotting potential (Lawton et al., 2009).

These minerals attach themselves to the skin and form a film-like structure which protects it mechanically against external physical or chemical agents. By absorbing the skin’s secretions, these minerals leave a water-poor medium that does not support the development of bacteria; thus resulting in a refreshing sensation. It is important to note that the application of palygorskite and sepiolite as dermatological protectors are widely discouraged following claims surrounding their carcinogenic effect when inhaled. Recent studies reveal palygorskite as neither toxic nor dangerous (McConnochie, et al., 1993, Santaren & Alvarez, 1994, Governa, et al., 1995).

2.10.2 Clay minerals in cosmetics
Based on the high adsorption potentials of substances such as toxins and greases, clay minerals are applied as active principles in cosmetics (Williams & Hillier, 2014). Often times they are applied during inflammatory processes such as boils, acne, ulcers and to modify physical appearance as well as preserve the physico-chemical conditions of the skin (Lopez-Galindo et al., 2007). Clay minerals are used as antiperspirants in creams, powders, and emulsions. They also support skin opacity, remove shine and cover blemishes (Lopez-Galindo & Viseras, 2004). Kaolinite, smectites, talc and palygorskite are some of the clay minerals used in cosmetics. Micas nevertheless based on their reflectance and iridescence is used in eye shadows and lipsticks. It is important to note
that the use of fibrous clay minerals in solid preparations is dangerous due to the high tendency of a carcinogenic effect when inhaled (Carretero, 2002).

2.10.3 Application of clay minerals as excipients
Excipients refer to substances introduced into certain pharmaceutical formulations for the purpose of: improving its organoleptic characteristics such as taste, smell and colour. Also excipients enhance physico-chemical properties such as viscosity; facilitating the pharmaceutical formulations preparation; and promoting the pharmaceutical formulation’s disintegration when orally administered in the form of pills, capsules (Carretero, 2002).

Palygorskite, kaolinite, talc and smectites are the major clay minerals used as excipients. Some of the reasons for their usage includes but not limited to (i) lubricants to ease the manufacture of pills – Talc being a 2:1 clay mineral held by weak van der Waals forces, soft and unctuous is used during tablet formation. Adhesion of the powder to the compression pistons is prevented by the lubricating nature of talc (Carretero, 2002). (ii) agents to aid disintegration, based on their ability to increase in volume in the presence of water (smectites), or the dispersion of sepiolite fibres and palygorskite, which support the liberation of the drug of the pharmaceutical formulation when it gets into the stomach, (iii) inert bases for cosmetics (palygorskite, talc, smectite, kaolinite) and (iv) emulsifying, polar gel and thickening agents based on their colloidal features and thixotropic properties (palygorskite, smectites, kaolinite and talc) and to avoid the segregation of the pharmaceutical formulation’s components and the formulation of a sediment that is difficult to redistribute (Carretero & Pozo, 2009).

Most clay minerals used as excipients are known to influence the liberation and stability of drug bioavailability (Carretero, 2002). The liberation of antibiotics such as amoxicillin, ampicillin, cyclamycin, analgesics (e.g. paracetamol), and anxiolytics (e.g. diazepam) is affected by clay minerals (White & Hen, 1983). This action occurs either in the pharmaceutical formulations themselves or in the gastrointestinal tract. There is a harmful effect of mineral excipients on the stability of drugs which is as a result of the interaction between the drug and the mineral. The interaction often times results in speeding the rate of degradation of the drug which invariably leads to loss of therapeutic activity and increased health risk. The degradation of absorbed digoxin,
a cardiovascular tonic by montmorillonite is a typical example (Iwuagwu & Aloko, 1992). Likewise, the degradation of hydrocortisone and dexamethasone when absorbed by palygorskite and sepiolite (Porubcan et al., 1979).

To avoid rapid metabolism, clay minerals are used for encapsulation of highly reactive compounds – such as Resveratrol, a polyphenol known for having antioxidant properties – that impede the growth of malignant cancer cells (Forteza et al., 1988). There is an emerging use of halloysite as a carrier for gene-therapy drugs. Here, functionalised halloysite tubes are used as paths for drug delivery via endocytosis (cells enveloping halloysite nanotubes), thus supporting antitumor activity (Vergaro et al., 2012). There is on-going research for the collection and treatment of circulating tumor cells from blood using halloysite as a substrate.

2.11 Clay minerals in spas

The application of clay minerals in spas is widely spread. Generally, they are mixed with water for geotherapy, mixed with sea water or minero-medicinal water and then matured (pelotherapy), or mixed with paraffin (paramuds) (Shi et al., 2011). A single or mixture of clay minerals such as smectites and kaolinite are mostly applied with occasional use of chlorite, sepiolite, palygorskite and others. Often times, trace minerals such as calcite, quartz, dolomite and feldspar are seen alongside many other minerals.

Some of the characteristic features of these clay minerals that support their usage in spas include: (1) their high absorption/adsorption capacity which makes them capable of eliminating toxins and grease from the skin, (2) their high cation exchange capacity which allows the exchange of nutrients such as K⁺, Na⁺, Ca²⁺, Mg²⁺ while the clay remains in touch with the skin, (3) plastic potentials for easy application and attachment to the skin during treatment, (4) rheological properties for the formation of a viscous and consistent paste, (5) softness and grain size (6) similar pH to that of the skin to avoid irritation, (7) high heat-retention capacity to help sustain the heat required during the treatment of chronic rheumatic inflammations, sports traumatisms and other dermatological issues (Ferrand & Yvon, 1991, Summa & Tateo, 1998).

The mobility of toxic elements such as As, Pb, Hg, Cd, Se, Sb, Cu, Zn is highly considered in the clay-water mix used in spas to avoid intoxications. The exchangeability of the toxic elements as
well as the nature of bonding to the minerals is widely considered (Carretero, 2002). Some of the ways of applying clay minerals for therapeutic purposes in spas are discussed below.

2.11.1 Geotherapy
This involves the mixture of one or more clay minerals with water and its direct application upon the skin, leaving a consistent layer of about 1 cm thick. It is the therapeutic use of clay materials usually natural earth that has different minerals in them. Geotherapy is mainly used for facial treatments, generally in the cold direct application on the skin (kaolinite or smectites mixed with water) (Summa & Tateo, 1998).

Depending on the area of the body to be treated, the application of clay minerals can be in the form of cataplasms or mud baths which are used for the treatment of dermatological infections such as boils, acne, ulcers, abscess, seborrhea as well as in the alleviation of pains caused by chronic rheumatic inflammations (Britschka, et al., 2007, Arab & Alshikh, 2012). This method stimulates perspiration and sebaceous secretions which flow more easily in a fluid state. Sodium, Chloro, potassium and urea are eliminated during perspiration, resulting in the activation of metabolic change and the excretion of catabolites (Arab & Alshikh, 2012).

2.11.2 Cataplasms and mud baths
Cataplasm refers to a poultice or plaster moist mass that is often warm and medicated usually spread over the skin to treat an inflamed, aching or painful area (Lotti & Ghersetich, 1999). This is often used when the mud is applied to a fraction of the body. When the treatment is for inflamed areas, the mud to use must have a temperature lower to that of the body. In such circumstance, the clays and water mixtures results in a cooling sensation of the region being treated, thus an anti-inflammatory agent since it absorbs the heat given off by the inflammation.

Mud baths are complex detox therapies that help to remove toxins and impurities from the body, thus leaving the skin refreshed and rejuvenated. The volcanic ash helps to exfoliate the skin. Often time’s mud baths are used when the area under treatment is extensive and are executed by submerging part of the body in a bowl or bath containing a mixture of clays and water (Lim, 2016). It application to a large extent had enhanced the clinical conditions of patients with knee osteoarthritis.
2.11.3 Pelotherapy
Pelotherapy is the application of thermal muds “peloids” for recovering muscle-bone-skin pathologies (Carretero, 2002). It is the most widely practiced of all the clay pastes in spas and beauty therapy which is linked to the maturation process that results in enhancement of the therapeutic capacity of the final peloid administered to patients. Peloids (thermal muds) are ‘natural products made up of a mixture of mineral water (sea water and salt-lake water included), with organic or inorganic matter generated from biologic or geologic processes or both’ which are used for therapeutic purposes in the forms of baths at temperatures approaching 45 – 50 °C (Tateo & et al., 2009, Williams, et al., 2009, Monasterio, et al., 2015). Alternatively, peloids could be defined as ‘matured mud or muddy suspension with healing and/or cosmetic properties composed of a complex mixture of fine-grained materials (mineral and/or organic), with mineral water, sea water, salt-lake water, and commonly organic compounds from biological metabolic activity’ (Fernando et al., 2007). The therapeutic effect of peloids is linked with the thermal treatment, water chemistry, and clay properties.

Peloids could either be natural or sensustrictu. Natural peloids are those whose muddy dispersion is maturated at the place of their occurrence and possible use for healing or cosmetic. The sensustrictu on the other hand are those that have the maturation of the muddy dispersion occurring inside spas and laboratories by artificial processes and scientific modifications such as the incorporation of natural functional compounds like antioxidants, anti-cellulite properties. In most cases the maturing processes alter some of the properties of the clay minerals. For instance, properties such as absorption capacity, plasticity and cooling index tend to increase while a decrease is noticed on the grain size. Usually, peloids portray a stimulatory antiphlogistic and analgesic action and as such should have appropriate properties: hydric degree, consistency, adhesiveness, heat capacity, cooling rate, exchange capacity, ease of handling and pleasant sensation when applied to the skin (Gomes et al., 2013).

On the basis of the composition of peloids, three major categories are identified namely, essentially inorganic peloids; essentially organic peloids; and mixed peloids. Considering the origin and composition of both solid and liquid phases, natural peloids are referred to with names like fango,
loam, sapropel, peat, biofilm. The composition, properties, preparation and maturation of peloids are critical factors to be considered in pelotherapy (Carretero, 2002). Nevertheless, the mode of preparation of peloids varies from town to town as some spas’ use of naturally matured clays collected from spring basins. Others use artificial ponds where natural ‘virgin’ clays are mixed with mineral water or seawater. The properties of the clay-based peloids are affected by the composition of the raw clay, water composition and mixing conditions.

Maturation is a process that involves both the multiplicity of mineral waters and materials, including clay minerals, inorganic solutes, microorganisms, biogenic components and varying physico-chemical conditions such as temperature and pH. In simple terms, maturation is a period of time usually in months that allows chemical equilibration between the clay and water (Veniale et al., 2005). During this process, organic substances together with macro- and micronutrient elements contained in the water are absorbed by the peloids which are later released when applied to the human body. The maturation process often times help to improve certain physical properties of the mud such as the heat-retention capacity, rheology and adherence. In the same fashion, noticeable alterations are seen in the mineralogy, geochemistry and organic compounds.

During pelotherapy, the mixture of clays and water is applied hot within a temperature range of (40 – 45°C) for a period of 20 – 30mins usually in layers of about 1 and 2 cm (Williams & Hillier, 2014). An impermeable material is used to cover the mixture to help conserve the heat. The heat is transferred through the skin to the intravenous areas before it gets to the inner part of the body by conduction and convection via the bloodstream. Deleterious and harmful pathogens that may have been released from a particular patient are destroyed during the process of rematuration (Carretero, 2002). Peloids generally produce a sensation of heat in the area being treated, vasodilation, perspiration and the stimulation of cardiac and respiratory frequency (Carretero et al., 2006).

In general, peloids exhibit a stimulatory, antiphlogistic, analgesic action and are recommended for chronic rheumatic processes, degenerative osteoarthritis in any part of the body, dysendocrine arthropathies, spondilo-arthritis ankylopoietic, spondylosis, and so on (Carretero, 2002). They are widely used for the sequelae of osteoarticular injuries, fractures, dislocations, dermatological diseases and the like (Carretero, 2002).
2.12 Clay minerals in aesthetic medicine
Clay minerals via invasive cosmetic treatments are predictable ingredients that enhance the physical appearance of humans. In aesthetic medicine (to clean and moisturise the skin and to combat compact lipodystrophies, acne and cellulite), the use of clay minerals ranges from its use as cosmetic products, excipients, pelotherapy, geotherapy and paramuds (Carretero, 2002). Often times in geotherapy, facial treatments are done using kaolinite or smectites mixed with water. Nevertheless, in the form of facial masks, dermatological diseases such as spots, acne and seborrhea are treated using a hot mixture of clays and water. This procedure enhances perspiration and sebaceous secretions due to the easy flow when in fluid state while also opening the pylosebaceous orifices. Sodium, urea and potassium are discharged during perspiration thus giving rise to metabolic change and excretion of catabolites. Clay minerals such as kaolinite, smectites, talc and palygorskite are used, although use of the last two is recommended only in liquid preparations - creams, emulsions, etc. (Arab & Alshikh, 2012).

Peloids in aesthetic medicine serve the same purpose as geotherapy with the benefit of contributing nutrients obtained during the maturing process. Due to the impermeability of paramuds and the subsequent inability of generated perspiration during their usage, paramuds supports the moisturisation of the skin (Arab & Alshikh, 2012). The moisturing effect takes place as the perspiration soaks into the upper layers of the epidermis. In addition, the application of paramuds leaves the skin in a state of hyper-porous, thereby allowing absorption of cosmetic materials by the corneous layer down to the inner most layers of the epidermis. Paramuds also helps to stop the growth of cellulite since they are anti-inflammatory and stimulate venous and lymphatic circulation in the area of application (Carretero, 2002).

2.13 Clays in pottery applications
Clays are of great importance in pottery and are baked at very high temperatures to become hard. In pottery applications, clays are used together with some additives to further enhance their properties when worked and fired. Some of the clays used in pottery for the purpose of this review are discussed from section 2.1.3.1 to section 2.1.3.4.
2.13.1 Kaolin
Kaolin, or china clay, is pretty scarce in nature but the purest of all clays which makes it highly sought after by potters (Claeyssens, 2009). It is less common, unlike other types of clays; deposits are found in Europe, England, Asia and North America. Kaolin is an essential requisite in the making of pure white porcelain or china. Several kinds of kaolin are naturally available. The majority is the secondary kaolin that is applied as mined or washed to remove unwanted minerals such as quartz, feldspar or iron oxide. The residual kaolin due to the large amounts of non-clay minerals they contain, require thorough washing before being used (Norton, 1976).

Kaolin clays are used for porcelain, based on the purity of their mineral components. They appear very light in colour. In moist state, they are light grey and near-white and then, white when fired (Norton, 1976). The chemical composition of kaolin can be linked with the formula of the mineral kaolinite (Al$_2$O$_3$.2SiO$_2$.2H$_2$O) (Rhodes, 1996).

Kaolin is extremely refractory clay and has a melting point which is above 1800 °C. When used as a single material, kaolin hardly shapes into objects because of its poor plasticity (Van Olphen, 1974). It refractoriness, also makes it difficult to mature by firing to a hard, dense object. Generally, it is highly problematic to work with kaolin by itself; rather it is mixed with additives to both increase its workability and to lower the kiln temperature required to produce a hard; dense product. As a result of its relatively coarse grain structure, it has a low shrinkage and little dry strength (Olupot et al., 2006).

2.13.2 Ball clays
Ball clays are secondary or transported type of clays commonly found in stratified layers, often alternating with layers of coal and with other types of clay. They are fine grained in nature, more fusible, and more plastic. They are not as pure as kaolin but comparatively free from iron and other mineral impurities and burns to a light gray or light buff colour (Norton, 1976). Ball clays are really used as a complete body due to their excessive shrinkage, rather are combined in clay bodies. Thus, adjusting the mixture toward practical, workable clay; adding plasticity, better green and enhanced casting properties (Rhodes, 1996). It is highly plastic. Although not so pure as kaolin, ball clay is relatively free from iron and other mineral impurities and burns to a light gray or light buff colour (Norton, 1976). When fired to about 1800 °C it tightens into a dense structure.
They are highly recommended as additives to aid the plasticity of kaolin in the manufacture of white wares. In a situation where white body is needed, approximately 15% of ball clay can be added to a clay body; more than this quantity in a white ware body results in a gray, off-white, or buff colour (Rhodes, 1996). The existence of ball clay in a porcelain body decreases its translucence (Rhodes, 1996). Natural ball clays are usually dark gray which is as a result of the presence of carbonaceous material. This carbon contents are gotten rid-off during firing without interfering with the final fired colour of the clay. The plasticity of ball clays increases with a higher content of carbon.

2.13.3 Fire clays
Fire clays unlike ball clay and kaolin are neither popular nor in high demand because of their resistance to heat. While some fire clays possess high plasticity, others lack plasticity, with varying colour when fired (Norton, 1976). Clays that resist deformation up to about 1500 °C are considered to be pure and iron free, thus are called fire clay. In cases where iron-bearing minerals are present, when fired they burn to a buff or brownish colour with darker splotches (Rhodes, 1996). They comprise mainly kaolinite, halloysite, and/or diaspora (Van Olphen, 1974).

Fire clays are mostly applied in the manufacture of fire brick and other refractory parts for kilns, furnaces, boilers, and melting pots. Industries such as steel, copper, and other metallurgical industries could not operate without fire brick furnaces in which high-temperature smelting is done (Virta, 2002). They are also used in stoneware bodies to create a desirable roughness and texture due to the presence of dark iron spots. Fire clay is also useful in mudding in kiln doors, making clay pots for pyrometric cones, and for wadding under kiln shelves (Rhodes, 1996).

2.13.4 Stoneware and earthenware clays
Stoneware clays are plastic clays which mature or become vitreous at 2100 °F to 2372 °F. They have varying colours, plasticity and fired colour ranges from a very light gray or buff to a darker gray or brown (Rhodes, 1996). Stoneware clays are secondary clays; hard with a dense surface. They are known to bond effectively with their glazes, tough and forgiving during throwing and firing. Based on their quality, they are applied mostly in industrial ceramics and functional applications such as dinnerware.
Earthenware clays are the most commonly found in nature and is known to have the lowest firing temperature. This is on the account that they contain iron and other mineral impurities in high quantity which makes them tight and hard-fired at about 950 to 1100 °C. In the natural state such clay due to iron oxide appears red, brown, greenish, or gray. When fired, there is a change in colour, red or tan to brown or black subject to the clay and the condition of the firing. A large number of potteries around the globe are made of earthenware clay, and it is also the common raw material for brick, tile, drain tile, roof tile, and other heavy clay products. They have a low shrink factor, porosity and a high tendency to stain easily.

Table 2.2: Clay minerals associated with geophagic, cosmetic/medicinal and pottery applications (Williams, 2005).

