KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, GHANA

HEALTH IMPLICATIONS OF SOME GEOPHAGIC CLAYS ON GROWTH PERFORMANCE, HAEMATO-BIOCHEMICAL PROFILE AND HISTOLOGY OF FEMALE SPRAGUE DAWLEY RATS

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JAMES MANKUBASI ZAKARI

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JAMES MANKUBASI ZAKARI (B.Ed Health Sciences Education)

A Thesis submitted to the Department of Materials Engineering,

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in partial fulfillment of the requirements for the degree

of

MASTERS OF SCIENCE IN ENVIRONMENTAL RESOURCES MANAGEMENT.

JANUARY, 2015

DECLARATION

I hereby declare that this work is the result of my own and it has not been submitted in part or whole for any other degree else where. Specific references and sources of information used have been duly acknowledged.

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ABSTRACT

Geophagy is the purposeful or deliberate consumption of earth and clay deposits by animals, including man. It is a special type of pica, which is defined as the craving and subsequent consumption of non-food substances. The study was conducted to determine the health implications of some geophahic clays on growth performance, biochemical profile and histology of female Sprague dawley rats. Twenty-four female Sprague dawley rats were used for the experiment. They were divided into four groups of six and were housed in metal cages. Each group was weighed before commencement and at the end of the experiment. The powdered clay samples were dissolved in distilled water to form suspensions. Group 1 was used as the control and received 1ml of distilled water and feed *ad libitum* while test groups received varied doses of 0.1g, 0.3g, and 1.0g representing (0.2ml, 0.6ml and 1ml) of 40g/1000ml per body weight of the rats. The clay doses were administered once daily for 28 days and the effects on body weigh, organ weight, haematology, histology and serum biochemical parameters were evaluated. X'ray fluorescence (XRF) results showed that dominant elements in the clay were silicon, aluminum, iron potassium and magnesium oxides. Concentrations of Vanadium, Asernic and Barium in the clay samples was higher than the various RDAs but very negligible. It was also found that lead concentration in clay was 0.03mg (25ppm) higher than the RDA of 0.01ppm. These heavy metals present could be the cause of necrosis and other morphological changes in the histology results. The mineralogical composition of some geophagic clay samples was investigated using X'ray diffractometry (XRD). The XRD results showed that geophagic clays consisted mainly quartz and kaolinite. With respect to growth performance, the control group gained mean weight of (39.89g), and test groups of 0.1g/kg/b.wt, 0.3g/kg/b.wt and 1.0g/kg/b.wt gained corresponding body weights of 159.75g, 34.86g and -69.96g which were significantly different from control more especially those fed on the highest dose of 1ml of the suspension.The values of liver, kidney, stomach, heart, lungs, spleen, ovaries and uterus were not statistically different (P> 0.05) between the controls and test groups. The mean values for Red blood cells (RBC), Haemoglobin (HB), Haematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular Haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and White Blood Cells (WBC) were statistically similar but mean values of Lymphocytes and Neutrophils were statistically different among the treatment means. The Albumin, Globulin, Protein, ALT, AST, GGT, TBIL and DBIL were not affected by the treatments but ALP and INBIL were significantly influenced by the treatments. Significant differences were not observed for the Cholesterol, Triglycerides, HDL and VLDL levels recorded. Kidney function parameters considered were also not significantly different (P>0.05). Although some clay elements were slightly higher in concentrations than their RDAs this did not affect the biochemical parameters. However, some selected organs subjected to histology studies did show signs of defects which correlates well with the chemical results, hence eating clays which contain negligible anounts of heavy metals could be harmful to human in the long term.