<table>
<thead>
<tr>
<th>Clay minerals</th>
<th>General formula</th>
<th>Application areas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaolinite Group – kaolinite, dickite and nacrite.</td>
<td>Al₂Si₂O₅(OH)</td>
<td>Pottery, cosmetics, geophagy</td>
</tr>
<tr>
<td>Montmorillonite/Smectite Group – pyrophyllite, talc, vermiculite, saponite, montmorillonite, sauconite and nontronite.</td>
<td>(Ca, Na, H)(Al, Mg, Fe, Zn)₂(Si, Al)₄O₁₀(OH)₂-XH₂O</td>
<td>Geophagy, cosmetics, paint</td>
</tr>
<tr>
<td>Illite/mica Group</td>
<td>(K, H) Al₂(Si, Al)₄O₁₀(OH)₂-XH₂O</td>
<td>Pottery</td>
</tr>
<tr>
<td>Chlorite Group – Amesite, chamosite, Delessite, Nimite etc.</td>
<td>X₄⁺₆Y₄O₁₀(OH, O)₈</td>
<td></td>
</tr>
</tbody>
</table>

2.14 Harmful effects of clays and clay minerals
Taking into account of how clay and its associated clay minerals are uncontrollably consumed in geophagic practices, the challenges are inevitable. For geophagists, a total blockage of the lower intestine which can only be remedied by surgery is a major risk associated with eating of clay (Norton, 1976). Nutrient deficiencies could also result from eating clays. There are cases of detrimental effects on the human teeth and gums and on the digestive system, excessive nutrients, poisoning, and parasitic invasions (Padilla & de la Torre, 2006).

It is important to mention that studies have shown that geophagy often has adverse effects which result from the potential ingestion of parasites (Williams et al., 2009) and/or harmful microorganisms and toxic compounds. Moreover, the anti-nutritional effects of clay may be
exerted due to their high adsorption capacity. Consequently, it can cause anaemia by binding to iron (Kawai et al., 2009, Yanai et al., 2009). Previous studies in aesthetic medicine indicate that peloids occasionally contain hazardous elements such as arsenic (As), cadmium (Cd), lead (Pb), asbestos minerals, free silica and Iron-oxy-hydroxides that may be released during application. These ions find their way to the skin and blood stream. The radioactive elements can result in the production of harmful ionizing radiations that could reside in the lung tissues for a long time (Vreca & Dolenec, 2005, Pozo et al., 2009).

2.15 Conclusion
The beneficial effects on human health, pharmaceutical formulations, aesthetic medicine and spas based on the therapeutic use of clay minerals cannot be over emphasized. Their applications could be as active principles orally administered in pharmaceutical formulations for purposes of gastrointestinal protectors, osmotic oral laxatives or for topical purposes (cosmetics, dermatological protectors) and as excipients: (lubricants, emulsifiers, inert bases). The wide use of these clay minerals is as a result of their unique characteristics such as high specific area, high absorption/adsorption potential, rheology, chemical inertness as well as low toxicity for the patient being treated.

As excipients, clay minerals tend to react with drugs thereby affecting the bioavailability of the active principle in the area of liberation and stability. Geotherapy, pelotherapy and paramuds are applied based on their cation exchange capacity, absorption/adsorption capacity, plasticity, grain size, and rheology and cooling index in aesthetic medicine and spas.

In spas, their usage is related to the treatment of dermatological diseases and alleviation of pains linked to chronic rheumatic inflammations. In the area of aesthetic medicine, they are principally used in the moisturisation of the skin and to tackle compact lipodystrophies, acne and cellulite. Most geophagic clays due to their size, shape and nature result in intestinal perforation of the GIT. This condition is hazardous to human health. The kaolinitic geophagic clays on account of their potential to absorb water from the human gastrointestinal tract are used as anti-diarrheal medications. Smectitic clays on the other hand are natural intestinal detoxifier based on their ability to absorb toxins from the gastrointestinal tract. The quartz particles in geophagic clays results in
damaging the dental enamel of geophagic individuals due to mastication which is due to its hardness being greater than that of hydroxylapatite - a calcium phosphate mineral with a Mohr scale hardness of 5 that constitutes the bulk of the dental enamel. Other associated health risks linked with geophagia include increased prevalence of intestinal helminths especially *Trichuris* and *Ascaris* species. There is a great need for beneficiation exercise of geophagic clays containing quartz with the aim of reducing the coarse angular nature. The intake of geophagic materials pose risk of dangers associated with silica (Si) and lead (Pb) poisoning. Following the increasing adverse effects resulting from the excessive consumption of clay minerals, it is crucial to mention that geophagic practice be discouraged.
References


Mahaney, W. C., Hancock, R., Aufreiter, S., & Huffman, M. A. (1996). Geochemistry and clay mineralogy of termite mound soil and the role of geophagy in chimpanzees of the Mahale


Seim, G. L., Ahn, C. I., Bodis, M. S., Luwedde, F., Miller, D. D., Hillier, S., Young, S. L.


CHAPTER THREE


Abstract
Geophagia, the intentional ingestion of soil has both useful and negative health effects due to the biomass, physicochemical, mineralogical and geochemical composition of the gritty material. In a few circumstances, clayey soil is viewed as a supplement to healthful deficiencies resulting from a less than stellar eating routine. Geophagy has been of interest to researchers and analysts due to continued habitual practice by diverse communities globally in spite of reports contraindicating its purported health benefits. The gritty materials remain an area of interest considering the nature in which they are obtained, transported and stored. Geophagic clayey soil from chosen casual markets in South Africa (including samples originally from Nigeria) were investigated and characterised utilising XRF, XRD, electrical conductivity and for pH to survey the nutritious qualities that support the continued utilisation by mostly females and pregnant ladies in various parts of South Africa. XRD results of the analysis carried out on the six representative samples obtained revealed that the clay material is mainly composed of kaolinite with minor palygorskite, illite, Amesite, Gupeite, Hematite and Magnetite. Quartz was the major non-clay constituent identified. The results from the XRF analysis on the samples portray average values of major elements such as SiO$_2$ (54.02%), Al$_2$O$_3$ (35.45%), Fe$_2$O$_3$ (6.73%), K$_2$O (2.76%), MgO (1.16%) with MnO, Na$_2$O and P$_2$O$_5$ falling below 0.5%. Titanium was a major heavy metal identified from the samples which may result in heavy metal toxicity. The potential medicinal application of these clayey soils is supported by the kaolinite contents; in contrast the trace elements are pointers of probable adverse effects on humans.

Key words: Clayey soils, Geophagy, Kaolinite, Mineralogy, Quartz.

3.1 Introduction
Clay is a naturally occurring material composed of fine-grained minerals, which are for the most part plastic at proper water contents and will solidify when dried or fired (Nayak & Singh, 2007). Clay possesses varying chemical composition depending on both the physical and geographical occurrence. Characteristic clay minerals are well known to humanity from time immemorial. As a result of their affordability, abundance in diverse regions of the globe, high sorption potential for ion exchange, clay minerals are applied as strong adsorbents (Guggenheim & Martin, 1995).

Although clay minerals share an essential arrangement of structural and chemical characteristics, each clay mineral has its own unique properties that decide how it will associate with other synthetic species. The variation in both chemistry and structure, among the different clays prompt their applications in different fields. Often time clay minerals are classified by the differences in their layered structures. Some of the classes of clay include smectites (montmorillonite, saponite), mica (illite), kaolinite, serpentine, talc, vermiculite and sepiolite (Grim, 1968).

Clay is principally made of silica, alumina and water, oftentimes with considerable amounts of iron, alkalies and alkali earths (Shichi & Takagi, 2000). Basically, there are two structural units when looking at the atomic lattices of several clay minerals. One unit comprises of firmly pressed oxygen and hydroxyls in which aluminium, iron and magnesium atoms are inserted in an octahedral mix with the goal that they are equidistant from six oxygen or hydroxyls. The second unit is made of silica tetrahedrons that are orchestrated to shape a hexagonal system that is rehashed to form Si₄O₆(OH)₄ (Shichi & Takagi, 2000).

Previously, geophagy used to be peculiar amongst pregnant women and children in rural communities in African countries but recently, it has been gaining a global adoption. The implications of consuming geophagic clays in respect to their heavy metal content are still being studied. From the South African context, efforts had been made regarding geophagy as documented in the studies of (Saathoff et al., 2002), (Ekosse & Jumbam, 2010), (Ekosse et al., 2010), (Mpuchane et al., 2010), (Ngole et al., 2010), (Songca et al., 2010), (Mogongoa et al., 2011) and (Ngole & Ekosse, 2012) all pointing that geophagy is prevalent within the various provinces within the country. Most of the geophagists, particularly pregnant women, supported their practice on several factors, some of which include natural craving to alleviate early morning
illness, reducing the threat of infection as well as the assurance of the quality and quantity of breast milk for their unborn child (Johns & Duquette, 1991). Further claims include its potential to agitate appetite and curb vomiting during pregnancy.

Emphatically speaking, no explanation can basically substantiate the practice of geophagy as it has great benefits as well as adverse implications. Previous studies reveal that humans and some animals engage in geophagy due to certain nutrient deficiency (Young, 2010), and in other instances such as in responses to gastrointestinal disorders (Mitchell et al., 1977). Human geophagy is a global phenomenon that is seen amongst all races, ages, sexes, and social classes (Hunter, 1993). With immigrants and cross border trades amongst nations, geophagic clayey soils are sold in open markets in South Africa, rural dwellings in America, Asia and Europe (Crawford & Bodkin, 2011). Most geophagic clayey soils are highly retentive due to their fine-grained sizes, thus absorbing metal ions into their crystal lattices. The concentration levels of these elements determine their usefulness or harm when consumed by humans.

Several techniques such as scanning electron microscope, X-ray diffraction and infrared spectrometry, atomic absorption spectroscopy were used in identifying the specific clay minerals. The principal aim of the study is to determine both the physical and chemical makeup of various geophagic clays sold in different informal markets within South Africa irrespective of their sources with the goal of ascertaining their suitability for human consumption.

3.2 Experimental methods
3.2.1 Materials
The clay samples were obtained from selected informal open markets within South Africa as indicated in Table 1. The six representative samples (four originally from South Africa and two from Nigeria) were used as received from the vendors at the markets without any form of modification. Geophagic clay samples from South Africa were labelled GSA 1 – 4 while the samples from Nigeria were labelled GNG 1 – 2. To further enhance the surface area of the clayey soils, the samples were ground using mortar and pestle. Physical characterisation of the various samples was executed using standard methods as proposed by (Nwosu et al., 2013). The samples were digested prior to chemical characterisation by Atomic Absorption Spectrophotometer (AAS).
3.2.2 Digestion of clay samples
Various samples (2g) were weighed into a Teflon crucible and moistened with 100mL of 0.5M, 1M and 2M HCl acid. The mixtures were covered and placed on a shaker for 24 hours at 130 rpm. The solutions were filtered and the filtrates were stored in sterile bottles prior to being analysed for minerals using AAS.

3.2.3 Loss of ignition (LOI)
Each clay sample (10g) was weighed and put into a crucible before being transferred into a furnace (1000°C) for 3 hours. The representative fractions were then allowed to cool in a desiccator and re-weighed. Weight lost is the loss due to ignition, usually expressed as:

\[ \text{LOI} = \frac{\text{Weight loss} \times 100}{\text{weight of initial sample}} \]

3.2.4 Determination of oxides using X-ray fluorescence (XRF)
The clay sample (10g) was pelletised using a mould at very high pressure and then placed in the sample compartment of X-ray fluorescence (XRF; Rigaku ZSX PrismaII). This was to analyse the elemental compositions of the various minerals that make up the clay.

3.2.5 Determination of the mineral phases using X-ray diffractometer (XRD)
The XRD patterns of clay were obtained on a powder X-ray diffractometer Model Rigaku Ultima IV with CuKα radiation (40kV and 40mA) having a scanning speed of 0.04o/s. This was done at the Extractive Metallurgy Laboratory of the University of Johannesburg. The XRD aided the determination of the mineralogical composition of the material components and also qualitative and quantitative phase analysis of multiphase mixtures as described by (Kawai et al., 2009a). Representative bulk samples were crushed and homogenised to fine powder at approximately 10-15µm in size. Each reflection as displayed by the X-Ray diffraction corresponds to particular mineral. With the peaks well separated from each other, their heights are applied in the determination of the orientation of certain mineral in the mixture. The basis of clay mineral analysis by X-Ray diffraction is centred on the identification of various peaks and comparison of their relative heights. Different phase levels were identified as semi quantitative estimates based on the relative peak heights (Moore & Reynolds, 1989). The wavelength of 1.5406 was used to calculate the diffraction angles.
3.2.6 Determination of trace Elements Using Atomic Absorption Spectrophotometer (AAS)
The filtrate from the digestion of the clay samples was analysed for elemental quantitative analysis using AAS (Win Lab32). Each metal with the exception of silica (Si) and aluminium (Al) that required nitrous oxide flame were analysed using air acetylene flame and deionised water as blank.

3.3 Results
3.3.1 Physico-chemical characterization of geophagic clays
The physico-chemical characteristics of the studied geophagic clayey soils which provide information on their benefits and possible deleterious effects are summarized in Tables 1 and 2. The colour ranged from yellowish orange through to brownish and yellow to brownish grey. The greyish colour may be attributed to the presence of finely disseminated organic matter while, the yellowish to brownish colour could be linked to the presence of iron oxides or from mixing of green clay minerals and organic matter. Clayey soil pH was in the range of 4.47 to 6.36 with an average of 5.54. Considering all six samples, GSA 1 registered the highest pH while GNG 2 recorded the lowest 4.47. Electrical conductivity values ranged from 121.5μS/cm to 1028.5μS/cm with a mean of 671.1μS/cm. With respect to the loss of ignition (LOI) all four samples originally from South Africa (GSA 1 - 4) showed higher values compared to samples originally from Nigeria. There were noticeable textural differences, with GNG1 and GNG2 samples being sandy, whereas samples GSA 1 - 4 were more clay-rich. The latter appeared to consist of weathered shale or clay, while GNG1 and GNG2 probably represented alluvial sandy material.
Table 3.1: Physical characterization of geophagic clay samples

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>pH (H₂O)</th>
<th>LOI %</th>
<th>Electrical Conductivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSA 1</td>
<td>6.36</td>
<td>0.27</td>
<td>1028.50 µS/cm</td>
</tr>
<tr>
<td>GSA 2</td>
<td>5.76</td>
<td>0.63</td>
<td>908.50 µS/cm</td>
</tr>
<tr>
<td>GSA 3</td>
<td>5.73</td>
<td>0.34</td>
<td>822.00 µS/cm</td>
</tr>
<tr>
<td>GSA 4</td>
<td>6.26</td>
<td>0.24</td>
<td>974.50 µS/cm</td>
</tr>
<tr>
<td>GNG 1</td>
<td>4.67</td>
<td>0.23</td>
<td>121.5 µS/cm</td>
</tr>
<tr>
<td>GNG 2</td>
<td>4.47</td>
<td>0.17</td>
<td>171.6 µS/cm</td>
</tr>
</tbody>
</table>

GSA 1 - 4 (Clay samples obtained from informal markets: Midrand, Johannesburg CBD, Bree taxi rank, Hillbrow respectively), GNG 1 & 2 (Clay samples obtained from Johannesburg CBD, originally from Nigeria).

Table 3.2: Colour and macroscopic description of 6 geophagic samples from informal markets.

<table>
<thead>
<tr>
<th>Sample</th>
<th>(Hue value chroma)</th>
<th>Colour</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSA 1</td>
<td>10YR6/6</td>
<td>Dark Yellowish Orange</td>
<td>Clayey sand; disperses easily but does not slake.</td>
</tr>
<tr>
<td>GSA 2</td>
<td>10YR8/6</td>
<td>Pale Yellowish Orange</td>
<td>Coarse blocky material, soft and decomposed; strongly variegated and patchy in colour; Clayey/silty texture.</td>
</tr>
<tr>
<td>GSA 3</td>
<td>10YR4/2</td>
<td>Dark Yellowish Brown</td>
<td>Coarse platy structure; weathered shale; clayey; disperses easily but does not slake immediately.</td>
</tr>
<tr>
<td>GSA 4</td>
<td>10YR4/2</td>
<td>Dark Yellowish Brown</td>
<td>Coarse platy structure; weathered shale; clayey; disperses easily but does not slake immediately.</td>
</tr>
<tr>
<td>GNG 1</td>
<td>5Y5/4</td>
<td>Reddish Brown</td>
<td>Coarse blocky structure; sandy texture; contains fragments of carbonized wood; slakes immediately in water.</td>
</tr>
<tr>
<td>GNG 2</td>
<td>2.5Y6/2</td>
<td>Light Brownish Grey</td>
<td>Sandy material containing white inclusions and organic matter; slakes immediately in water.</td>
</tr>
</tbody>
</table>
Table 3.3: Chemical composition in oxide state of geophagic clayey soils obtained from open markets

<table>
<thead>
<tr>
<th>Chemical composition</th>
<th>GSA1 Weight (%)</th>
<th>GSA2 Weight (%)</th>
<th>GSA3 Weight (%)</th>
<th>GSA4 Weight (%)</th>
<th>GNG1 Weight (%)</th>
<th>GNG2 Weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al₂O₃</td>
<td>33.55</td>
<td>33.55</td>
<td>31.91</td>
<td>32.53</td>
<td>31.13</td>
<td>31.02</td>
</tr>
<tr>
<td>BaO</td>
<td>0.09</td>
<td>0.09</td>
<td>0.12</td>
<td>0.09</td>
<td>0.05</td>
<td>0.07</td>
</tr>
<tr>
<td>CaO</td>
<td>0.33</td>
<td>0.33</td>
<td>0.31</td>
<td>0.33</td>
<td>0.05</td>
<td>0.00</td>
</tr>
<tr>
<td>Cl</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>0.01</td>
<td>1.37</td>
<td>1.94</td>
</tr>
<tr>
<td>Cr₂O₃</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>CuO</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.00</td>
</tr>
<tr>
<td>Fe₂O₃</td>
<td>9.46</td>
<td>9.46</td>
<td>9.25</td>
<td>8.32</td>
<td>2.01</td>
<td>1.90</td>
</tr>
<tr>
<td>Ga₂O₃</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>K₂O</td>
<td>3.70</td>
<td>3.70</td>
<td>4.11</td>
<td>4.27</td>
<td>0.38</td>
<td>0.39</td>
</tr>
<tr>
<td>MgO</td>
<td>1.56</td>
<td>1.56</td>
<td>1.76</td>
<td>1.85</td>
<td>0.12</td>
<td>0.13</td>
</tr>
<tr>
<td>MnO</td>
<td>0.11</td>
<td>0.11</td>
<td>0.10</td>
<td>0.10</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Na₂O</td>
<td>0.40</td>
<td>0.40</td>
<td>0.56</td>
<td>0.53</td>
<td>0.90</td>
<td>1.28</td>
</tr>
<tr>
<td>Nb₂O₅</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>NiO</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>P₂O₅</td>
<td>0.08</td>
<td>0.08</td>
<td>0.06</td>
<td>0.07</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>PbO</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Rb₂O</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>SO₃</td>
<td>0.02</td>
<td>0.02</td>
<td>0.10</td>
<td>0.12</td>
<td>0.07</td>
<td>0.08</td>
</tr>
<tr>
<td>SiO₂</td>
<td>49.77</td>
<td>49.77</td>
<td>50.74</td>
<td>50.96</td>
<td>61.67</td>
<td>61.21</td>
</tr>
<tr>
<td>SrO</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>ThO₂</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>TiO₂</td>
<td>0.80</td>
<td>0.80</td>
<td>0.79</td>
<td>0.69</td>
<td>1.72</td>
<td>1.72</td>
</tr>
<tr>
<td>V₂O₅</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.28</td>
<td>0.00</td>
</tr>
<tr>
<td>Y₂O₃</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>ZnO</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>ZrO₂</td>
<td>0.02</td>
<td>0.02</td>
<td>0.04</td>
<td>0.03</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>TOTAL</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>
Table 3.4: Chemical composition in elemental state of geophagic clayey soils.

<table>
<thead>
<tr>
<th>Chemical composition</th>
<th>GSA1 Weight (%)</th>
<th>GSA2 Weight (%)</th>
<th>GSA3 Weight (%)</th>
<th>GSA4 Weight (%)</th>
<th>GNG1 Weight (%)</th>
<th>GNG2 Weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Si</td>
<td>23.26</td>
<td>24.05</td>
<td>23.72</td>
<td>23.82</td>
<td>28.83</td>
<td>28.61</td>
</tr>
<tr>
<td>Al</td>
<td>17.75</td>
<td>16.48</td>
<td>16.89</td>
<td>17.22</td>
<td>16.48</td>
<td>16.42</td>
</tr>
<tr>
<td>Fe</td>
<td>6.61</td>
<td>6.17</td>
<td>6.47</td>
<td>5.82</td>
<td>1.41</td>
<td>1.33</td>
</tr>
<tr>
<td>K</td>
<td>3.07</td>
<td>3.59</td>
<td>3.41</td>
<td>3.55</td>
<td>0.32</td>
<td>0.32</td>
</tr>
<tr>
<td>Mg</td>
<td>0.94</td>
<td>1.12</td>
<td>1.06</td>
<td>1.11</td>
<td>0.07</td>
<td>0.08</td>
</tr>
<tr>
<td>Ti</td>
<td>0.48</td>
<td>0.50</td>
<td>0.47</td>
<td>0.41</td>
<td>1.03</td>
<td>1.03</td>
</tr>
<tr>
<td>Na</td>
<td>0.30</td>
<td>0.54</td>
<td>0.42</td>
<td>0.39</td>
<td>0.66</td>
<td>0.95</td>
</tr>
<tr>
<td>Ca</td>
<td>0.24</td>
<td>0.26</td>
<td>0.22</td>
<td>0.23</td>
<td>0.04</td>
<td>1.94</td>
</tr>
<tr>
<td>Mn</td>
<td>0.08</td>
<td>0.11</td>
<td>0.08</td>
<td>0.08</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table 3.5: Trace element composition of geophagic clayey soils.

<table>
<thead>
<tr>
<th>Trace Element</th>
<th>GSA1 mg/L</th>
<th>GSA2 mg/L</th>
<th>GSA3 mg/L</th>
<th>GSA4 mg/L</th>
<th>GNG1 mg/L</th>
<th>GNG2 mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>0.26</td>
<td>0.21</td>
<td>0.20</td>
<td>0.17</td>
<td>0.15</td>
<td>0.16</td>
</tr>
<tr>
<td>Pb</td>
<td>0.20</td>
<td>0.24</td>
<td>0.26</td>
<td>0.18</td>
<td>0.18</td>
<td>0.09</td>
</tr>
<tr>
<td>Zn</td>
<td>0.33</td>
<td>0.33</td>
<td>0.21</td>
<td>0.56</td>
<td>0.09</td>
<td>0.29</td>
</tr>
<tr>
<td>Fe</td>
<td>81.37</td>
<td>63.53</td>
<td>37.82</td>
<td>39.08</td>
<td>5.18</td>
<td>1.66</td>
</tr>
<tr>
<td>Co</td>
<td>0.13</td>
<td>0.09</td>
<td>0.05</td>
<td>0.05</td>
<td>0.01</td>
<td>0.00</td>
</tr>
<tr>
<td>Mn</td>
<td>6.05</td>
<td>6.54</td>
<td>5.68</td>
<td>5.14</td>
<td>1.23</td>
<td>1.24</td>
</tr>
<tr>
<td>Cr</td>
<td>0.01</td>
<td>0.12</td>
<td>0.07</td>
<td>0.04</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

3.4 Discussion
3.4.1 Colour
Colour as a physical property of clays aids in describing specific characteristics, such as mineralogical composition, age and soil processes (chemical alteration, carbonate accumulation, the presence of humified organic matter). The colour and texture of the clay often have an influence on the type of soil being consumed by the geophagists (Brime, 1985a). White clay is mainly made up of kaolin; while yellowish and reddish clays are composed of iron, which could be a source of
iron supplement (Reilly & Henry, 2000). However, most geophagic soils contain mainly ferrous-oxide iron which apparently is not easily absorbed by the body (Abrahams & Parsons, 1997a).

With the aid of a Munsell colour chat, the predominant form of iron oxide in clays could be easily determined. Thus, a deep red (5YR) on a Munsell colour chat is indicative of soils whose iron oxide mineral is hematite (Fe₂O₃), while soils having goethite [FeO(OH)] as the main iron oxide mineral are yellowish brown (2.5YR–7.5YR). Soils containing lepidocrocite, another form of iron oxyhydroxide, are a distinctive orange colour (7.5YR) (Mogongoa et al., 2011, Fernández-Caliani & Cantano, 2010). A previous study asserted that the inclination of clayey soils towards reddish or brownish is based on the supposition that such clays contain more iron (Young et al., 2008). In most cases, iron in haem form (Fe²⁺) is readily absorbed by the body unlike the non-haem form (Fe³⁺), with best possible uptake occurring in the duodenum (Ngole et al., 2010).

The colour (hue values) of the geophagic soils ranged from yellowish (10YR) to brownish with samples GSA 1-4 being yellowish brown suggesting the presence of Magnetite, Hematite or goethite FeO(OH) while GNG1 and GNG2 were brownish grey in appearance which could infer the presence of oxidised Fe³⁺ and Mn⁴⁺. The consumption of the clays for iron supplementation may be acceptable to some extent (Bain et al., 2011b, Mogongoa et al., 2011). Nevertheless, it is important to note that the reddish or yellowish shade of clays could be used to deduce the presence of Fe but not its quantity or bioavailability (Seim et al., 2016). The study of Ngole et al., (2010) in Cameroon supports the consumption of samples GSA 1-4 as most brownish or reddish clays have more palatable earthy aroma that emanates from them usually after very light showers of rain, and they are less sticky.

3.4.2 Texture
Based on previous studies, the attitude of most geophagists towards the feel of clay they consume revealed a preference for clays that leave little or no undesirable sensation while in the mouth (Diko & Diko, 2013). Textural analyses results revealed that the soil samples were texturally dominated by clay size particle with some silt and very fine sand particles. Based on these outcomes, it seems that these geophagic soils possess high surface area, which permits them to adsorb much more water; since adsorption is a function of surface area. This property therefore
may support the medicinal hypotheses as they tend to curtail diarrhea and other gastrointestinal ailments.

Consumers of geophagic clays prefer those that are soft, silky and powdery. Samples GNG 1 – 2 when compared to samples GSA1 – 4 will not suit the pleasure of the individual due to the feel of coarseness and sand grains (Van Onselen et al., 2015). Geophagic clays that are gritty comprise silt and fine sand particles of quartz and feldspars which may destroy dental enamels of an individual (via grinding, cracking, splitting and breakage during mastication); because quartz (harness 7) scratches the dental enamel, which is the main inorganic component of the human tooth and dominated by hydroxyl apatite (Ca$_5$(PO$_4$)$_3$(OH), a calcium phosphate mineral), of hardness 5 on the Mohr’s scale. Previous studies indicated medium sized sand (250-500 μm) to have severe dental damage in hominid species (Diko & Diko, 2013, Ngole et al., 2010). Perforation of the sigmoid colon has been reported in some geophagic individuals (Ngole-Jeme & Ekosse, 2015a). Foreign bodies in the small intestines can cause perforation and the most important consideration of these bodies is the size, shape and nature of the object (Woywodt & Kiss, 2002, Lohn et al., 2000).

3.4.3 pH
The taste of geophagic clays is largely influenced by their pH and dissolved salt contents. A more acidic soil generally, leaves a sour taste when consumed (Nfor et al., 2014). The control of excessive secretion of saliva during pregnancy among women in Kenya and Nigeria via the ingestion of clay had been reported (Diko & Ekoosse, 2014). The application of geophagic clays during pregnancy as reported by some women in reducing the secretion of saliva could be linked to the sour taste of the clays (Ibeanu et al., 1997b).

The pH values of all the studied clay samples, irrespective of their sources, lie within the acidic to moderately acidic range as they are generally below 7, thus imparting a sour taste to the individual when consumed. The pH values of samples GNG1 and GNG2 conform to the studies of (Diko & Diko, 2014). Based on their very strong acidic level, the clays could prevent excessive secretion of saliva as well as reduce nausea during pregnancy in women. Due to the acidity (pH = 2) of the gastric juice found within the stomach, there could be possible chemical reactions
involving clay minerals and organic matter in the geophagic clayey soil (Diko & Ekosse, 2014). However, residence time of ingested material in the stomach, being approximately 2 hours, is inadequate for any significant reaction to occur.

With a pH of about 8 in the duodenal and intestinal sections of the gastrointestinal tract (GIT) (Oomen et al., 2000), noticeable chemical alterations may occur within some of the clay-size particles when consumed. However, with the silica dominated silt and sand-sized particles, alteration may not occur. These unaltered silt and sand-sized particles could seep through the GIT and possibly be stuck in the diverticulitis of the sigmoid colon. Similar remarks have been reported by (Oomen et al., 2000) in a boy after extended consumption of sandy material. Due to the abrasive nature of these silica-rich particles, possible lacerations, and eventual rupturing of the colon may occur (perforation of the sigmoid colon).

The solubility of Fe and other cations in the GIT increases with a decrease in pH (Jahanshahee et al., 2004b). With most of the studied clays being acidic, their ingestion has higher tendencies of preventing the stomach pH from falling to levels that are favourable for the dissolution of Fe thus reducing their bioavailability to the geophagic individual even when there is a high concentration of Fe in the ingested soil.

3.4.4 Electrical conductivity (EC)
The quantity of dissolved salts in soils could be determined based on electrical conductivity (Young et al., 2008). All the studied geophagic soils exhibited very high EC, indicating that the amount of dissolved salts contained in them is high. This is a possible indicator that the taste of the samples could in addition to pH have been influenced by the salt content. These higher values will result in the clays absorbing diarrhea-causing enterotoxins while also protecting the gastrointestinal epithelium. Studies conducted by (Ngole et al., 2006), revealed that there is a relationship between flocculation, soil pH and salts. The flocculation of geophagic soil could influence their ability to coat and create a shield in intestinal mucosa thus protecting the intestines from the acidic gastric juice (Goldberg & Forster, 1990). The observed high dissolved salts in the studied geophagic soils are, however likely to influence the degree of flocculation that may occur.
3.4.5 Loss of ignition (LOI)
The loss on ignition values is indicative that the clayey soil samples had lower carbonaceous matter and higher mineral matter contents. The degree of organic matter in the samples was basically low, inferring a low pathogen load, thus, the danger of bacterial infection including those like *Escherichia coli*, *Enterobacter histolytica*, and helminths infection such as those of *Ascaris lumbricoides* and *Strongyloides stercoralis* as a result of consuming the clays is slim. Nutrient scarcity due to low organic matter will result in diarrhea-causing pathogen not surviving.

From a toxicity or nutritional stand point, not all trace elements analysed are of biological significance, but were all reported for the sake of completeness. Trace elements of interest based on human nutrition such as copper (Cu), manganese (Mn), lead (Pb) and zinc (Zn) were in varying compositions ranging from moderate to high when compared with the usual range in mineral soils. The same is true of trace elements such as chromium (Cr) and lead (Pb), which are often linked with toxicity. The level of zinc was highest in GSA4 with 0.56 ppm and lowest in sample from Nigeria – GNG1 with 0.09 ppm. The level of copper, lead and manganese were lower than the values found for mineral soils as reported by (Mahaney *et al*., 2016). The amount of Pb in all the samples exceeded the recommended EPA (Environmental Protection Agency) standard levels of about 0.01 mg/L.

Elemental analysis results (Table 3.3) revealed that the average SiO$_2$ values ranged from 49.77% - 61.67% with the GNG 1 samples recording the highest value while GSA 1 and GSA 2 both shared lowest value. Mean Al$_2$O$_3$ values also ranged from 31.02% in the GNG 2 samples to 33.55 % in both GSA 1 and 2 samples respectively. GNG 2 showed the lowest Fe$_2$O$_3$ content of 1.90% while GSA 4 had the highest of 8.32%. CaO concentrations in all the samples were less than 0.4%. Average TiO$_2$ values were in the range of 0.69% - 1.72%. On the other hand, GNG 1 and 2 both showed lower concentrations of K$_2$O with GSA 4 having the highest of 4.27%. Na$_2$O content was highest (1.28%) in GNG 2 samples. There were trace amounts of both MgO and K$_2$O in the clays.

3.4.6 Physico-chemical characterization
Lower amount of MgO and K$_2$O in the samples may point to lack of expandable clays (Kabata-Pendias, 2010). The various concentrations are indicative that the clays are hydrated siliceous aluminosilicate. From the investigations, most of the oxides infer that bulks of the samples were
containing more of silica oxide and alumina with other minerals occurring in trace amounts. The relatively high \(\text{Fe}_2\text{O}_3\) could be attributed to superficial oxidation while the low concentrations of Ti in the various clays may result in the inability to mineralise into neither anatase nor rutile. The \(\text{TiO}_2\) in the sampled clays have the tendencies of occurring as free Ti-oxides (Odewumi, 2012). In the octahedral sheet of kaolinite, some Ti may have substituted for Al since no \(\text{Al}^{3+}\) substitution in the tetrahedral sheet would take place due to the \(\text{Al}_2\text{O}_3\) values in the clays (31.02 - 33.55 wt.%) being below that of pure kaolinite which is 39.49 wt.% (Dolcater et al., 1970).

The concentrations of aluminium within the studied samples were in the range of (16.42 – 17.75 wt. %), whereas a daily recommended values for Aluminium is 0.03 pmm (Ekosse, 2001). The amount of major oxides such as \(\text{Fe}_2\text{O}_3\), \(\text{TiO}_2\) and \(\text{MnO}\) exceed the required values for human body function. The samples originally from South Africa (GSA1-4), however, were more iron-rich than any of the samples, with ferric oxide ranging from 8.32 to 9.46%. There is a strong likelihood of the geophagic clays introducing heavy metals such as Fe, Zn, Pb, Cu, Ti and Mn in the gastro-intestinal system of the geophagists. This might impact negatively on the consumer by increasing the pH of the gastro-intestinal tract (World Health Organisation, 2008).

Pregnant women from the city of Johannesburg may suffer from liver damage due to high concentration of iron in all the studied samples. Despite iron (Fe) providing supplement for those who lack iron in their blood, a high level of Fe in the blood could result in hemochromatosis and possible death (Ekosse & Mulaba-Bafibiandi, 2008). Besides, the consumption of iron rich lateritic soils may influence negatively on the utilisation of copper, zinc and selenium in the body (World Health Organisation, 2008).

The highest concentration of chromium was recorded in sample GSA2 as shown in Table 4 (0.12mg/L) which is above the World Health Organization (WHO) recommended value of 0.05 ppm (Brand et al., 2009). Chromium (Cr) has been reported to be responsible for lung cancer and death (World Health Organisation, 2008).

Sample GSA3 had the highest concentration of lead (Pb) with 0.26mg/L while GNG2 had the lowest concentration of 0.09mg/L. Given that the daily recommended value for lead (Pb) is
0.01ppm (Shanker & Venkateswarlu, 2011), sample GSA3 poses a problem as it is higher than the maximum recommended limit while GNG2 is 0.08mg/mL higher than what is recommended. Previous studies revealed that exposure to Lead may cause a decline in the intelligence of children and as well as cancer in adults (World Health Organisation, 2008). Consumption of these clayey samples over a long time may result in serious health effects such as coma, seizure, the dysfunction of the kidney, liver and heart of the consumers (Wang et al., 2009). Multiple organs in the human body are being is been targeted by Lead (Pb) due to its systemic toxicity which can cause cardiovascular, renal, gastro-intestinal and haematological effects (Nkansah et al., 2016). Neurotoxicity, carcinogenicity and reproductive failures in adults are some of the effects of exposure to Lead (Pb) as contained in the data from the European Food Safety Authority (EFSA) (Nkansah et al., 2016).

Zinc (Zn) concentration in the studied samples was highest in sample GSA4 with 0.56mg/L Zn in the clay. A minimal concentration of 0.29mg/L was observed in sample GNG2. Having a recommended value for Zinc (Zn) as 0.01 (Herreros et al., 2008), excessive consumption of these clays may lead to unusual drowsiness, nausea/vomiting, diarrhea and growth retardation of an unborn baby (World Health Organisation, 2008). Zinc plays a vital role in cellular metabolism and is a major component of body tissues and fluids (Scherz & Kirchhoff, 2006). Most catalytic activities of enzymes require Zinc. Also, it is required during immune function, wound healing, protein synthesis, DNA synthesis and cell division. Zinc ions are effective antimicrobial agents even at low concentrations (Soetan et al., 2010). Zinc is required for proper sense of taste and smell and supports normal growth and development during pregnancy, childhood, and adolescence (Osredkar, 2012). There are claims of Zinc having antioxidant properties, which may protect against accelerated aging and helps speed up the healing process after an injury; however, studies differ as to its effectiveness (Bhattacharya et al., 2016).

3.4.7 Mineral phases
There was a degree of consistency from the results of the bulk chemical analysis with those of the mineral analyses of the bulk soils (Table 3.6 and figures 3.1 – 3.6). The identification of minerals based on diagnostic peaks was aided by the International Centre for Diffraction Data (ICDD) reference numbers and the crystals system, d-values, and peak intensity of the various minerals.
The X-Ray diffraction analysis of the different geophagic clays revealed Kaolinite as the major clay mineral alongside muscovite, Amesite and Fayalite.

Quartz was the major non-clay mineral identified. With the high quartz content in the clays, a possible formation of kaolinite could have been through the alteration of feldspatic sandstones rich in quartz (Osredkar, 2012). The significant level of quartz in the soils may pose a threat to the health of the geophagic individual. This is because quartz is harder than dental enamel which could be easily destroyed during mastication of clays. Another problem linked with the quartz is that when ingested, it passes through the gastrointestinal tract unaltered and gets deposited in the colon. Due to the abrasive nature of the materials, laceration or rupturing of the colon may occur thus leading to perforation.