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This work is dedicated to my family. To my parents Lariba Kpandana (Wudanzoori) and Zakari Mankubasi affectionatedly called (Nya) all of blessed memory; who thought it wise enrolling me into school. For their hard work and consistent prayers, unflinching love and support for my education raised me to this pedestal. To my wife, Ruth Lardi for her constant prayer support, unconditional love, encouragement and a heart to serve always. Also to my beautiful queens; Miriam, Betty, Rhoda, Philipa, Hilda and Blessing, for their prayers and patience for denying them the fatherly love during this period. To Rev. John Mankubasi and my brothers especially Isaac D. Mankuyi for their enormous financial support, love, and encouragement when times were difficult to continue. May the good Lord replenish whatever resources they expended on my education.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Geophagy, according to Eugene (2010), is the practice of eating clay, chalk and other forms of earth that generally are not considered part of a normal diet. Pica is the scientific term for craving and subsequent consumption of non food items (Young, 2010). The clay soil commonly consumed by geophagic persons contains several mineral nutrients including magnesium, zinc, copper, manganese, silicon, and iron, as well as some toxic mineral elements such as lead and aluminium (Ekosse *et al.*, 2010).

According to Diamond (1998), several researchers found geophagy in various species of living things, including mammals, birds, reptiles, butterflies and Isopods. Scientists are interested in geophagy in human because its effects and results are still unclear. Moreover, there is still controversy over whether minerals deficiencies of iron, calcium and zinc cause pica or a consequence of its practice (Solyom *et al.*, 1991, Boyle and Mackey,1999).

There have been various hypotheses advanced to explain geophagic behaviour. The first one is the hunger hypotheses which posits that people consume non-food substances because they do not have anything else to eat. The second is the micronutrient deficiencies which states that people eat non-food substances in an attempt to increase micronutrient intake of iron, zinc or calcium (Wiley and Katz, 1998). The third hypothesis states that pica is motivated by an attempt to mitigate the harmful effects of plant chemical or microbes (Young, 2010).

Several reasons have been advanced to justify the practice; some of which are cultural and others medicinal (Tateo *et al.*, 2001; Carretero, 2002; Ekosse *et al.*, 2010).

Geophagy has been a tradition in many cultures in different parts of the world. It is highly prevalent in Ghana, especially among pregnant and lactating women, for various reasons including traditional or cultural activities which take place during pregnancy, religious ceremonies or as a remedy for disease (Vermeer, 1971). Clay consumption is however, expected to have some physiological and pathological effects owing to the fact that clay contains considerable amounts of organic materials, including many live microorganisms that could have significant effects on consumers (Eugene, 2010).

Gelfand (1945) speculated that the origins of geophagy may be based on the fertility of the earth. Thus, women ate soil before, during and after pregnancy because of the soil's fertility, with their children being encouraged to eat soil in order to ensure their future fertility. Gelfand (1945), belief in the religious and medical powers of soil may have been other early reasons why earth was consumed.

The ingested geophagic clayey soils could supplement nutrients and minerals, and serve as homeopathic remedy for common ailments (Reilly and Henry, 2000; Gomes and Silva, 2007). The consumption of geophagic clayey soils also has drawbacks which impact negatively on human health, and some of which include anaemia, microbial infections, helminthiasis, intestinal obstruction, dental abrasion and heavy metal poisoning (Geissler *et al*., 1998; Kawai *et al.*, 2009). Furthermore, some bacteria, pesticides, and some radionuclides in soil may affect human body (Simons, 1998). Besides, geophagy is an important risk factor for orally acquired nematode infections in African children (Glickman *et al.*, 1999). Though, the consumption of geophagic clayey soil is very prevalent in the developing countries, including Ghana, there is however very limited documentation of research on the mineral contents and chemical compositions of the soils which are being consumed. Whilst geophagy originated in the tropics, the practice subsequently spread to become almost a worldwide phenomenon. Reviews by (Laufer, 1930; Cooper, 1957; Anell and LagerCrantz, 1958; Halsted, 1968; and Hunter, 1973) indicate the widespread practice and diversity of deliberate soil consumption.

Review of the literature by Anell and LagerCrantz, (1958) mapped the distribution of geophagy in Africa and clearly indicate that geophagy is not limited to any particular age group, race, sex, geographic region or time period. In addition, ingestion of kaolin, also known as dirt chalk or white chalk was found to be relatively common type of pica found in Central Georgia. (Grigsby *et al.*,1999). Generally, geophagy was found to be most common in under developed countries where many people are poor and suffer from malnutrition, but it is not confined to them (Oliver, 1997).

A group of researchers also reported that geophagy in human is commonly found worldwide in several continents including Africa, South Africa, Guatemala, New Guinea, Philippines and Thailand and the Americas (Hunter, 1973; Abrahams and Parsons, 1997). Not only in the under developing countries but also in some areas of developed countries.

It has been observed that clay soils ingested by geophagic persons around the world vary in colour from whitish, creamy, greyish, yellowish, to reddish (Hunter, 1993; Woywodt and Kiss, 2002; Stoke, 2006; Ekosse *et al*., 2010). In West Africa, particularly Ghana and Togo, geophagia involves ingestion of a creamy-white loamy clayey soil locally known in Ghana as '*ayelo'* in Ga or '*shire*' in Akan and '*farin kasa'* in Hausa, specially mined and processed for local markets (Tano-Debrah and Bruce-Baiden, 2010). Some of this clay products is mined from a clay mining town called *Anfoega* in the Volta Region of Ghana. It is locally known in *Anfoega* as '*eye'* implying a dominantly white clay. The freshly mined wet semi-solid clay soil is moulded into lumps ranging from about 20g to over 200g a piece, sun dried and sold in markets for ceramic, traditional and cultural applications, and to a small extent, for ingestion.

Generally pica and geophagia prevalence in Ghana is reported to be 28% and 48%, respectively among women of reproductive age (Tayie *et al.*, 1999; Stokes, 2006).

The focus of this study was therefore to mineralogically and geochemically analyse some geophagic clayey soils from Kumasi markets in the physical state in which they are ingested, in order to determine their health effects. It is anticipated that the findings of the study could establish baseline mineralogical and chemical compositions of geophagic clayey soils in Kumasi markets and contribute to the renewed and recently rekindled research interests and intellectual debates on geophagic practice within the broader scientific community (Sheppard, 1998,Williams and Haydel, 2010; Finkelman *et al.*, 2005).

1.2 Statement of the problem

According to Fennelly (2010), people ingest earth to gain minerals lacking in their diets. This theory helps account for earth-eating among pregnant women, whose nutritional needs are greater. Fennelly, (2010) found that soils sold in Ghanaian markets to pregnant African women are richer in iron and copper than the dietary supplement pills made by pharmaceutical companies specifically for perinatal use.

Although, there is evidence of geophagy in many places around the world, the danger to humans when ingesting earth can be significant.The consumption of geophagic clays have been critised as unhygienic (contaminated with faecal matter from animals and sewage), exposing consumers to toxic constituents in the soils such as heavy metals and parasites.

There are also distinct forms of bacteria that exist naturally in the soil that can cause severe illness. Interactions between the ingested soil and the gastrointestinal fluids may result in the liberation of some of the toxic chemicals contained in the soils (Woywodt and Kiss, 2002).

One of the most immediate health threats from geophagy is intestinal blockage, a condition in which the ingested material forms a thick blockage in the digestive tract (Eugene, 2010).

The amounts of clay consumed by people ranges from one gram to three hundred grams, in one day. A very high consumption rate of 150 grams in 15 minutes was reported by Vermeer and Frate, (1979). The huge amounts consumed could lead to adverse health conditions if the elemental contents of the clay exceed the requirements of the body per day.

1.3 Objectives of the study

1.3. Primary objective

The main objective of the study was to assess the health implications of some geophagic clays on growth performance, haemato-biochemical profile and histology of female Sprague dawley rats.

1.3.2 Sub - objectives

- To determine the chemical and mineralogical components of the clay samples.
- To determine the effects of clay on biochemical parameters of rats.
- To identify any positive or negative implications of clay consumption on health.