**Table 3.6: Mineral phases**

<table>
<thead>
<tr>
<th>Mineral</th>
<th>ICCD card</th>
<th>GSA1</th>
<th>GSA2</th>
<th>GSA3</th>
<th>GSA4</th>
<th>GNG1</th>
<th>GNG2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaolinite</td>
<td>01-083-0971</td>
<td>82.40</td>
<td>-</td>
<td>39.24</td>
<td>96.96</td>
<td>61.72</td>
<td>-</td>
</tr>
<tr>
<td>Quartz</td>
<td>01-083-2468</td>
<td>8.30</td>
<td>57.06</td>
<td>-</td>
<td>-</td>
<td>25.94</td>
<td>38.73</td>
</tr>
<tr>
<td>Hematite</td>
<td>01-072-6231</td>
<td>5.50</td>
<td>1.70</td>
<td>0.18</td>
<td>0.07</td>
<td>0.65</td>
<td>0.18</td>
</tr>
<tr>
<td>Muscovite</td>
<td>01-076-2572</td>
<td>-</td>
<td>41.09</td>
<td>58.31</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Magnetite</td>
<td>01-079-0416</td>
<td>1.20</td>
<td>0.11</td>
<td>0.83</td>
<td>0.26</td>
<td>0.27</td>
<td>2.91</td>
</tr>
<tr>
<td>Amesite</td>
<td>01-076-0534</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>55.27</td>
</tr>
<tr>
<td>Fayalite</td>
<td>01-070-1861</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4.78</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gupeite</td>
<td>03-065-9131</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.91</td>
</tr>
<tr>
<td>Others</td>
<td>2.60</td>
<td>0.04</td>
<td>1.44</td>
<td>2.71</td>
<td>6.64</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
Figures 3.1-3.6 summarize the results of the mineral phases of the different geophagic clay samples using XRD. The geophagic clay samples originally from South Africa had lesser quartz content than the samples originally from Nigeria. The quartz content of the clay samples is directly related to the silica content of the clays and the samples from Nigeria had higher silica content than the samples from South Africa (see tables 3.3 and 3.4).

**Figure 3.1:** X-ray diffractogram of geophagic soil (GSA1) indicating mineral peaks (Note: K = Kaolinite, Q = Quartz, M = Muscovite)
Figure 3.2: X-ray diffractogram of geophagic soil (GSA2) indicating mineral peaks
(Note: P = Plagioclase M = Muscovite)

Figure 3.3: X-ray diffractogram of geophagic soil (GSA3) indicating mineral peaks
(Note: K = Kaolinite M = Muscovite)
Figure 3.4: X-ray diffractogram of geophagic soil (GSA4) indicating mineral peaks
(Note: K=Kaolinite H=Hypothetical silica p= Pseudobrookite)

Figure 3.5: X-ray diffractogram of geophagic soil (GNG1) indicating mineral peak
(Note: K=Kaolinite Q = Quartz)
Figure 3.6: X-ray diffractogram of geophagic soil (GNG2) indicating mineral peaks (Note: A = Amesite Q = Quartz)

3.5 Conclusion
This study has established the chemical and mineralogical composition of geophagic clayey soils sold in selected South African informal markets. XRD results of the analysis established that the clay materials are mainly composed of kaolinite with minor palygorskite, illite, Amesite, Gupeite, Hematite and Magnetite. Quartz was the major non-clay constituent identified which poses a threat to human dental enamel. Titanium was a major heavy metal identified from the samples which may result in heavy metal toxicity. The oxidation state of the iron which was 3 indicates that the iron and aluminium are major components of the clay matrix.

Excessive consumption of these clays will result in constipation. Nevertheless, the potential medicinal application of these clayey soils is supported by the kaolinite contents, magnesium and potassium in contrast; the trace elements are pointers of probable adverse effects on humans.
3.6 Acknowledgment
The authors thank the University of Johannesburg for providing facilities used for the XRD, XRF and AAS analysis.

References


bentonites and kaolin for ceramic applications. *Journal of Applied Sciences*, 8, pp. 1021-1027.


CHAPTER FOUR

4.0 THE EFFECTS OF AN ACIDIC ENVIRONMENT ON SELECTED GEOPHAGIC CLAYEY SAMPLES

Abstract

Geophagia, the deliberate ingestion of non-food lithospheric substances, is a major form of pica in most African cultures. A common lithospheric pica substance ingested in the central business district of Johannesburg, Gauteng Province, South Africa, is a yellowish-brown clay soil. Women of reproductive age usually ingest this clay. Alluring flavour, alleviation of nausea during pregnancy, and absorption of toxins in the gastrointestinal tract are some of the reasons why people engage in geophagia. There are claims that geophagia to a certain extent helps to replenish mineral nutrients in malnourished individuals. The study of the acid extractable mineral contents of some selected geophagic clays was done to provide information on the mineral contents that could possibly be absorbed in the gastrointestinal tract. Results showed that the clay soil was very dry with an average moisture content of 0.31 ± 0.01%. The concentrations of the minerals in the acid extract were aluminium, iron, copper, lead, zinc, cobalt, and nickel. Nickel was detected in neither the water extracts nor acid extracts of sample GNG1. Sample GNG2 also did not have detectable cobalt in either of the two extracts. Based on the percentage extractable data, aluminium was the most extractable metal. Based on the Recommended Daily Allowance (RDA), the clayey soil samples liberated very high amounts of nickel and lead which could pose a danger to the body.

Key words: Clay, geophagia, mineral, pregnancy, trace elements.

Prepared for publication as: Okereafor, G. U; Mulaba-Bafubiandi, A.F; and Mavumengwana, V. (2016). The effects of an acidic environment on selected geophagic clayey samples
4.1 Introduction
Pica in humans involves the persistent ingestion of unsuitable substances having little or no nutritional value (Myaruhucha, 2009, Tayie et al., 2013). Examples of such non-food substances include soil, clay, ice, burnt matches, soot, charcoal, cigarette ashes, and baking soda (Kushner & Retelny, 2005). Globally, geophagia, which is the deliberate consumption of lithospheric substances, particularly clayey soil, is the most common form of pica (Ekosse et al., 2010). The clay soil usually ingested by geophagists contains quite a lot of mineral nutrients such as copper, iron, magnesium, manganese, silicon, and zinc, as well as some toxic mineral elements including lead and aluminium (Ekosse et al., 2010).

Geophagists from previous studies, ingest a variety of clay soil that vary in colour; some of which are whitish, creamy, greyish, yellowish, and reddish (Hunter, 1993, Woywodt & Kiss, 2002, Stokes, 2006, Ekosse et al., 2010). In South Africa, particularly in the Limpopo, Mpumalanga and Kwazulu Natal provinces, geophagia encompasses the ingestion of a reddish-brown clay soil locally known as Umhlabathi (soil) amongst the Zulus. Most of the clay products are mined from hills and mountains, river beds, valleys, excavation sites and termitaria within Limpopo (Polokwane and Sekhukhune) and Free State (Qwaqwa and Mangaung) provinces in South Africa. The clays are mined using selective digging, hand grabbing and picking techniques. After which the clays are processed through sieving, slurrying, grinding and pounding. Beneficiation techniques such as baking, burning and boiling are used to ensure the clays are more edible as well as to reduce their microbial load (Ekosse et al., 2010).

Generally, an increasing prevalence of pica and geophagia has been reported in South Africa among women of reproductive age (Tayie et al., 2013, Mathee, 2014, Mathee et al., 2014). Botswana, Malawi, Nigeria, Swaziland, Ghana, Togo, Zambia, and Zimbabwe are a few of the other African countries where geophagia has been reported (Walker et al., 1997, Dominy et al., 2004, Ekosse & Jumbam, 2010). Beyond Africa, geophagia has been reported in Australia, China, Germany, Haiti, India, Mexico, Middle East, Latin America, and Turkey (Vermeer & Frate, 1979, Ekosse et al., 2010). From previous studies, the average daily clay soil ingestion among women in Johannesburg was reported as 70g, or in a range between 40 - 100g which was higher than the 50g average reported among women in rural Holmes County, Mississippi (Nwafor, 2008, Tayie et al.,
The clay soils ingested in Johannesburg are the shale type composed of 54.02% silicate, 35.45% aluminium oxide, 6.73% iron oxide, 2.76% potassium oxide, 1.02% sodium oxide, 1.28% calcium oxide, 1.16% magnesium oxide, 1.72% titanium oxide, and trace amounts of other oxides (Okereafor et al., 2016).

Several reasons supporting geophagia have been reported. The consumption of clay has been applied as a therapy for diarrhoea and stomach distress apparently to absorb bacterial toxins related with gastro-intestinal ache; it has also been used to relieve nausea and the toxaemia of pregnancy (Hunter, 1993, Tayie & Lartey, 1999, Dominy et al., 2004, Diko & Diko, 2014). Alternatively, geophagia has been associated with lead poisoning, hyperkalemia, phosphorous intoxication, dental injury and other undesirable effects including low bone mineralisation (Kutalek et al., 2010, Phipps et al., 2012, Tayie et al., 2013, Fosso-Kankeu et al., 2015). The associations between geophagia and anaemia have been reported in several studies (Stokes, 2006, Abrahams, 2012). Zinc deficiency has been perceived to support pica in young children (Chen et al., 1985).

Notwithstanding the contradictory reports, a lot of evidence points to some dietary benefits of geophagia. While some researchers have guessed that geophagia during pregnancy may prompt iron deficiency and anaemia, others have specified that geophagia may, in fact, be an adaptation to iron deficiency (Stokes, 2006, Young et al., 2007). The high prevalence of geophagia among anaemic pregnant women sparks a quest for knowledge of the potentially available mineral contents of the lithospheric substances ingested (Tayie et al., 2013). This necessitates mineral analysis of the soil types usually ingested. The different clayey soil ingested in the Central Business District of Johannesburg, Gauteng Province South Africa as a potential source of iron and other mineral nutrients has not been studied. This study seeks to bridge this knowledge gap.

The use of hydrochloric acid (HCl) extraction in this study is based on the proposition that the acid soluble fraction of the minerals in the clay soil is the component that is possibly available for absorption in the gastrointestinal tract (Geissler et al., 1998, Hooda et al., 2002, Tayie et al., 2013). A similar approach has been adopted in previous studies to examine lithospheric pica materials consumed by pregnant women in Australia, India, Kenya, Uganda, and Tanzania (Geissler et al., 1998, Dominy et al., 2004). The extraction process is aimed at simulating the acidic conditions in
the human stomach using 0.1M HCl, pH 1.0, and the rhythmic movement that takes place in the stomach for a period of about 3 hours (Guerra et al., 2012).

4.2 Methods

4.2.1 Clay soil samples
Six representative clay soil lumps were purchased via a systematic random sampling technique from selected informal markets within the metros of Johannesburg. Four of these clayey soil samples are known to be originally from South Africa and designated GSA 1-4 while the other two are from Nigeria GNG1-2. The clay soil lumps from each market were crushed and pulverised in a mortar into a fine composite powdered sample.

The composite powdered sample from each market was mixed thoroughly and then partitioned into laboratory samples in several clean 100g size polythene bags, sealed and then stored in a cold room until needed. Analytical samples were taken from these bags for analyses. Market samples were not combined but kept separate for analysis.

4.2.2 Hydrochloric acid extraction of minerals
Approximately 30g of each clay sample was extracted with 200 ml 0.1 M HCl solution in a conical flask (Geissler et al., 1998, Dominy et al., 2004). The extraction was achieved by a slow agitation of the mixture in the conical flask for 2 hours on a Magnetic Hotplate stirrer FMH (model STR-MH-180, Biochemical Medical Scientific cc., Cape Town, South Africa) at 37°C and 120rpm. The crude extract was then filtered through a No. 1 ashless 11.0μm Whatman grade filter paper. The filtrates were analysed for mineral contents while the residues were oven dried for 2 hours, crushed and pelletised before being analysed also for mineral composition.

4.2.3 Mineral Analysis
Each solution was analysed for the minerals: zinc, iron, magnesium, nickel, aluminium, copper, silica and lead, using an Atomic Absorption Spectrophotometer (Model iCE 3000 series, Thermo Scientific) with wavelength and lamp settings specific for each mineral element. The various solutions from the acid extract were analysed in duplicate for the above minerals.
4.2.4 Determination of oxides using X-ray fluorescence (XRF)
10g each of both crude and oven dried acid treated clay sample were pelletised using a mould at very high pressure and then placed in the sample partition of X-ray fluorescence (XRF; Rigaku ZSX PrismusII). This was to analyse the elemental compositions of the various minerals that are left in the clays before and after treatment with acid.

4.2.5 Loss of Ignition
For expression of the mineral content on dry weight basis, the moisture content of the clay samples was determined using the air-drought oven approach (Arlington, 1995). About 2.0g of each clay sample was weighed and put into a crucible before being transferred into a furnace at 120 ºC for 3 hours. The representative fractions were then allowed to cool in a desiccator and re-weighed using an electronic digital weighing scale. The moisture determination was done in duplicate.

4.3 Results
The clayey soil lumps consumed by geophagists were found to be very dry with a mean moisture content of 0.31 ± 0.01% despite being obtained from the open market. The general chemical composition of the clayey soils categorised by source are shown in Table 4.1. In decreasing order of abundance, some of the selected minerals in the samples before treatment with acid were aluminium, zinc, copper, lead, cobalt, and nickel.

Results from the acid extracts however, did not follow this sequence of abundance (Table 4.3). The concentrations of the minerals in the acid extract from most to least were aluminium, iron, copper, lead, zinc, cobalt, and nickel. Nickel was not detected in either the water extracts or acid extracts of sample GNG1. Alternatively, sample GNG2 also did not have detectable cobalt in both extracts. Based on the percentage extractable, aluminium was the most extractable. Based on the Recommended Daily Allowance (RDA), the clayey soil samples contained very high amount of nickel and lead.
Table 4.1: Chemical composition of solid clay samples

<table>
<thead>
<tr>
<th>Chemical composition</th>
<th>GSA1 Weight (%)</th>
<th>GSA2 Weight (%)</th>
<th>GSA3 Weight (%)</th>
<th>GSA4 Weight (%)</th>
<th>GNG1 Weight (%)</th>
<th>GNG2 Weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>SiO₂</td>
<td>50.47</td>
<td>50.11</td>
<td>50.96</td>
<td>50.89</td>
<td>50.54</td>
<td>49.93</td>
</tr>
<tr>
<td>Al₂O₃</td>
<td>31.55</td>
<td>31.36</td>
<td>31.32</td>
<td>30.74</td>
<td>31.61</td>
<td>31.43</td>
</tr>
<tr>
<td>Fe₂O₃</td>
<td>9.86</td>
<td>9.71</td>
<td>9.51</td>
<td>9.46</td>
<td>9.18</td>
<td>9.04</td>
</tr>
<tr>
<td>MgO</td>
<td>1.52</td>
<td>1.40</td>
<td>1.58</td>
<td>1.46</td>
<td>1.56</td>
<td>1.44</td>
</tr>
<tr>
<td>MnO</td>
<td>0.11</td>
<td>0.07</td>
<td>0.11</td>
<td>0.08</td>
<td>0.10</td>
<td>0.07</td>
</tr>
<tr>
<td>CaO</td>
<td>0.31</td>
<td>0.15</td>
<td>0.27</td>
<td>0.13</td>
<td>0.20</td>
<td>0.14</td>
</tr>
<tr>
<td>Na₂O</td>
<td>0.48</td>
<td>0.44</td>
<td>0.69</td>
<td>0.57</td>
<td>0.56</td>
<td>0.40</td>
</tr>
<tr>
<td>K₂O</td>
<td>4.50</td>
<td>4.31</td>
<td>4.52</td>
<td>4.40</td>
<td>4.71</td>
<td>4.45</td>
</tr>
<tr>
<td>TiO₂</td>
<td>0.90</td>
<td>0.86</td>
<td>0.88</td>
<td>0.85</td>
<td>0.79</td>
<td>0.74</td>
</tr>
</tbody>
</table>

+ Crude clay sample
++ Acid treated clay
**ND** None detected
Table 4.2: Trace element contents of clayey soil before treatment with hydrochloric acid

<table>
<thead>
<tr>
<th>Trace elements</th>
<th>GSA1</th>
<th>GSA2</th>
<th>GSA3</th>
<th>GSA4</th>
<th>GNG1</th>
<th>GNG2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni</td>
<td>0.04</td>
<td>0.09</td>
<td>0.02</td>
<td>0.01</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Al</td>
<td>1.27</td>
<td>1.48</td>
<td>0.71</td>
<td>0.94</td>
<td>2.35</td>
<td>2.55</td>
</tr>
<tr>
<td>Zn</td>
<td>0.33</td>
<td>0.33</td>
<td>0.21</td>
<td>0.56</td>
<td>0.09</td>
<td>0.29</td>
</tr>
<tr>
<td>Cu</td>
<td>0.26</td>
<td>0.21</td>
<td>0.20</td>
<td>0.17</td>
<td>0.15</td>
<td>0.16</td>
</tr>
<tr>
<td>Fe</td>
<td>1.21</td>
<td>3.48</td>
<td>1.06</td>
<td>1.35</td>
<td>2.12</td>
<td>1.83</td>
</tr>
<tr>
<td>Pb</td>
<td>0.20</td>
<td>0.24</td>
<td>0.26</td>
<td>0.18</td>
<td>0.18</td>
<td>0.09</td>
</tr>
<tr>
<td>Co</td>
<td>0.13</td>
<td>0.09</td>
<td>0.05</td>
<td>0.05</td>
<td>0.01</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND Not detected

Table 4.3: Acid extractable mineral contents of clayey soil ingested by geophagists in Johannesburg.

<table>
<thead>
<tr>
<th>Trace Elements</th>
<th>GSA1</th>
<th>GSA2</th>
<th>GSA3</th>
<th>GSA4</th>
<th>GNG1</th>
<th>GNG2</th>
<th>RDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni</td>
<td>0.06</td>
<td>0.15</td>
<td>0.06</td>
<td>0.03</td>
<td>ND</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Al</td>
<td>3.52</td>
<td>1.99</td>
<td>1.45</td>
<td>2.61</td>
<td>3.91</td>
<td>3.62</td>
<td>3.00</td>
</tr>
<tr>
<td>Zn</td>
<td>0.16</td>
<td>0.36</td>
<td>0.12</td>
<td>0.15</td>
<td>0.10</td>
<td>0.10</td>
<td>3.00</td>
</tr>
<tr>
<td>Cu</td>
<td>0.17</td>
<td>0.18</td>
<td>2.72</td>
<td>0.12</td>
<td>0.26</td>
<td>1.44</td>
<td>2.00</td>
</tr>
<tr>
<td>Fe</td>
<td>1.67</td>
<td>5.92</td>
<td>1.21</td>
<td>1.64</td>
<td>4.38</td>
<td>2.21</td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>0.48</td>
<td>0.54</td>
<td>0.39</td>
<td>0.46</td>
<td>0.08</td>
<td>0.16</td>
<td>0.01</td>
</tr>
<tr>
<td>Co</td>
<td>0.21</td>
<td>0.12</td>
<td>0.09</td>
<td>0.81</td>
<td>0.03</td>
<td>ND</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Recommended daily allowance adapted from World Health Organisation (World Health Organisation, 1996).

ND Not detected

N/A Not available

RDA Recommended daily allowance

4.4 Discussion

This study gives first-hand information on mineral contents of selected clayey soils consumed by geophagists particularly in the Johannesburg Central Business District area of Gauteng province, South Africa, and the acid extractable fractions which are possibly available for absorption in the human body.
There is a significant amount of iron that is expelled during simulated stomach conditions. It is, however, crucial to note that absorption from non-heam iron is usually low under normal conditions but could increase considerably under anaemic conditions (Tayie et al., 2013). Following the very concentration of iron in virtually all the clay samples, pregnant women are posed to having liver damage. Irrespective of iron (Fe) supplementing for the lack iron in the blood, a high level often results in hemochromatosis and possible death (World Health Organisation, 2008). In addition, the continued ingestion of iron rich lateric soils may impact adversely on the utilisation of minerals such as copper, zinc and selenium in the body (Brand et al., 2009, Okereafor et al., 2016).

A profound observation in this study is that the toxic heavy metals, nickel and cobalt, were not detected in the acid extracts GNG 1 and 2 respectively. Despite reports of nickel and cobalt poisoning in children ingesting heavy metal contaminated soils, the type of clays under investigation are not potential source of nickel or cobalt (Ekosse & Jumbam, 2010).

A disturbing finding from this study is that most of the clayey soil samples contained aluminium in the range of 1.48 – 2.55mg/L per 10g, with a corresponding acid extractable fraction of 1.99 – 3.91mg/L. Normal dietary intake of aluminium is reported to be between 3 - 20 mg/day of which only about 15μg is usually absorbed and then eliminated through the kidneys (Mahan et al., 2007, Willhite et al., 2012). The detected acid extractable fraction of aluminium in this study was above the recommended dietary intake. Within this area of study therefore, clay geophagia may pose a significant risk to aluminium toxicity. Excessive ingestion of aluminium could result in bone mineralisation problems and intestinal malfunction (Willhite et al., 2012, Tayie et al., 2013). There are reports on links between aluminium ingestion and age-related Alzheimer’s disease (Flaten, 2001, Dobbs, 2009, Walton, 2012). Nevertheless, due to the beneficial immunological features of aluminium-rich clays, geophagia had been reported to enhance the secretory and immune system of female monkeys (Gupta & Rost, 2000, Tano-Debrah & Bruce-Baiden, 2010).

The lead content of the different clayey soil samples, despite being not as high as aluminium, was the most acid extractable. Compared to the RDA values, the acid extractable fraction of the lead content, which is the amount potentially available for absorption, was very high. The ability of
lead to inhibit or mimic the actions of calcium (which can affect calcium-dependent or related processes) and to interact with proteins (including those with sulfhydryl, amine, phosphate and carboxyl groups) may affect several biological processes in the body (ATSDR, 2007). Excessive lead also results in long-term harm in adults, including increased risk of high blood pressure and kidney damage (Dooyema et al., 2012). Exposure of pregnant women to high levels of lead could cause miscarriages, stillbirths, premature births and low birth weight, as well as minor malformations (Gilbert-Barness, 2010, Okereafor et al., 2016).

Only a small fraction of the small amount of zinc in the clay was acid extractable. The acid extractable content could potentially contribute 12% of the RDA of 8 mg/day for women of reproductive age (Giudici et al., 2013, Tayie et al., 2013). Daily zinc loss is between 2 - 3mg which implies that the small amount of acid extractable zinc from the clay soil could be of value especially in zinc deficiency prone communities where geophagia is practiced (Mahan et al., 2007).

The dietary value that is linked with nickel is quite vast considering the small amounts of the mineral that is required by humans on a daily basis. Nickel plays a vital role in assisting the body absorb the iron it needs as well as prevent conditions such as anaemia (Das et al., 2008). The extractable nickel content from the studied samples exceeds the RDA. Previous investigations revealed that excessive nickel content often result in allergic reactions, digestion problems, high red blood cell counts, kidney failure and possibly, lung cancer (Duda-Chodak & Blaszczyk, 2008, Okereafor et al., 2016).

4.4.1 Strengths and limitations
This study presents the first reports on the mineralogical compositions of some of the geophagic clayey soils ingested in Johannesburg area of Gauteng Province, determined using a state of the heart instrument, atomic absorption spectrophotometry. Beside the total mineral contents, reported in this study are also the acid extractable fractions to provide information on potentially available contents to the human body. Despite having some of the mineral contents as acid extractable, a major setback is that, it does not ascertain that all the liberated minerals may be bioavailable in the
gastrointestinal tract. Nevertheless, reliable information on the mineral contents of the clayey soil ingested by geophagists in South Africa was established in this study.

4.5 Conclusions
The minerals in the studied geophagic clayey soil samples obtained from selected informal markets in the central business district area of Johannesburg, South Africa were only sparingly acid extractable. On account of the different RDA’s, iron is the mineral nutrient that was significant in the studied clayey soils, while aluminium and lead poisoning have known undesirable effects that come with their ingestion.

The potential harmful health effects of geophagia in relation to some clayey soils cannot be overemphasised and more research is needed to fully appreciate their benefits and possible detrimental effects. However, as a precaution, education of pica especially during pregnancy should be encouraged at prenatal counselling.
References


CHAPTER FIVE

5.0 CLAY MINERALS AND CLAYEY SOILS AS POSSIBLE MICROORGANISMS REPOSITORY

Abstract
Clay minerals are one of the most abundant and crucial structural materials of the Earth’s crust and they play a significant role in many aspects of life. The present study aims to critically investigate the presence of microorganisms within different clayey soil samples. Microorganisms are ubiquitous by nature due to their natural ability to adapt to different habitats. Bacteria and Archaea are the most abundant microorganisms in the soil and serve many important roles like nitrogen fixation. Fungi are also an integral part of the soil micro flora catering other organisms as food sources, and facilitating as layer organisms and beneficial relationships with plants and other organisms. The continued evolution of man and subsequent application of clayey soils in areas such as pottery, cosmetics, engineering and geophagia leaves one with the question of man’s safety considering that some of the microorganisms are harmful. Soil samples with high amount of fine particles and colloidal substances were studied while taking into account the identified hydrated aluminosilicates and their roles in bioavailability. The study also investigated into the possible types of microbial flora associated with each type of clayey soil as well as their functions within the human body. Considering the diverse origin of these microorganisms, their modes of attachment to clayey soils (with the aim of minimising their transmission to human beings) was also discussed.

Key words: Clay soil, Clay minerals, Microorganisms, Geophagia.

5.1 Introduction
Generally, due to the composition of various organic matters, mineral particles, metal oxides and microorganisms, soils are regarded as highly complex heterogeneous systems. Clay refers to naturally occurring materials that are composed primarily of fine-grained minerals, which are basically “plastic” at appropriate water contents and harden when dried or fired (Ekosse et al., 2010a, Gillott, 2012a). Clays are common products of the weathering and hydrothermal alteration of various rocks, which are also precipitates from aqueous fluids. They are the finest fraction of a sediment or soil in the classification of clay, silt and sand. Clays are composed of minerals that are chiefly inorganic with most of the minerals being hydrous silicates containing aluminium, potassium and some other cations (Heckroodt, 1991). The distinct feature of a clay body is as a result of the minerals it contains which also determine the quality and application of such clay.

Certain portions of the geological structure of the earth especially along river beds are naturally composed of Clay and clay minerals such as Aluminosilicate, Kaolinite, and Montmorillonite (Chamley, 2013). These clay minerals besides forming the soil in which plants grow, constitute major components in a variety of products and applications such as animal feed, oil absorbents, cosmetics, pharmaceuticals, antibacterial agents and wastewater treatment. Clay minerals often possess a glue-like property that supports attachment of microorganisms and their nutrients. When in contact with clayey soils, bacteria tend to attach to the clay surface via physical and chemical interactions expedited by cell envelope structures and the diversity of mineral surface, functionality and crystallography. When bacteria and minerals are separated by some finite distance and by adhesion forces, the cumulative effects of interfacial forces determine the interactions (Mahaney et al., 2000a).

On account that most clay minerals are common in low-temperature environments where microorganisms strive, the interactions between microbes and clays are crucial to a variety of surficial processes. These interactions have the potential to contribute significantly to global cycles of various processes, which imperatively serve as links between the geosphere and life. In natural soils, particularly in subsoil and rocks, microbes are abundant and remain in constant contact with clay minerals (Fletcher, 1996). The interaction between microorganisms and clay minerals often results in dissolution, refinement and transformation, reduction of trace elements incorporated in
the clay minerals and uptake of trace elements by the production of siderophores and chelators as well as the promotion or reduction of absorbance of trace elements on clay minerals (Edwards et al., 2004). The survival time of most microbes within a clay body is mainly dependent on the available clay minerals.

Most metabolic processes in microorganisms are supported by certain heavy metals present within clayey soils. This is because over time, microorganisms have developed several mechanisms by which they can immobilise, mobilise or transform heavy metals into ions. These mechanisms are potential bioremediation strategies (Lee & Fein, 2000). Microbes that could strive under metal-stressed environment can be used as bioremediation agents (Nies, 1999). Many metal resistant bacteria have been isolated from contaminated soils, waters and sediments. Microorganisms tend to attach themselves to minerals but are limited by certain factors such as pH, ionic strength and temperature. There is a display of higher affinity for corundum surfaces than for quartz surfaces by bacteria such as Bacillus subtilis when pH and ionic strength are lowered (Khan et al., 2009). For example, the attachment of Bacillus polymyxa onto hematite is higher than onto quartz which is due to hematite being more hydrophobic (Yee et al., 2000). The cell wall structure of microorganisms also account for their presence within certain clay minerals and clayey soils. B. subtilis (Gram-positive bacterium) was observed to occur more than Pseudomonas mendocina (Gram-negative bacterium) in a soil with Fe-coated quartz surfaces (Shashikala & Raichur, 2002).

Clay minerals have also been claimed to have a positive impact on the growth and the metabolic activity of a variety of microorganisms ranging from bacteria to fungi. The growth of Achromobacter sp., Agrobacterium radiobacter, Bacillus subtilis, Bacillus megaterium, Escherichia coli (E. coli), Escherichia intermedia, Proteus vulgaris, Pseudomonas striata and Pseudomonas aeruginosa were stimulated by both montmorillonite and kaolinite (Ams et al., 2004). Bentonite has beneficial effects on the growth of soil micro flora in liquid media while in some cases it shortened the lag phase of E. coli.

It is important to note that the presence of microbes within clayey soil particles may influence layer charge, exchangeable cation, cation exchange capacity (CEC), swelling and the rheological
properties of clay minerals (Marcos, 2003; Marchuk, 2013). In most cases, these changes are beneficial, whereas others are harmful. These interactions often give rise to unique mineral assemblages by the production of bio signatures in ancient rock records.

Therefore, the present study is geared towards investigating the presence of microbes associated with different clayey soils with a view of linking their availability to specific mineralogical properties of the soil.

5.2 Materials and methods

5.2.1 Clay samples and study area
For the present study, samples of geophagic clays were obtained from various vendors within selected informal markets in Johannesburg area of South Africa. The samples were collected from exposed trays and put in labelled sterilised polyethylene bags and transported to the Mineral Processing and Technology Research Centre of the University of Johannesburg.

5.2.2 Soil analysis
From the various rocky samples, crushed clay samples were obtained using sterile mortar and pestle. A portion of the pulverised clay samples was then sieved to attain a grain size of less than 2mm. Physical characteristics such as colour, bulk density, specific gravity and water holding capacity (WHC) were assessed alongside chemical characteristics such as pH and electrical conductivity. Mineralogical and elemental compositions of the samples were done using SEM, XRF while the phase distributions were carried out using XRD.

5.2.3 Digestion of clay samples
2g of the various samples were weighed into a Teflon crucible and moistened with 100mL of 0.5M, 1M and 2M HCl acid. The mixtures were covered and placed on a shaker for 24 hours at 130rpm. The solutions were filtered and the filtrates were stored in sterile bottles prior to being analyse for minerals using Atomic absorption spectrophotometer.
5.2.4 Loss of ignition (LOI)
10g of each clay sample was weighed and put into a crucible before being put into a furnace at 1000°C for 3 hours. The representative fractions were then allowed to cool in a desiccator and re-weighed. Loss of ignition was then determined using the expression below.

\[ LOI = \frac{\text{Weight loss} \times 100}{\text{Weight of initial sample}} \]

5.2.5 Determination of oxides using XRF
10g of the clay sample were pelletized using a mould at very high pressure and then placed in the sample compartment of X-ray fluorescence (XRF; Rigaku ZSX PrismusII). This was to analyse the chemical compositions of the various minerals that make up the clay.

5.2.6 Determination of the mineral phases using X-ray diffractometer (XRD)
The XRD patterns of clay were obtained on a powder X-ray diffractometer Model Rigaku Ultima IV with CuKα radiation (40kV and 40mA) with a scanning speed of 0.04°/s. This was done at the Extractive Metallurgy Laboratory of the University of Johannesburg. The XRD aided the determination of the mineralogical composition of the material components and also qualitative and quantitative phase analysis of multiphase mixtures as described by Stotzky & Rem, (1966). Representative bulk samples were crushed and homogenised to fine powder at approximately 10-15µm in size. Each reflection as displayed by the X-Ray diffraction corresponding to a particular mineral. With the peaks well separated from each other, their heights are applied in the determination of the orientation of certain mineral in the mixture. The basis of clay mineral analysis by X-Ray diffraction is based on the identification of various peaks and comparison of their relative heights. Different phase levels were identified as semi quantitative estimates based on the relative peak heights (Moore & Reynolds Jr., 1997). The wavelength of 1.5406 was used to calculate the diffraction angles.

5.2.7 Determination of trace elements using atomic absorption spectrophotometer (AAS)
Using an atomic absorption spectrophotometer (AAS; Thermo Scientific, iCE 3000 series), all the filtrates from the digestion of the clay samples were analysed for elemental quantitative analysis. Each metal with the exception of Si and Al that required nitrous oxide flame were analysed using air acetylene flame and deionised water as blank.
5.2.8 Culturing of microorganisms from soil sample
Pulverised clay samples were used for isolation of microorganisms. Nutrient agar media was used for initial isolation and cultivation of microorganisms. One gram of individual clay sample was weighed and put into a test-tube containing 9ml NaCl solution. A 100µL was then inoculated onto the plates (Brime, 1985b). The plates were then incubated for 24 hours at 37°C for bacteria and 4-7 days for fungi at 25°C. Subsequently, the plates were observed for possible growth and pure cultures isolated. The isolated colonies were characterised using morphological, physiological and biological tests (Cheesbrough, 2005).

5.2.9 Isolation of Bacterial and Fungal Isolates
The identification of different colonies and sub culturing for purity was done on fresh nutrient agar plates. The plates were incubated at 37°C for 24 hours for bacteria and 4 days at 26°C for fungi. The isolated colonies were cultured in plates repeatedly by subjecting to streak plate method. Colonies were picked from purity plates and stocked on corresponding agar slants for bacteria and fungi.

5.2.10 Morphological and Biochemical Characterization of the Bacteria Isolates
The morphological characterisation of bacteria isolates using Gram's stain, spore stain and motility involved the growing of cells at 65°C in modified Luria Broth (mLB) medium (krishnaveni, 2013). The culture was then sampled and monitored for microbial spectrophotometrically by measuring the absorbance at 600nm (Zeigler, 2001) after 4, 12, 24 and 48 hour intervals. Biochemical properties of the isolates were carried out using specific procedures as described by Perfumo et al., 2007. The results were interpreted using Bergey’s Manual of Systematic Bacteriology (Banerjee et al., 2013).

5.3 Results and discussion
5.3.1 Physical characterisation
Four parameters (pH, electrical conductivity (EC), loss-on-ignition and colour) as shown in Table 5.1 were used for the characterisation studies. From the identified colour using naked eyes, samples (GSA1-4) were dominantly yellowish in appearance inferring the possibility of Fe which could be Magnetite or Hematite.
With respect to the pH, all the studied samples were below the pH of 7 which could influence the increase in the availability of micronutrients such as manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), and boron (B). The low soil pH would generally support a number of bacteria. GNG1 and GNG2 both showed higher EC values indicating the presence of higher amounts of dissolved salts. These higher values will result in the clays being favourable abode for microbes as most minerals required are readily available. The various LOI values are indicative that the clayey soil samples had lower carbonaceous matter and higher mineral contents.

**Table 5.1:** Physical characterization of geophagic clayey samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Original Source</th>
<th>pH</th>
<th>Electrical Conductivity</th>
<th>LOI %</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSA 1</td>
<td>South Africa</td>
<td>6.36</td>
<td>1028.50 µS</td>
<td>0.27</td>
<td>Yellowish Orange</td>
</tr>
<tr>
<td>GSA 2</td>
<td>South Africa</td>
<td>5.76</td>
<td>908.50 µS</td>
<td>0.63</td>
<td>Yellowish Orange</td>
</tr>
<tr>
<td>GSA 3</td>
<td>South Africa</td>
<td>5.73</td>
<td>822.00 µS</td>
<td>0.34</td>
<td>Yellowish Brown</td>
</tr>
<tr>
<td>GSA 4</td>
<td>South Africa</td>
<td>6.26</td>
<td>974.50 µS</td>
<td>0.24</td>
<td>Yellowish Brown</td>
</tr>
<tr>
<td>GNG 1</td>
<td>Nigeria</td>
<td>4.67</td>
<td>12.15 mS</td>
<td>0.23</td>
<td>Brownish Grey</td>
</tr>
<tr>
<td>GNG 2</td>
<td>Nigeria</td>
<td>4.47</td>
<td>17.16 mS</td>
<td>0.17</td>
<td>Brownish Grey</td>
</tr>
</tbody>
</table>

5.3.2 Chemical Characterisation (XRF)

The data given in Table 5.2 shows the chemical composition of the representative clay samples in their oxide states. From the results (Table 5.2) it is indicative that the various clay samples are composed of SiO$_2$ content of 49.77% to 61.67%; Al$_2$O$_3$ values of 31.02% to 33.55%; Fe$_2$O$_3$ values of 1.90% to 9.46%; K$_2$O values of 0.38% to 4.27%; MgO values of 0.12% to 1.85%; TiO$_2$ values of 0.69% to 1.72%; Na$_2$O values of 0.40% to 1.28%; CaO values of 0.05% to 0.33%. The concentrations are indicative that the clays are hydrated siliceous aluminosilicates. It is imperative that a bulk of the samples contain more of Silica oxide and alumina with other minerals occurring in trace amounts. The observed chemical compositions of the samples are indicative of a better striving environment for microorganisms.