1.4 Justification

Information from the study would be useful to medical practitioners, nutritionists and dieticians, health promoters and other interested non-governmental stakeholders. The study is expected to provide chemical and mineralogical properties of the clay, elemental composition to create awareness of the presence of heavy metals and their toxicity to the human body. The results could contribute to literature and provide information for the education of people who process, distribute and consume the clay.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Geophagy is a very complex behavior that involves a large number of academic disciplines such as soil science, anthropology, medicine, and environmental geology (Utara, 2002). Scholars from these diverse fields offered a range of hypotheses why earth is consumed yet, even after much investigation, geophagy remains an enigma for many reasons (Young, 2010)

Generally, the craving and purposeful consumption of substances that the consumer does not define as food for more than one month is regarded as pica (Hunter and de Kleine, 1984). However, the accidental consumption of a clod of earth would not be considered pica, nor would the exploratory mouthing behaviours of children. The consumption of “holy earth”, such as that from Esquipulas, Guatemala, by Catholics would also not be considered as it is motivated by religious belief, (Hunter and Kleine, 1984). Young (2011), asserts to the fact that, ingestion of tiny amounts of earth during solemn occasions such as oaths, mourning, and tests of innocence or for religious purposes also were excluded.

Pica originates from the Latin word for Magpie, a species of bird that has a habit of eating or carrying away all manner of extraneous objects Solyom *et al.*, 1991; Moore and Sear, 1994; Eastwood, 1997). Young *et al.* (2008) have described many different types of pica in literature such as ingestion of baby powder, charcoal, calcium hydroxide (lime), ash, uncooked starch and ice.

Table 2.1: Greek words for some specific forms of Pica

Greek Word	Substannce Ingested
Amylophagia	Starch
Cautopyrelophagia	Matches
Coniophagia	Dust from Ventian blind
Geomelophagia	Potatoes
Geophagia	Clay, dirt
Gooberphagia	Peanuts
Lectophagia	Lettuce
Lithophagia	Stones, pebbles. Rocks
Pagophagia	Ice
Stachtophagia	Ashes from cigarettes
Trichophagia	Hair
Xylophagia	Wood toothpicks

Source: From “Pica”, Iron Deficiency, and the Medical History.

According to Woywodt and Kiss, (2002) deliberate ingestion of soil by human beings is referred to as geophagia and has long been a source of fascination and puzzlement. Hunter and Kleine, (1984) equated the cravings for pica with those for tobacco, alcohol and recreational drugs. However, the motivation and consequences of this behavior remain unclear.

Many studies had been done about geophagy in various aspects, for example, geophagy as nutritional purposes (Abrahams, 1997) as medicinal purposes (Abraham and Parsons, 1996; Abraham, 1997), even the geophagy related to cultural or traditional beliefs also had been studied (Simon, 1998). Some of the researchers also studied the chemical and physical aspects of pica soil (Abrahams and Parsons, 1997; Mahaney *et al.*, 2000). According to

(Jones and Hanson, 1985; Kreulen, 1985; Krishnamani and Mahaney, 2000), geophagy has also been documented throughout the animal kingdom.

Earth eating continues throughout the world today (Young, 2010; 2011). Geophagists are highly selective about the earth they consume; many identify suitable earth by smelling it after it has been dampened (Geissler *et al.*,1997). Geophagists regularly expressed preferences for earth that was clay-like or smooth rather than gritty or sandy (Vermeer and Frate, 1979). Individuals sometimes went to great lengths to obtain clay-rich earth. They were willing to walk many kilometers to reach a site where a deposit of appropriate clay occurred (Forsyth and Benoit, 1989). Even among clay-rich earths, there were explicit favorites. For example, one husband who dug clay for his wife from a deposit that was closer to home and less public than her preferred site; after she tasted it, she sent him back to get the exact clay she craved (Finger, 1993).

2.2 The Three main hypothesis regarding geophagy

According to Young *et al.* (2008) geophagy is put into three major groups concerning the physiological causes of pica; such as hunger, micronutrient deficiency and protection from toxins and pathogens.