As described by Staley *et al.*, (1989), the higher levels of silicate in all the samples could be linked with the crystalline phase quartz that combines with Alumina in the aluminosilicate structure. The high content of SiO$_2$ and Al$_2$O$_3$ coupled with low content of CaO is a pointer to the samples being kaolinite in nature. In addition, it is a reflection of very intense weathering of primary minerals.
Following the studies of (Ekosse & Ngole, 2012) and (Murray, 1960), samples GSA 1-4 could in addition to being consumed be applied in brick making while GNG1-2 will function better in ceramics based on their SiO$_2$ content. The lower content level of MgO and K$_2$O indicate lack of expandable clays.

Table 5.2: Major oxides analysis of clayey soil samples.

<table>
<thead>
<tr>
<th>Chemical composition</th>
<th>GSA1 Weight (%)</th>
<th>GSA2 Weight (%)</th>
<th>GSA3 Weight (%)</th>
<th>GSA4 Weight (%)</th>
<th>GNG1 Weight (%)</th>
<th>GNG2 Weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SiO$_2$</td>
<td>49.77</td>
<td>49.77</td>
<td>50.74</td>
<td>50.96</td>
<td>61.67</td>
<td>61.21</td>
</tr>
<tr>
<td>Al$_2$O$_3$</td>
<td>33.55</td>
<td>33.55</td>
<td>31.91</td>
<td>32.53</td>
<td>31.13</td>
<td>31.02</td>
</tr>
<tr>
<td>Fe$_2$O$_3$</td>
<td>9.46</td>
<td>9.46</td>
<td>9.25</td>
<td>8.32</td>
<td>2.01</td>
<td>1.90</td>
</tr>
<tr>
<td>K$_2$O</td>
<td>3.70</td>
<td>3.70</td>
<td>4.11</td>
<td>4.27</td>
<td>0.38</td>
<td>0.39</td>
</tr>
<tr>
<td>MgO</td>
<td>1.56</td>
<td>1.56</td>
<td>1.76</td>
<td>1.85</td>
<td>0.12</td>
<td>0.13</td>
</tr>
<tr>
<td>TiO$_2$</td>
<td>0.80</td>
<td>0.80</td>
<td>0.79</td>
<td>0.69</td>
<td>1.72</td>
<td>1.72</td>
</tr>
<tr>
<td>Na$_2$O</td>
<td>0.40</td>
<td>0.40</td>
<td>0.56</td>
<td>0.53</td>
<td>0.90</td>
<td>1.28</td>
</tr>
<tr>
<td>CaO</td>
<td>0.33</td>
<td>0.33</td>
<td>0.31</td>
<td>0.33</td>
<td>0.05</td>
<td>0.00</td>
</tr>
<tr>
<td>MnO</td>
<td>0.11</td>
<td>0.11</td>
<td>0.10</td>
<td>0.10</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table 5.3: Trace Element composition of clayey soil samples.

<table>
<thead>
<tr>
<th>Trace Element</th>
<th>GSA1 mg/L</th>
<th>GSA2 mg/L</th>
<th>GSA3 mg/L</th>
<th>GSA4 mg/L</th>
<th>GNG1 mg/L</th>
<th>GNG2 mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>0.26</td>
<td>0.21</td>
<td>0.20</td>
<td>0.17</td>
<td>0.15</td>
<td>0.16</td>
</tr>
<tr>
<td>Pb</td>
<td>0.20</td>
<td>0.24</td>
<td>0.26</td>
<td>0.18</td>
<td>0.18</td>
<td>0.09</td>
</tr>
<tr>
<td>Zn</td>
<td>0.33</td>
<td>0.33</td>
<td>0.21</td>
<td>0.56</td>
<td>0.09</td>
<td>0.29</td>
</tr>
<tr>
<td>Fe</td>
<td>81.37</td>
<td>63.53</td>
<td>37.82</td>
<td>39.08</td>
<td>5.18</td>
<td>1.66</td>
</tr>
<tr>
<td>Co</td>
<td>0.13</td>
<td>0.09</td>
<td>0.05</td>
<td>0.05</td>
<td>0.01</td>
<td>0.00</td>
</tr>
<tr>
<td>Mn</td>
<td>6.05</td>
<td>6.54</td>
<td>5.68</td>
<td>5.14</td>
<td>1.23</td>
<td>1.24</td>
</tr>
<tr>
<td>Cr</td>
<td>0.01</td>
<td>0.12</td>
<td>0.07</td>
<td>0.04</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>
5.3.3 Mineralogical characterisation (XRD)
X-ray Diffraction patterns were obtained for the various representative samples (Figure 5.1) whilst semi-quantitative mineralogical results are provided in Table 5.4. Kaolinite, muscovite, fayalite, Ilmenite, amesite, gupeite, hematite, pseudobrookite and magnetite were some of the minerals associated with the clay samples. The dominant minerals were kaolinite and quartz. Both samples GSA3 and GSA4 showed the absence of quartz whereas others had it (8 - 57 wt. %). Similarly, all the samples with the exception of GSA 2 and GNG2 were extremely rich in kaolinite (39 – 97 wt. %). Quartz (57%) and muscovite (41%) were dominant in GSA2. The major minerals in GNG2 were Amesite (55%) and quartz (39%). Hematite was present from trace to only a few percent in all the samples. Ilmenite was found only in sample GNG1. A large number of the sampled clays are rich in kaolin which according to several studies (Singer & Sonja, 1971, Wilson, 2003b) are applied in the treatment of diarrhoea and mild intestinal upsets.

Table 5.4: Mineralogical composition of clayey soil samples.

<table>
<thead>
<tr>
<th>Mineral</th>
<th>ICCD card</th>
<th>GSA1</th>
<th>GSA2</th>
<th>GSA3</th>
<th>GSA4</th>
<th>GNG1</th>
<th>GNG2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaolinite</td>
<td>01-083-0971</td>
<td>82.40</td>
<td>-</td>
<td>39.24</td>
<td>96.96</td>
<td>61.72</td>
<td>-</td>
</tr>
<tr>
<td>Quartz</td>
<td>01-083-2468</td>
<td>8.30</td>
<td>57.06</td>
<td>-</td>
<td>-</td>
<td>25.94</td>
<td>38.73</td>
</tr>
<tr>
<td>Hematite</td>
<td>01-072-6231</td>
<td>5.50</td>
<td>1.70</td>
<td>0.18</td>
<td>0.07</td>
<td>0.65</td>
<td>0.18</td>
</tr>
<tr>
<td>Muscovite</td>
<td>01-076-2572</td>
<td>-</td>
<td>41.09</td>
<td>58.31</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Magnetite</td>
<td>01-079-0416</td>
<td>1.20</td>
<td>0.11</td>
<td>0.83</td>
<td>0.26</td>
<td>0.27</td>
<td>2.91</td>
</tr>
<tr>
<td>Amesite</td>
<td>01-076-0534</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>55.27</td>
</tr>
<tr>
<td>Fayalite</td>
<td>01-070-1861</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4.78</td>
<td>-</td>
</tr>
<tr>
<td>Gupeite</td>
<td>03-065-9131</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.91</td>
</tr>
<tr>
<td>Others</td>
<td>-</td>
<td>2.60</td>
<td>0.04</td>
<td>1.44</td>
<td>2.71</td>
<td>6.64</td>
<td>0.00</td>
</tr>
<tr>
<td>TOTAL</td>
<td>-</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
Figure 5.1: X-ray diffractogram of representative clayey soil indicating mineral peaks (Note: K = Kaolinite, Q = Quartz, M = Muscovite)

5.3.4 Identification of bacterial isolates

The sub-cultured isolates were sent to Inqaba Biotec (Pretoria) for sequencing studies that aided in the identification of the isolates. Briefly, the 16S target regions of the isolates were amplified using the following 16S universal primers: 27F: 5’AGAGTTTGATCMTGGCTCAG 3’ and 1492R: 5’CGGTACCTTACGACTT 3’. The PCR products were gel extracted, purified and sequenced in the forward and reverse directions on the ABI PRISMTM 3500xl Genetic analyzer. Sequences were analyzed using CLC work bench 7 followed by a BLAST search provided by NCBI.

Out of the 373 isolates that were identified with the various samples, Gram positive bacteria were more distinct (71.04%) compared to Gram negative bacteria (28.96%). Fifty-seven percent of the Gram positive bacteria were non-spore formers. *Bacillus amyloliquefaciens* was the more prominent isolate. *Bacillus megaterium* and *Bacillus halodurans* were also significantly accounted for in the clay samples (Table 5.5). On account of the Gram negative bacteria, several species
including *Achromobacter, Acinetobacter, Actinobacillus, Agrobacterium* and *Enterobacter* were isolated (Table 5.5).

**Table 5.5: Bacteria associated with the clay samples**

<table>
<thead>
<tr>
<th>Gram positive bacterial species</th>
<th>No. of Isolates</th>
<th>% Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus amyloliquefaciens</em></td>
<td>27</td>
<td>7.2</td>
</tr>
<tr>
<td><em>B. megaterium</em></td>
<td>18</td>
<td>4.8</td>
</tr>
<tr>
<td><em>B. halodurans</em></td>
<td>14</td>
<td>3.8</td>
</tr>
<tr>
<td><em>B. thuringiensis</em></td>
<td>6</td>
<td>1.6</td>
</tr>
<tr>
<td><em>B. coagulans</em></td>
<td>5</td>
<td>1.3</td>
</tr>
<tr>
<td>Unclassified <em>Bacillus</em> species</td>
<td>43</td>
<td>11.5</td>
</tr>
<tr>
<td>Non-spore forming</td>
<td>152</td>
<td>40.8</td>
</tr>
</tbody>
</table>

**Gram negative bacterial species**

<table>
<thead>
<tr>
<th>Non-spore forming bacterial species</th>
<th>No. of Isolates</th>
<th>% Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Achromobacter</em> spp.</td>
<td>4</td>
<td>1.1</td>
</tr>
<tr>
<td><em>Acinetobacter</em> spp.</td>
<td>12</td>
<td>3.2</td>
</tr>
<tr>
<td><em>Actinobacillus</em> spp.</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td><em>Agrobacterium</em> spp.</td>
<td>3</td>
<td>0.8</td>
</tr>
<tr>
<td><em>Enterobacter</em> spp.</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td><em>Pseudomonas</em> spp.</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>Unclassified</td>
<td>85</td>
<td>22.8</td>
</tr>
</tbody>
</table>

**Table 5.6: Diversity of bacterial phylum identified from the clay samples**

<table>
<thead>
<tr>
<th>Phylum</th>
<th>GSA1</th>
<th>GSA2</th>
<th>GSA3</th>
<th>GSA4</th>
<th>GNG1</th>
<th>GNG2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Firmicutes</td>
<td>4.7</td>
<td>4.1</td>
<td>4.5</td>
<td>3.9</td>
<td>3.6</td>
<td>3.0</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>9.2</td>
<td>4.7</td>
<td>5.8</td>
<td>7.2</td>
<td>4.1</td>
<td>6.3</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>1.8</td>
<td>1.9</td>
<td>1.1</td>
<td>1.0</td>
<td>1.3</td>
<td>2.8</td>
</tr>
<tr>
<td>Unclassified</td>
<td>81.8</td>
<td>85.7</td>
<td>84.9</td>
<td>84.3</td>
<td>85.6</td>
<td>84.9</td>
</tr>
<tr>
<td>Acidobacteria</td>
<td>1.2</td>
<td>1.3</td>
<td>1.3</td>
<td>0.9</td>
<td>1.4</td>
<td>1.1</td>
</tr>
<tr>
<td>Plantomycetes</td>
<td>0.2</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>1.2</td>
<td>1.4</td>
</tr>
<tr>
<td>gemmatimonadetes</td>
<td>1.1</td>
<td>1.5</td>
<td>1.5</td>
<td>1.8</td>
<td>2.8</td>
<td>0.5</td>
</tr>
</tbody>
</table>
5.3.5 Pathogenicity and toxicity of identified microorganisms

On account that the clay samples being studied are often consumed by humans, the potential of affecting the health of geophagists cannot be ignored with the dominance of uncultured bacterium. (Table 5.8) contains a summary of selected microorganisms with the potential to harm clay consumers. More potentially harmful microorganisms were recorded in the clays which could be attributed to the clay being sourced from informal markets, where storage and handling may have contributed to their high level of contamination. Most of the identified microorganisms were basically opportunistic microorganisms and are likely to infect immunocompromised individuals such as children, unborn babies (exposed through pregnant women), HIV infected persons and the elderly. They cause damage to their host via invasion, colonisation and growth, and several strategies to establish virulence, the relative ability of a pathogen to cause disease in the host.

Table 5.7: Microbial classes composition detected in the clay samples

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>GSA1 Total sequence%</th>
<th>GSA2 Total sequence%</th>
<th>GSA3 Total sequence%</th>
<th>GSA4 Total sequence%</th>
<th>GNG1 Total sequence%</th>
<th>GNG2 Total sequence%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unknown</td>
<td>65.7</td>
<td>79.2</td>
<td>77.8</td>
<td>79.7</td>
<td>87.8</td>
<td>75.8</td>
</tr>
<tr>
<td>Tracheophyta</td>
<td>0.2</td>
<td>0.7</td>
<td>1.5</td>
<td>0.3</td>
<td>1.3</td>
<td>1.8</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>12.3</td>
<td>7.1</td>
<td>9.3</td>
<td>6.2</td>
<td>4.4</td>
<td>3.5</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>16.7</td>
<td>6.6</td>
<td>8.2</td>
<td>12.5</td>
<td>3.1</td>
<td>10.8</td>
</tr>
<tr>
<td>Bacterioidets</td>
<td>1.8</td>
<td>1.3</td>
<td>1.7</td>
<td>0.4</td>
<td>1.5</td>
<td>1.6</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>3.3</td>
<td>5.1</td>
<td>1.5</td>
<td>0.9</td>
<td>1.9</td>
<td>6.5</td>
</tr>
</tbody>
</table>

5.3.5 Pathogenicity and toxicity of identified microorganisms

(Table 5.8) contains a summary of selected microorganisms with the potential to harm clay consumers. More potentially harmful microorganisms were recorded in the clays which could be attributed to the clay being sourced from informal markets, where storage and handling may have contributed to their high level of contamination. Most of the identified microorganisms were basically opportunistic microorganisms and are likely to infect immunocompromised individuals such as children, unborn babies (exposed through pregnant women), HIV infected persons and the elderly. They cause damage to their host via invasion, colonisation and growth, and several strategies to establish virulence, the relative ability of a pathogen to cause disease in the host.
Enterobacter spp. Bacteraemia, respiratory tract infections, UTI.

Pseudomonas spp. Causes infection and bacteremia in immunocompromised hosts such as HIV/AIDS, cystic fibrosis, bronchiectasis, and severe chronic obstructive pulmonary disease, burns, malignancy, diabetes mellitus, bronchopneumonia, endocarditis, osteomyelitis, urinary tract infections, gastrointestinal infections, and meningitis.

B. megaterium Pneumonia, septicaemia, corneal infections, meningitis, food poisoning, eye infection, lung infection.

B. coagulans

B. thuringiensis

5.4 Conclusion
From this study, irrespective of their sources it is clear that most of the clay samples serve as repositories for microorganisms of different species. Considering the high level of microbes within the clays, it becomes necessary that especially humans take caution in the application of clays as most of the microorganisms are seen as opportunistic pathogens. As repositories for microorganisms, most of the microorganisms could have been introduced to the clay during transportation to market place, handling and storage. The moisture content of the clays suggests that the microorganisms will survive as long as they are not inactivated by other treatments.

Geophagic clays support mineral supplementation to the geophagists in one hand and on the other remove nutrients from the human body rather than releasing minerals for absorption in the body. The consumption of clay can potentially reduce bio-available nutrient absorption in the human diet and lead to micronutrient deficiencies. Regular consumption of these clays has the likelihood of enhancing a mother’s immune system, which invariably benefit the foetus by receiving passive immunity from the mother. Based on the microbial load of the studied clays, parasitic infections resulting from transmission of Ascaris lumbricoides, and other major toxigenic bacteria, causative agents of gas gangrene, tetanus and botulism and geohelminth infections could easily be transmitted to humans. Nevertheless, some of the soil-borne Gram-positive bacteria may be useful in bioremediation and other biotechnological applications due to their ability to synthesise a wide range of value-added products, such as flocculants, surfactants and pigments.
References


Mahaney, W. C., Milner, M. W., Hs, M., Hancock, R., Aufreiter, S., Reich, M. & Wink, M.  

Marchuk, A., 2013. Effect of cations on structural stability of salt-affected soils (Doctoral  
dissertation).

Marcos, N., 2003. Bentonite-iron interactions in natural occurrences and in laboratory–the  
effects of the interaction on the properties of bentonite: a literature survey. *Posiva  

Moore, D. M. & Reynolds Jr., R. C. 1997. X-ray diffraction and the identification and  
analysis of clay minerals, New York, Oxford University Press.


biotechnology*, 51, pp. 730-750.


approaches for bioremediation of hydrocarbon-contaminated soils. *Chemosphere*, pp. 179- 
184.


Bacteriology, Baltimore, Williams and Wilkins Co.

547-563.


CHAPTER SIX

6.0 GC×GC-TOFMS ANALYSIS OF VOLATILE FATTY ACIDS PRODUCED BY *Lactobacillus fermentum* WHEN IN CONTACT WITH GEOPHAGIC CLAY.

Abstract
Probiotics are microorganisms that produce volatile fatty acids (VFAs), they are usually found in the large intestines and provide a number of benefits to humans. Most of the time, geophagic clay eaten by humans ends up in the large intestines as it is is not be absorbed by the body, except for the minerals they carry. These clays contain different minerals which may cause harm to humans or react with other minerals, VFAs or VFA producing bacteria to cause harm. The effect of geophagic clays on the probiotic- *Lactobacillus fermentum* was studied to understand if they affect the production of VFAs by the probiotic or react with the probiotic to produce altered VFAs. GC×GC-TOFMS analysis was done on the culture media of probiotics alone and the supernatant of the probiotic and clay combination. The results revealed the production of different known VFAs (acetic acid and propanoic acid) which are beneficial to humans, other volatiles (oxalic acid, pyridine, and a host of others which are not beneficial to humans and numerous unknown volatiles by *L. fermentum* alone and in combination with geophagic clay samples.

**Keywords:** Geophagic clay, GC×GC-TOFMS, *L. fermentum*, Probiotics, Volatile fatty acids.

6.1 Introduction
Probiotics are live microorganisms believed to be beneficial to the host organism. According to the currently adopted definition by FAO/WHO, probiotics are living microorganisms, which when administered in adequate amounts; offer the hosts a health benefit (Sanders, 2008). Probiotics directly or indirectly affect the host beneficially; this may include enhanced barrier function, modulation of the mucosal immune system, production of antimicrobial agents, enhancement of digestion and absorption of food and alteration of the intestinal microflora (Jean et al., 2003; Hemaiswarya et al., 2013).

Volatile Fatty Acids (VFAs) are important biological products in the human colon and in the rumen of farm animals. They are substances typically formed in the intestines when food is digested. They are made up of a chain of carbon molecules, and are formed as a result of oxidation. They are produced by the bacterial degradation of carbohydrates and proteins that escape digestion and absorption in the small intestine (Cummings et al., 1987; Morrison et al., 2004). Examples of VFAs include: Formic acid (Methanoic acid), Acetic acid (Ethanoic acid), Propionic acid, Butyric acid and Valeric acid (Pentanoic acid) (Prochazka, 2008). VFAs act as mediators in the inflammatory process in the large intestine. They are produced from fermentation processes and stimulate water absorption and mineral uptake, as such, they have been noted in preventing mineral deficiency and to hasten recovery from diarrhoea. Acetate can be used as an energy source and induces an insulin response, while propionate may reduce hepatic cholesterol synthesis (Wasielewska & Zygmunt, 2015).

Gas chromatography (GC) has become broadly accepted as a dependable means for the quantitative analysis of complex mixtures of fatty acids. The method dates back to the early 1950s with the finding that short-chain fatty acids can be separated by vapor-phase chromatography (James & Martin 1952, Quehenberger et al., 2011). The first critical progression to the initial procedure followed shortly thereafter when it was shown that converting the fatty acids to methyl esters improved the physical properties of long-chain fatty acids for analysis by GC (James & Martin 1956; Quehenberger et al., 2011). Since then, major improvements have been made in both methodology and technology, and GC coupled with mass spectrometry (GC-MS) has become a
routine procedure with broad application to biochemical, biomedical, forensic, agricultural, environmental and industrial research (Quehenberger et al., 2011).

GC×GC is a technique used for complete analysis and characterization of complex samples (Adahchour et al., 2006; Cordero et al., 2006; Vial et al., 2011). GC×GC-TOFMS affords better selectivity and sensitivity compared to one-dimensional gas chromatography mass spectrometry (Schoenmakers et al., 2003). The comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometric detection (GC×GC-TOFMS) is the most advanced analytical technique of high sensitivity and selectivity (Hoskovec et al., 2012).

The focus on the effect of geophagic clay on probiotic was undertaken to ascertain if the consumption of this clay has any adverse or beneficial effects on human health. Probiotics are known to be found in the large intestines and some of the consumed clays which are not absorbed by the body end up in the large intestines. These clays contain minerals which, when in contact with the probiotics or even the VFAs produced by the probiotics, may react and produce alternative volatile compounds with either beneficial or detrimental effects, alter the metabolism of the probiotics to produce modified VFA’s, inhibit the growth of probiotics which may result in adverse effects on the physiology of the large colon.

6.2 Materials and methods
6.2.1 Sample Collection
Geophagic clay samples (six samples) were obtained from different informal markets around Johannesburg CBD. Of the six samples, four were originally from South Africa (labelled GSA 1-4) and two were originally from Nigeria (labelled GNG 1-2). The different samples were homogenised under sterile conditions using a laboratory mortar and pestle and the samples were stored in sterile zip-lock bags. The probiotic Lactobacillus fermentum (ATCC 9338) used in this study was purchased from MediMark.

6.2.2 Sample Preparation
The probiotic Lactobacillus fermentum (L. fermentum) was inoculated into nutrient broth of pH 7 and incubated at 37°C for 24 hours. This was used as starter culture. 50mL of nutrient broth was prepared in different Schott bottles. The clay samples were weighed as follows: 2g, 4g, 6g, 8g, 10g
into McCortney bottles and autoclaved to make them sterile. 100 µL from each of the probiotic starter cultures was transferred into a Schott bottle containing 50 mL broth, this served as positive control.

100 µL of the starter culture was transferred into another Schott bottle containing 50 mL of nutrient broth and 2g of one of the clay samples was added; and so on until the 10 g of clay sample. Different clay samples (2g) were weighed into Schott bottles of 50 mL broth without adding the probiotic; these served as negative controls for all clay samples. The samples were incubated for 24 hours at 37 °C with constant swirling.

24 hours thereafter, the samples were removed from the incubator. 20mL of each of the samples were measured into clean sterile 50 mL falcon tubes and centrifuged. The supernatant (200uL) was put into 50 mL clean sterile falcon tubes, and analytical grade chloroform (200 µL) was added (1:1 v/v) and vigorously shaken for 5 minutes using a vortex to achieve liquid-liquid extraction. The mixture was separated into two phases and the chloroform phase was collected using a micro pipette and put into GC vials and immediately analysed on a GCxGC-TOFMS.

6.2.3 Instrumental Conditions
The sample was analyzed using a cryo jet Pegasus 4D GC×GC-TOFMS (Leco Corporation St. Joseph, MI, USA). Samples were run in a two-dimensional mode. Sample injection volume was 1 µL with helium as the carrier gas at a flow rate of 1 mL/min. The inlet temperature was set at 280 °C and a split mode injection type was used with a ratio of 10. The first dimension column was a Restek Rtx-5siLMS (30 m × 250 µm × 0.25 µm d.f.) and the temperature profile was 10 °C/min from 50 to 300 °C with a hold for 5 min at 300 °C. The second dimension column was Restek Rxi17siLMS (1 m × 250 µm × 0.25 µm d.f.) and the temperature profile was the same as the first dimension except for the temperature offset of +10 °C. The second dimension separation time was set to 4 seconds and controlled with an internal cryo-jet-modulator creating a hot/cold pulse. This instrument utilizes electron impact ionization (EI). The temperature of ion source was set at 250 °C. The detector voltage was set at 1564V and the filament bias at −70 V. The mass range collected was 40 to 550 m/z with an acquisition rate of 200 spectra/s (Ralston-Hooper et al., 2008). The data generated was analyzed using ChromaTOF® software.
6.3 Results
GC×GC-TOFMS analysis was done to determine the production of VFAs by the probiotic *L. fermentum* alone and when in contact with geophagic clay. The results obtained as shown in Table 5.1 showed that the production of VFAs by *L. fermentum* differed depending on the clay sample present. These differences could be due to the presence of different elements in the clay and their reaction with the probiotics. The generation of altered VFAs could be as a result of the probiotics being either strained by the heavy metals present in the clay samples or due to the fact that the minerals in the clay render the nutrients unavailable for proper bacterial metabolism. The negative controls (clay samples alone) did not produce VFAs as expected. Table 6.1 is a representation of the known compounds/volatiles only as the data from the GC×GC-TOFMS gave numerous unknown compounds.
Table 6.1: Results showing the effects of geophagic clay on *Lactobacillus fermentum* probiotic.

<table>
<thead>
<tr>
<th><em>L. fermentum</em></th>
<th>+ 0g clay VFAs produced</th>
<th>+ 2g GNG1 clay VFAs produced</th>
<th>+ 2g GSA1 clay VFAs produced</th>
<th>+ 4g GNG1 clay VFAs produced</th>
<th>+ 4g GSA1 clay VFAs produced</th>
<th>+ 6g GNG1 clay VFAs produced</th>
<th>+ 6g GSA1 clay VFAs produced</th>
<th>+ 8g GNG1 clay VFAs produced</th>
<th>+ 8g GSA1 clay VFAs produced</th>
<th>+ 10g GNG1 clay VFAs produced</th>
<th>+ 10g GSA1 clay VFAs produced</th>
<th>+ 2g GNG2 clay VFAs produced</th>
<th>+ 2g GSA2 clay VFAs produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓ Formic acid methyl ester ✓ Propanoic acid ✓ Isobutyric acid ✓ Isovaleric acid</td>
<td>✓ Ethyl chloride (chloroethane) ✓ Methyl methacrylate (Methyl 2-methylpropenoate) ✓ Tetrahydropyran Z-10-dodecenoate</td>
<td>✓ Amylene hydrate (tert-amyl alcohol) ✓ Methyl formate ✓ Amylene hydrate (tert-amyl alcohol)</td>
<td>✓ Propenoic acid, anhydride ✓ Amylene hydrate (tert-amyl alcohol) ✓ Pyridine ✓ Oxalic acid</td>
<td>✓ Methyl formate ✓ Propanoic acid ✓ Propanedioic acid, ester</td>
<td>✓ Methyl formate ✓ Propanoic acid ✓ Propanedioic acid, ester</td>
<td>✓ Methyl formate ✓ Propanoic acid ✓ Propanedioic acid, ester</td>
<td>✓ Propenoic acid, anhydride ✓ Amylene hydrate (tert-amyl alcohol) ✓ Pyridine ✓ Oxalic acid</td>
<td>✓ Methyl formate ✓ Acetic acid ✓ Amylene hydrate (tert-amyl alcohol) ✓ Trichloroacetic acid, hexyl ester</td>
<td>✓ Methyl formate ✓ Acetic acid ✓ Amylene hydrate (tert-amyl alcohol) ✓ Trichloroacetic acid, hexyl ester</td>
<td>✓ Methyl formate ✓ Acetic acid ✓ Amylene hydrate (tert-amyl alcohol) ✓ Trichloroacetic acid, hexyl ester</td>
<td>✓ Methyl formate ✓ Acetic acid ✓ Amylene hydrate (tert-amyl alcohol) ✓ Trichloroacetic acid, hexyl ester</td>
<td>✓ Methyl formate ✓ Acetic acid ✓ Amylene hydrate (tert-amyl alcohol) ✓ Trichloroacetic acid, hexyl ester</td>
<td></td>
</tr>
</tbody>
</table>
| + 4g GNG2 clay | ✓ Propanoic acid  
✓ Isobutyric acid  
✓ Pyridine | + 4g GSA2 clay | ✓ Propanoic acid  
✓ Amylene hydrate ( tert- amyl alcohol)  
✓ Methyl methacrylate (Methyl 2-methylpropenoate) |
| + 6g GNG2 clay | ✓ Methyl formate  
✓ Acetoin  
✓ Pyridine | + 6g GSA2 clay | ✓ Methyl formate  
✓ Ethyl chloride ( chloroethane)  
✓ Acetic anhydride  
✓ Methyl methacrylate (Methyl 2-methylpropenoate) |
| + 8g GNG2 clay | ✓ Oxalic acid  
✓ Pyridine | + 8g GSA2 clay | ✓ Ethyl chloride ( chloroethane)  
✓ Propanoic acid, 2-hydroxy-, ethyl ester  
✓ Propanoic acid, 2-methyl-, anhydride  
✓ Methyl thioacetate  
✓ Methyl methacrylate (Methyl 2-methylpropenoate) |
| + 10g GNG2 clay | ✓ Methyl formate  
✓ Propanoic acid, 2-oxo- | + 10g GSA2 clay | ✓ Ethyl chloride ( chloroethane)  
✓ Amylene hydrate ( tert- amyl alcohol)  
✓ Oxalic acid |
| + 2g GSA3 clay | ✓ Ethyl chloride ( chloroethane)  
✓ Propanoic acid, 2-oxo-, ethyl ester  
✓ Amylene hydrate ( tert- amyl alcohol)  
✓ Hexadecanoic acid, methyl ester  
✓ Decanoic acid, methyl ester | + 2g GSA4 clay | ✓ Ethyl chloride ( chloroethane)  
✓ Methyl methacrylate (Methyl 2-methylpropenoate) |
| + 4g GSA3 clay | ✓ 2-Oxo-n-valeric acid  
✓ Trichloroacetic acid, hexyl ester | + 4g GSA4 clay | ✓ Ethyl chloride ( chloroethane)  
✓ Binapacryl |
<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ 6g GSA3 clay</td>
<td>✓ Amylene hydrate (tert-amyl alcohol) ✓ Methyl methacrylate (Methyl 2-methylpropenoate)</td>
<td></td>
<td>✓ Methyl methacrylate (Methyl 2-methylpropenoate) ✓ Carbonyl sulfide ✓ Ethyl chloride (chloroethane) ✓ Acetic acid ✓ Amylene hydrate (tert-amyl alcohol) ✓ Carbon Tetrachloride ✓ Methyl methacrylate (Methyl 2-methylpropenoate) ✓ Methyl tetradecanoate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ 8g GSA3 clay</td>
<td>✓ Tetrahydropyran Z-10-dodecenoate ✓ Amylene hydrate (tert-amyl alcohol) ✓ Methyl methacrylate (Methyl 2-methylpropenoate)</td>
</tr>
<tr>
<td>+ 10g GSA3 clay</td>
<td>✓ Acetic acid ✓ Amylene hydrate (tert-amyl alcohol) ✓ Methyl methacrylate (Methyl 2-methylpropenoate)</td>
<td></td>
<td>✓ Methyl formate ✓ Ethyl chloride (chloroethane) ✓ Guanidine ✓ Thioacetic acid ✓ Amylene hydrate (tert-amyl alcohol) ✓ Trichloroacetic acid, hexyl ester</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ 8g GSA4 clay</td>
<td></td>
</tr>
</tbody>
</table>
6.4 Discussions
The GCxGC-TOFMS results showed that *L. fermentum* probiotic produced the following known VFAs: Formic acid methyl ester, Propanoic acid, Isobutyric acid, Isovaleric acid and numerous unknown VFAs. With the addition of the different geophagic clays at different measurements, we observed a change in the VFAs produced when the different quantities of clay come into contact with the probiotic. Aside the VFAs produced; there was production of other chemical compounds which may have resulted from a reaction between different minerals present in the clay and the VFAs produced by the probiotic.

The results of the different measurements of the GNG 1 clay were as follows: 2g GNG 1 clay when added to *L. fermentum* produced ethyl chloride (chloroethane), Methyl methacrylate (methyl 2-methylpropenoate) and tetrahydropryn Z-10-dodecenoate. These compounds are not VFAs but ethyl chloride is known to have anesthetic effect and inhibit bacteria (Burney *et al.*, 2006) and methyl methacrylate, the methyl ester of methacrylic acid is used as “cement” by orthopedic doctors to treat bone dislocations (Lee & Rhee, 2009) while tetrahydropryn Z-10-dodecenoate has no known use(s). Methyl methacrylate is produced by a reaction which involves oxidative hydrogenation of isobutyric acid (Lee & Rhee, 2009) (which was produced by the probiotic) hence, it is possible that some minerals in the clay could have either coordinated to the isobutyric acid to enhance a spontaneous rearrangement or influence its conversion to methyl methacrylate.

Incubation of 4g GNG 1 clay with *L. fermentum* produced amylene hydrate (tert-amyl alcohol), propanoic acid which is a VFA is known to be produced by an oxidative reaction from propionaldehyde in the presence of manganese or sometimes cobalt ions. Propanoic acid has antibacterial and anti-inflammatory activities and it is sometimes used for food preservation and as food additive (Dhall, 2016); and Pyridine which is known to have toxic effects on humans.

*L. fermentum* incubated with 6g GNG 1 clay was observed to produce methyl formate, tert-amyl alcohol and pyridine. Increasing the amount of GNG 1 clay to 8g produced propenoic acid anhydride which is corrosive to humans, tert-amyl alcohol and pyridine which is toxic to humans together with oxalic acid which alters the pattern of ruminal fermentation and the bacterial community (Benbati *et al.*, 2013). Interestingly, the more the amount of GNG 1 clay was increased,
the VFAs produced were limited to only tert-amyl alcohol and pyridine. Since no more than 10g of clay was used, it would be interesting to see if the types of VFAs produced either become constant or they are not produced at all and also to observe what happens when less than 2g of clay is used.

Varying the amounts of GNG2, GSA1, GSA2, clays (from 2g to 10g) in the presence of *L. fermentum* was observed to produce most of the VFAs noted for GNG1 clay except for the production of dicyclobutyl ester and 2,2,4-trimethyl-1,3-dioxolane as additional extra VFAs seen when 2g GNG 2 clay was incubated with the probiotic. Interestingly, 2,2,4-trimethyl-1,3-dioxolane has been shown to be a volatile thermal decomposition product of soy samples with no apparent nutritional value (Artz *et al.*, 2001). Methyl thioacetate and acetic anhydride (anhydride of acetic acid) were two additionally different VFAs produced during incubation with 6g and 8g of GSA2. Brewer’s has been one of a few microorganisms known to produce methyl thioacetate as a metabolic product from methyl mercaptan. Although methyl thioacetate has no known nutritional value, it has however, found a number of applications in beer and cheese-making industries despite its foul smell (Matsui & Amaha, 1981).

Further analysis on GSA3 and 4 clays showed that although most of the volatiles as seen in the incubation of other clays with the probiotic were produced (Table 6.1), four interesting additional volatiles namely, methyl tetradecanoate, binapacryl, carbon tetrachloride and guanidine were also detected due to these clays at 4, 6 and 10g. While, carbon tetrachloride has a number of applications (e.g. solvent in chemical synthetic reactions) in the chemical industry with no known nutritional value, its detection is intriguing as it can be toxic. Guanidine on the other hand is a well-known metabolite of protein metabolism that is usually found in urine, its detection and possible formation may be due to a number of protein metabolism processes coupled to the minerals found in this type of clay. The most interesting detection is that of binapacryl, is another well-known fungicide (anti-fungal agent) which attains its fungicidal activity when it is transformed into the toxic 2-(butan-2-yl)-4,6-dinitrophenol (Wearing, 2014). There have not been studies indicating the inhibition of probiotics by 2-(butan-2-yl)-4,6-dinitrophenol. Since most fungicides tend to affect bacteria as well, further studies are therefore needed to evaluate the effects of binapacryl on probiotics.
The results observed showed that there is a change in the production of VFAs by *L. fermentum* when in contact with clay. At different measurements of the clay, the results showed the production of volatiles which are different from the ones produced by the probiotic. Some of these volatiles were by-products of a reaction between the elements in the clay and the VFAs produced by the probiotic. It is also important to consider that since *L. fermentum* is a microorganism, it is possible that with the introduction of the clay, the organism might start producing secondary metabolites to defend itself against foreign substances (in this case the clay minerals) and some of these secondary metabolites may have reacted with the clay minerals to produce the alternative volatiles seen in Table 6.1.

**6.5 Conclusion**

From the results observed in this study, it can be concluded that the ingestion of clay by humans may have adverse side effects based on some of the volatiles produced as seen in the results of this study. Although some of the volatiles are VFAs (for example acetic acid and propanoic acid) and have beneficial effects on humans, some are detrimental to human health (pyridine) hence consumption of this clay over a long period of time can lead to adverse health conditions.
References


CHAPTER SEVEN

7.0 GENERAL DISCUSSION AND CONCLUSION

7.1 General discussion

There are increasing interests in the occurrence of human geophagia (Finkelman et al., 2005, Ekosse & Jumbam, 2010). The ingestion of clayey soil as reported in previous studies amongst other things is to alleviate stomach pains and diarrhoea, prevent morning sickness and also act as a binding agent of toxins and bacteria in the intestines. More precisely, a need to pinpoint prospective health hazards which may emanate from this habit has begun (Young et al., 2007, Ekosse et al., 2010). Geophagists in the Johannesburg central business district of Gauteng province, South Africa are probably at risk of exposure to potentially harmful biological and non-biological soil contents. Some of these health hazards include heavy metal poisoning as well as human pathogenic bacteria and other parasites.

From this study, it was revealed that most of the soils consumed contain several levels of mineral nutrients, including calcium, copper, magnesium, manganese, phosphorus, potassium and zinc. These mineral nutrients varied with soil types (see chapter three).