2.2.1 The hunger hypothesis

The hunger hypothesis posits that people consume non-food substances because they do not have anything else to eat (Laufer,1930; Bateson and Lebroy, 1978). This hypothesis proposes that there is no benefit to eating earth. Instead, people do so either because they have no food to eat. According to Wiley and Katz (1998), earth is supposedly consumed to ease hunger pains when no other food is available. On the contrary, David Livingstone's observations on geophagia in Africa refuted poverty as a possible explanation for the practice Woywodt and

Kiss, (2002), but observed that the practice was common amongst the slaves, the contended workers as well as the poor and that the abundance of food did not prevent it.

2.2.2 The Micronutrient Deficiency Hypothesis

The micronutrient hypothesis posits that people with micronutrient deficiencies eat non-food substances in an attempt to increase micronutrient intake of iron (Hunter, 1973), zinc (Smith and Halsted, 1970), or calcium (Wiley and Katz, 1998). According to Abrahams (1997) in humans however, investigators have largely discounted the hypothesis that geophagy is a physiological response to a need for nutrients, such as iron. Severance *et al.* (1988) and Reid (1992) stated that, the tendency to infer that anaemia elicits soil consumption is confounded by the fact that geophagy often leads to, rather than correct, iron, zinc, or potassium deficiencies.

Halsted (1968), suggested that it was an attractive theory that the earth eater was instinctively seeking some nutrient, such as a mineral, that was lacking in his diet. For example, the substance that many geophagists consume was clay which had high calcium content. This had been invoked to explain why pregnant women might crave for clay. However, no objective or controlled data were available to indicate that geophagia occurred because certain minerals were lacking in the diet. Another version of this hypothesis is that a micronutrient deficiency causes disturbed taste sensitivities or malfunctioning of appetite-regulating brain enzymes that cause non-food substances to become appealing (von Bonsdorff, 1977). In this scenario, pica is a consequence of micronutrient deficiency, but not an attempt to remedy it.

2.2.3 The protection hypothesis

The protection hypothesis states that pica is motivated by an attempt to mitigate the harmful effects of plant chemicals or microbes (Johns, 1986; Profet,1992). Under this hypothesis, there are two mechanisms by which geophagic earth may be protective:

First by reducing the permeability of the gut wall to toxins and pathogens and by binding directly to toxins and pathogens (Young, 2010). It is proposed that pica substances protect by both adsorbing pathogens and toxins within the gut lumen or by coating the surface of the intestinal endothelium, thereby rendering it less permeable to toxins and pathogens. The intestinal mucosal layer acts as a physical barrier between ingesta and the bloodstream by filtering out large molecules, as well as a chemical barrier by maintaining a pH gradient. Geophagic earth especially, if it is clay-rich, can bind with and thereby reinforce the protective mucosal layer and enhance mucosal secretion, thereby reducing permeability of the intestinal walls (Gonzalez *et al.,* 2004).

The second mechanism involves binding directly to toxins, parasites and other pathogens. This can either render them unabsorbable by the gut or inhibit their respiration (Hladik and Gueguen, 1974). According to this hypothesis, overt gastrointestinal distress, which can be the result of exposure to either toxins or pathogens (Simjee, 2007), also trigger pica.

Additionally, this hypothesis implies that pica substances would be ingested during periods of rapid growth, i.e., the times of greatest need for protection from toxins and microbes. Under this hypothesis, childhood and pregnancy, especially early pregnancy which is the critical period of organogenesis (Moore and Persaud, 1998), are the periods when pica most likely would occur (Flaxman and Sherman, 2000).

Pregnant women, who are immunologically suppressed (Formby, 1995; Fessler, 2002), also may need protection from substances that would normally be harmless. Nutrients for the foetus come from the mother’s diet, stored nutrients in the mother’s bones and tissues and synthesis of certain nutrients in the placenta. Sometimes, a mother requires vitamin or mineral supplements especially iron, calcium and folate while some women require vitamin D (Willis, 1990).

Iron is important in the body, as it is the main component of haemoglobin which carries oxygen through the body. Pregnant women should eat iron-rich foods to prevent iron deficiency.

Calcium is essential for maintaining the bone integrity of pregnant women and providing skeletal development of the foetus.

Magnesium is also an essential mineral and is needed for bone, protein and fatty acid formation, making new cells, activating B Vitamins and forming Adenosine triphosphate (ATP) (Njoki and Kiprono, 2009).