With the pH of the samples being acidic (pH varied from 4.47 to 6.36), there are likelihoods for malabsorption of nutrients within the GIT. This is due to the fact that the enzymes responsible for absorption are not functional in an acidic environment. However, a substantial amount of the mineral nutrients found in the studied soils are bioavailable. Thus, they will contribute to mineral nutrition and alleviation of mineral deficiencies, such as iron, iodine and zinc, predominant in South Africa (see chapter four).

All geophagic soils in this study had microorganisms, of which quantities apparently depend on the area where soil samples were collected, handled and stored. Despite the particle size and closely packed nature of the studied clayey soil samples, a high microbial load was observed. This suggests that there is a potential health hazard for the geophagists, principally on the grounds that the soils were not processed in any manner such as heating to reduce their microbial load. The presence of such numerous uncultured bacteria may provide new gateways for further research on the microbial aspects and importance of microbes in geophagic practicing.
Based on some of the volatiles produced when the clays are in contact with probiotics as evidence in the results of this study, deleterious effects may result on a prolong consumption.

7.2 Recommendations
This study emphasizes the relevance of geophagic clayey soil as a rich source of important minerals. However, the consumption of clay materials may impart negatively on the geophagists. The results from this study establish the hypothesis that due to mode of collection, transportation and exposure to airborne contaminants at informal markets, clayey soils possess harmful microorganisms as well as contain toxic heavy metals. Therefore, the assessment of the geophagic soil composition in combination with the free-living soil microorganisms that serves as biological indicators of soil health may provide explicit knowledge into the risks that these soils potentially harbour.

Generally, the ingestion of clayey soils particularly by women should be moderate if not avoidable. Pretreatment of clays using heat (smoking and baking) may be adopted to ensure microbial safety.
References
APPENDIX

Possible Heavy Metals’ Transportation and Ground Erosion during Heavy Rainfall in the Rural Mhangweni (Tzaneen, Limpopo, South Africa).

Okereafor G.U, Mulaba-Bafubiandi, A.F, Mavumengwana, V

1. University of Johannesburg, Mineral Processing and Technology Research Centre, Department of Metallurgy, School of Mining, Metallurgy and Chemical Engineering, Faculty of Engineering and the Built Environment, Johannesburg, South Africa.

2. University of Johannesburg, Department of Biotechnology and Food Technology, Faculty of Science, Johannesburg, South Africa.

E-mail: amulaba@uj.ac.za; guokereafor@gmail.com

Abstract

In South Africa, intense mining has contributed to the accumulation of heavy metals such as As, Cd, Cr, Pb, Zn, Ni and Cu in sediments around water sources and farms. The Mining sector despite its economic viability had impacted unfairly in many parts of the country resulting in an undesirable change in both the physical and biological nature of the ecosystem. The contamination of heavy metals remains an area of interest because most minerals - when mined, processed/beneficiated or smelted - generate solid waste in the form of fine particles, tailings, smelter slag, that are typically not properly disposed of during and after mining. These heavy metals have deleterious effects on the vegetation, water sources, livestock and humans as exposure to them could result in certain ailments such as deformity, kidney damage, cancer and miscarriage. Apparently, this affects the health and survival of humans and other living creatures. After heavy rainfall, sediments were transported over a vast area of land in Mhangweni village within the Tzaneen municipality (Limpopo Province) through surface runoff. Samples of the runoff water together with sediments were collected using a sterile bottle and subjected to certain laboratory studies. This was to ascertain the safety and danger it poses to the environment and humans due to possible heavy metals that may be contained therein. Water samples containing soil particles were collected and subjected to various geochemical analyses using XRF, XRD, SEM/EDS, AAS to determine possible toxicity on surrounding water sources and farms. Infra-red spectroscopy
revealed the solid particles contained in the water were composed of SiO$_2$ 46.8%, Al$_2$O$_3$ 27.4%, Fe$_2$O$_3$ 21%, TiO$_2$ 1.54%, Na$_2$O 0.6%, MgO 0.52% with corresponding mineral phases dominated by quartz followed by Sodium aluminosilicate and Magnesioferrite. The filtrate had a pH of 7.3 with very minimal trace elements. Iron was more distinct with 6.07mg/l. The observed high concentration of Iron may be linked to the odour and reddish-brown colour of the water which has a higher tendency of leaving stains on laundry, porcelain, dishes, utensils, glassware, sinks, fixtures and concrete. The general population of people within the study area are prone to having various health challenges resulting from excessive soluble heavy metal in their surrounding water.

**Key words:** Debris, health, Mine water, pollution, toxicity.

**Accepted for publication as:** Okereafor, G. U; Mulaba-Bafubiandi, A.F; and Mavumengwana, V. (2016). Possible Heavy Metals’ Transportation and Ground Erosion during Heavy Rainfall in the Rural Mhangweni (Tzaneen, Limpopo, South Africa). The South African Institute of Mining and Metallurgy 3rd Young Professional Conference 2017 - (SAIMM) Journal.
Introduction
The nature, characteristics and level of mine waste generated at specific mines differ greatly on the basis of the resource being mined, technology applied, and geology of the area. Mine operators endeavour to manage waste generated to enable them to handle the enormous volumes of waste produced as well as avert the discharge of contaminants into the surrounding environment. Despite South Africa’s legislation and regulation regarding waste management at mining sites, some level of contaminants are observed which are often uncontrolled. Mine waste generally refers to unwanted, currently uneconomic, solid and liquid materials found at or near mine sites. This waste contributes to one of the world’s major waste streams due to their composition of high concentrations of elements and compounds that impact negatively on humans and the ecosystem (Hudson-Edwards et al., 2011).

Nonetheless, based on market conditions and available extraction technology, certain variations in mineral content occur between ore and waste rock. There are a number of instances where material that was once considered waste has become a resource for modern mining operations (Rankin, 2011). There is a huge difference in the physical and chemical composition of mine waste materials, which also determines their potential for environmental contamination. These waste materials include overburdens, waste rocks, tailings, slags, mine water and gaseous wastes. Considering the nature of this waste, it poses great threat to humans, the surrounding environment and the entire ecosystem. Besides the health hazards, other challenging environmental impacts emanating from waste disposal at mine sites include the loss of tillable land following its conversion to a waste storage site, and the subsequent introduction of sediment, acidity, and other contaminants into the surrounding surface. The water running over exposed problematic or chemically reactive waste.

Heavy metals (HMs) are comprised in major component of most mine waste and their continued circulation in soil and water puts the world in a worrying state due to their poisonous and deleterious effects. HMs are natural components of the earth’s crust and may be referred to as metallic chemical elements (metals, metalloids, lanthanides and actinides) with a relatively high density (at least five times the specific gravity of water) and include Lead (Pb), Cadmium (Cd), Arsenic (As), Chromium (Cr), Thallium (Tl), Lead (Pb), Iron (Fe), Copper (Cu), Zinc (Zn), Nickel
Some of these HMs such as copper (Cu), chromium (Cr)(III), zinc (Zn), manganese (Mn), cobalt (Co) and molybdenum (Mo) - at minimal concentrations - are of biological importance. However long-term exposures and high concentrations leave deleterious impacts on a host of biomolecules. Importantly, mercury (Hg), cadmium (Cd), chromium (Cr)(VI), arsenic (As) and lead (Pb) are very toxic even at very low concentrations (Rahman & Singh, 2016).

Sediments are naturally vital constituents of an aquatic habitat. Sediments have the potential of retaining contaminants that are not bioavailable to humans (Nemati et al., 2011). The resuspension of sediments, desorption, and the redox reactions of sorptive substance are some of the processes through which pollutants may be released into water column. Thus, sediments besides being found in reservoir, also acts as probable source of pollutants in the marine system (Varol, 2011, Nemati et al., 2011). Some HMs are deposited along roadides during mining activities and exposure to them may result in diseases such as deformity, kidney mutilation, cancer, and miscarriage (Alomary & Belhadj, 2007). Within the scientific community, HMs contamination has remained a subject of continuous interest due to their toxicity (Varol, 2011, Morselli et al., 2003). Following the very slow rate of soil formation, the process of erosion is capable of destroying the productivity of land within a short time frame. In most cases, accelerated erosion washes away the upper layer (A-horizon) of soil in an area, thus exposing the inner layer (B-horizon) leaving it with the dreadful effects of rainfall surface runoff and gravitational forces.

A proper knowledge of the forms of soil pollutants and physicochemical properties provides a basis for careful soil management that reduces, to the barest minimum, the adverse effects of pollutants on the ecosystem. Heavy metals in soluble mineral states or in the form of an ionic solution get discharged as effluents and mine waste which contaminates groundwater by leaching. Some of these metals such as Zn, Pb, Fe, Cr, Al, get introduced to the food chain as they are absorbed by plants (Calace et al., 2002). The final consumption of these plants poses a threat to human health when their daily limits are exceeded. On the other hand these heavy metals have effects on the microbial activities of the soil (Wyszkowska & Wyszkowski, 2002). Based on the relevance of sediments in the body of water, investigation of metal content is required in assessing the extent of pollution in the marine environment (Alomary & Belhadj, 2007).
Water problems over the years have impacted negatively on the development of Mhangweni community in Limpopo Province. Water shortage is one of the nightmares of the households from this area. Due to irregular municipal water supply, households suffer greatly in the quest for portable water and they have to buy water; thus, as a result, residents of the Mhangweni community are affected financially. Following a heavy downpour, this paper reports the physio-chemical features of runoff water and sediments with the sole aim of assessing the distribution of HMs in surface runoff water and sediments from surrounding mining sites as well as the ecological risks of HMs in the study area.

Methodology

Sampling site
The sampling area is located within the Greater Tzaneen Municipality. Mhangweni is 34km away from Tzaneen. It is located in a lush, high precipitation, fertile region with tropical and subtropical agriculture taking place in a 20,000 km² district. Water and sediment samples were collected from down the valley.

Collection of Sample and pre-treatment
Using sterile bottles, both water and sediment samples were collected. Water samples were stored in an insulated ice box after sampling and transported to the laboratory for storage at 4°C prior to further analysis. Sediments were obtained from the water mixture by centrifugation and filtering. Collected sediments were oven dried at 100°C and then kept in polyethylene bottles for mineralogical and heavy metal analysis.

Experimental
Several techniques were adopted for the analysis of water and sediments: SEM/EDS, XRF, XRD and ICP-MS were conducted using standard procedures. Mineralogical phase analysis and morphological of the filtered sediments were carried out using an X-ray power diffractometer (XRD; Rigaku Ultima-IV X-ray diffractometer) and a scanning microscope (SEM; TESCAN Vega 3XMV) respectively. The samples were scanned from 10 to 80° at a rate of 2° min⁻¹. Elemental composition of the ore was determined by X-ray fluorescence (XRF; Rigaku ZSX PrismusII). Physicochemical analyses were carried out including physical analysis such as colour, odour while
chemical analyses conducted included pH, electrical conductivity, TDS in guidelines as stipulated by standard protocols of APHA (Clescerl et al., 1999).

**Results and discussion**

**pH of the solvent**
The pH of the solvent is a physical parameter that provides information regarding the acidity and alkalinity of the solvent. The pH of the raw sample before and after centrifugation was determined using Jenway model 3510 pH meter. The pH measurements revealed that the solvent was within the limit of standard drinking water. Before centrifugation and after centrifugation, pH values were 6.33 and 7.26 respectively. Such values will support the mobility and availability of heavy metals for plant uptake as they will not be toxic due to the fewer H+ ions.

**Electrical conductivity (EC)**
The concentration of ionic species contained in water determines the values of electrical conductivity. The electrical conductivity using a standard of 84µS and a Crison multimeter model MM 41 was 115.9µS. The revealed higher electrical conductivity (EC), suggests the presence of higher dissolved salt or ion concentration which means the sample is more conductive.

**Total dissolved solids (TDS)**
Using the expression 0.64 x EC, parameters for arriving at the total dissolved solid (TDS) which measures Total Dissolved Amount of Substance was obtained as 74.18mg/L. Higher concentration of salt in water or in soil adversely impacts the crop yield, create soil degradation and the likely pollution of groundwater. This parameter however did not deviate from the international standards.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Parameter</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Colour</td>
<td>Brownish</td>
</tr>
<tr>
<td>2</td>
<td>Odour</td>
<td>Odourless</td>
</tr>
<tr>
<td>3</td>
<td>pH</td>
<td>6.33 / 7.26</td>
</tr>
</tbody>
</table>
Characterisation of filtrate and sediments

The SEM micrograph shown in Figure 1 clarifies the entire surface morphology of the solid particles contained in the liquid mixture originally collected. They are more influenced by the greyish areas, noted to be silica oxide because it contributes about 40% of the total filtered residue from the liquid sample. The dark black spots were identified as copper oxides, which accounts for 2% of the sediment.

![Figure 2. SEM micrograph of sediments](image)

A typical mineralogical composition of the filtered sediments is shown in figure 3 as determined by XRD. The XRD results confirm the presence of Sodiumaluminosilicates (AlNa$_{12}$SiO$_5$), naturally occurring compounds of oxygen and silicon, and magnesioferrite (Mg(Fe$^{3+}$)$_2$O$_4$), a magnesium iron oxide mineral, along with peaks of quartz.
The partial compositional analysis of the sediments collected from the water sample is shown in Table II. As shown, SiO$_2$ (46.8%) was the most abundant compound found in the sediments. The oxides of Fe and Al were 21.0% and 27.4%, respectively. Oxides of Na, Mg, K, Mn and Cu were less than 1%. The amount of major oxides such as Fe$_2$O$_3$, TiO$_2$ and MnO exceed the acceptable values for human body function (World Health Organization, 2004).

Table II. Results of XRF Analysis detailing composition of sediments

<table>
<thead>
<tr>
<th>Composition</th>
<th>Na$_2$O</th>
<th>MgO</th>
<th>SiO$_2$</th>
<th>P$_2$O$_5$</th>
<th>K$_2$O</th>
<th>MnO</th>
<th>Fe$_2$O$_3$</th>
<th>CuO</th>
<th>ZnO</th>
<th>Al$_2$O$_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(%)</td>
<td>0.597</td>
<td>0.524</td>
<td>46.800</td>
<td>0.228</td>
<td>0.810</td>
<td>0.178</td>
<td>21.000</td>
<td>0.032</td>
<td>0.022</td>
<td>27.400</td>
</tr>
</tbody>
</table>
Table III. Results of ICP-MS Analysis on liquid sample showing heavy metal concentration.

<table>
<thead>
<tr>
<th>Element</th>
<th>Al</th>
<th>Zn</th>
<th>Fe</th>
<th>Na</th>
<th>Mg</th>
<th>Ca</th>
<th>Cu</th>
<th>Pb</th>
<th>Mn</th>
<th>Co</th>
<th>Ni</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc. (mg/L)</td>
<td>6.141</td>
<td>0.431</td>
<td>5.072</td>
<td>14.160</td>
<td>4.196</td>
<td>11.380</td>
<td>1.506</td>
<td>2.041</td>
<td>3.918</td>
<td>0.002</td>
<td>0.054</td>
</tr>
</tbody>
</table>

From the filtrate, ICP-MS as illustrated in Table III shows possible heavy metals that apparently exceed the maximum permissible level of the United States Environmental Protection Agency water composition (Praveena et al., 2015, Dubey & Ujjania, 2015). Aluminium (Al), Zinc (Zn), Iron (Fe), Copper (Cu), Lead (Pb) and Manganese (Mn) were all above the standard. There is a strong likelihood of the runoff water introducing heavy metals such as Fe, Zn, Pb, Cu, Ti and Mn into nearby rivers. Upon consumption by humans as drinking water, it gets into the gastro-intestinal system; this might impact negatively on the consumer by increasing the gastro-intestinal pH resulting in surface coating on the stomach (Ekosse & Mulaba-Bafibiandi, 2008).

Majority of the inhabitants within the area of Mhangweni are in danger of suffering from liver damage due to high concentration of iron in the water sample (sediments and filtrates). Despite Iron (Fe) acting as a supplement for those who lack iron in their blood, a high level of Fe in the blood could result in hemochromatosis and possible death (World Health Organization, 2004). Besides, the drinking of water with high iron content may negatively impact the utilisation of copper, zinc and selenium in the human body (Brand et al., 2009).

In the human body, the total aluminium concentration is approximately 9 ppm (dry mass) with an approximate daily intake of 5 mg, of which only a small fraction is absorbed. With the revealed high aluminium content, there is likelihood of this negatively affecting both plants and humans. Various ailments of the nervous system, such as Parkinson's disease, amyotrophic lateral sclerosis (Lou Gehrig's disease), and Alzheimer's disease as well as functional lung disorder may be experienced (Krewski et al., 2007).

As a trace element with dietary value, of approximately 2.3g, Zinc is contained in the human body. It helps in maintaining enzymatic processes and DNA replication. Only 20-40% of zinc present in food is being absorbed by the human body; thus many people drink mineral water rich in zinc.
Some of the indications of zinc deficiencies include loss of sense of taste and loss of appetite. Also the immune systems and enzyme systems of children may be negatively affected. Higher zinc content (4 – 8g) in the human body often results in symptoms such as nausea, vomiting, dizziness, colic, fever and diarrhoea. With a greater portion of the people within this area being economically disadvantaged and dependent water from streams, there is the risk of a mucous membrane infection from zinc chloride.

Lead and some of its compounds are largely toxic pollutants with the potential of limiting the synthesis of plant chlorophyll. Although, plants can take up high levels of lead up to 500 ppm from soils. Plant growth is often retarded by higher concentrations of Lead. Most of the crops within this area may suffer damage and reduced growth. Besides gaining access into the food chain via plant uptake, humans consume more lead in the water they drink. Considering the very high level of Lead shown in Table III, inhabitants are likely to have excess lead intake which may cause colic, skin pigmentation and paralysis. Females may experience menstrual irregularities, infertility and spontaneous miscarriage. Lead poisoning of the surrounding water may lead to lower IQs, behavioural changes and various concentration disorders in children.

**Conclusion**

This study revealed some physicochemical properties of water and sediments due to erosion after heavy rainfall in the village of Mhangweni, Limpopo (South Africa). Various anthropogenic activities around the area resulted in the alarming geochemical pollution of surface runoff water and sediments. The continuous increase of harmful chemicals from various mining activities put the surrounding farm lands and water bodies at risks of contamination from heavy metals, especially given their high concentrations. The repeated flow of such water into nearby rivers is harmful to aquatic organisms, vegetation and humans. There should be proper waste and mine management to guide against having tailings littered everywhere during and after mine operations. Considering that most of the heavy metal sources are anthropogenic in nature, their subsequent spread into the surroundings, (thus polluting new areas) could be controlled by remediation techniques such as excavation, stabilisation of the metals in the soil on site, and phytoremediation may be adopted for reducing the harmful effects of these metals.
Acknowledgments

We thank the Mineral Processing and Technology Research Centre of the University of Johannesburg for providing the resources used for conducting this study.
References