According to Ghorban (2008), geophagy during pregnancy has often been recommended as a means to increase the intake of some essential elements especially (Ca, Mg, Zn, Fe, Cu, Mn, and Se). In confirmation, Hunter and Kleine, (1984) had done the analysis of pica soil eaten by pregnant women in Central America and concluded that, geophagy in pregnancy provides a valuable supply of minerals and trace elements on an incremental, supplemental basis. The average rate of consumption of the holy clay (pica soil) tablets, supplied helpful amounts of calcium, potassium, iron, zinc, copper, nickel, manganese, cobalt, and selenium, all of which were vital to the healthy growth and development of human foetus.

A limited number of studies have tested these hypotheses, fewer have studied the health status of those practicing pica, and rarer still are studies that have correctly examined the physical, chemical, and mineralogical nature of the soils consumed.

In reviewing previous analytical work done on geophagic samples, there are a number of limitations that deserve special attention:

First, most published chemical analyses of geophagic earths are not useful for testing the nutritional hypothesis because they are confined typically to the total elemental content, without considering the extent to which these elements are biologically available (Wilson, 2003; Young *et al.*,2008). Human gut pH varies from pH 1–2 in the stomach to pH 7–8 in the small intestine, the site of the bulk of nutrient absorption. This fluctuation has major consequences for the bioavailability of elements, and must be considered when drawing conclusions about nutritional benefits.

Second, the amounts of the soils consumed need to be precisely specified; previous researchers have made calculations about intake based on amounts reported from another study, even if that study took place on a different continent, several decades in the past, or in a different age group (e.g., Hunter, 1973; Smith *et al.,* 1998).

Third, it is critical to establish the mineralogy of geophagic samples, especially in relation to the protection hypothesis; previous studies have been vague in the characterization of their mineralogy. For example, halloysite is identified specifically as the main clay mineral in the soils consumed by humans (Aufreiter *et al.,*1997) and various primates (Mahaney *et al.,*1993; Aufreiter *et al.,*2001). The distinction between different kaolin minerals could be important, as kaolinite and halloysite have different particle morphologies (flat platy vs. tubular or spheroidal), which could affect their dispersion or flocculation behaviour (Itami and Fujitani, 2005) as well as their viscosity and flow characteristics (Yuan and Murray, 1997).

These properties could be significant in affecting the ability of the clays to coat the gut wall, thereby acting as a barrier to harmful chemicals and microorganisms (Allen and Leonard, 1985) and as a stimulant to mucus secretion (Leonard *et al.,* 1994; Theodorou, *et al.,* 1994; Gonzalez *et al.,* 2004). Additionally, it may be inferred from some studies of geophagic soils that animals and humans can distinguish halloysitic from kaolinitic soils (Wilson, 2003), and knowledge of the exact mineralogy of the soil clays would provide useful information for testing this inference.

2.3 Mineralogy of Geophagic clay

In order to recognise the possible health benefits of consuming clay, it is important to understand its chemical properties and composition.Through understanding geophagy chemically, one will realise that it is "fit to eat."(Vermeer, 1971). Clays are formed by the gradual degradation of rock; this process is most commonly connected with sedimentary shales, mudstones and marine sediments in addition to various soils.

Clays have no geographic boundaries. Where there is soil, there usually exists clay. The properties of clay are dependent upon the environment in which they are found. Depending on the weather conditions, such as temperature, location, pressure, etc. and the composition, made from elements found within the clay from the surrounding environment, clays will be produced which have differing structural compositions and properties. Major clay groupings include kaolin, montmorillonite, halloysite, and allophane.

The most important property of clays is created by two of its common elements, aluminum and silicon. Due to the silica-aluminum ratio, which also takes part in determining the structure of clays, clays may absorb water and organic compounds. This is called the colloidal property of clay (Sposito, 1989). "Kaolinitic and montmorillonitic clays are those most frequently consumed by humans" (Wiley *et al.*, 1998)).

The former clay is made up of mostly aluminum and silicon, and it can only absorb organic compounds to their external surface. Conversely, montmorillonitic clays may absorb cations, water, and organic molecules into the inner layers of the clay. The absorption and structural properties of clay are the catalysts for the biological benefits of geophagy. According to

Churchman *et al.,* (1984) identifying certain minerals requires the use of supplementary treatment.

Several studies have highlighted the role of soil physicochemical properties on the chemical processes that may take place in soil. Though this role has been established in soils in their natural environment, no such studies have been made on soils in the gastrointestinal tract and yet they could have some of the answers to the reasons behind the consumption of soils or clays and the likely consequences of the practice. A survey by Mahaney and Krishnamani, (2003) revealed that most geophagic materials are not properly characterised in terms of their texture, Hydrogen ion concentration (pH), Electric conductivity (EC) and cation exchange capacity (CEC).

Studies conducted by Abrahams and Parsons, (1997) appraised three geophagical materials from the tropic countries: Thailand, Uganda, and Zaire. It was detected that Soil samples from Thailand were consumed by the female of Akha tribe in the north during pregnancy, menstruation, and after child bearing. Soil samples from Uganda and Zaire were purchased from a road side in Kampala and a market at Mudake respectively. In Uganda, soil was wrapped and advertised for its medicinal properties, for example, treatment for a variety of ailments, including coughs, stomach troubles, skin diseases, poisoning, and paralysis. In Zaire, soil was sold in a baked state and ground up before consumption could be supposedly alleviate a variety of ailment, for example, one-half teaspoonful of soil each day for 4 days was the recommended prescription for the treatment of smallpox.

They futher conducted a study on selected physical and chemical characteristics of the three soil samples. Physical characteristics were soil colour and soil texture. Chemical characteristics were hydrogen ion concentration (pH), organic matter (OM) content, and cation exchange capacity (CEC). Available nutrients were calcium, copper, iron, magnesium, manganese, and zinc were measured by atomic absorption spectrophotometry technique, potassium and sodium were measured by flame photometry technique, whereas, phosphorus was measured by visible spectrophotometry technique.

They concluded that iron was a mineral, which could be supplied to humans in significant amounts via ingested soil. They also commented that geophagy provided a direct link between the geochemistry of soil and human health (Mahaney *et al*., 2000).

According to Wilson (2003) and Young *et al.* (2008) these physicochemical properties may aid in the interpretation of physiological and nutritional reasons for the practice. Iron supplementation for example has been the most popular reason to justify geophagia among humans (Jones and Hanson, 1985; Dominy *et al*., 2004). But Reid (1992) and Severance *et al.* (1988) have shown that geophagia has in some cases resulted in, rather than correct iron (Fe), zinc (Zn), or potassium (K) deficiencies. Brouillard and Rateau, (1989) have attributed this occurrence to the cation exchange capacity (CEC) of the ingested soils, which is a physicochemical property.

The use of geophagic material for the control of diarrhoea (Mahaney *et al.*, 1996) is attributed to the surface area and water retention capacity of the soils or clays which are also physicochemical properties. These properties may dictate the inter-reactions between the ingested soil and other enzymes in the gastrointestinal tract (GIT) and consequently the ability of the soils to perform the very function for which they have been consumed (Hooda, 2004).

2.4. Geophagy: Ghanaian Perspective

In Ghana, Kaolin which is locally known as ‘Hyire’ is chalk-like substance and is very popular with pregnant and lactating mothers as well as herbalist. The chalk can be bought in ethnic shops and markets in the form of blocks, pellets and powders (i.e. a variety of sizes and differing content of minerals). There are no particular brands, batches or expiry dates. These “Hyire” samples are prepared by the native people depending on what the sample will be used for. Some of them are burnt in an oven whilst others are sun dried to have a good desired effect. Certain additives are added to the base material to enhance their potency.

It is traditionally consumed by pregnant women as a remedy of morning sickness. Pregnant and lactating women are able to satisfy the very different nutritional needs of their bodies by eating ‘hyire’.

Traditionally, herbalists or native doctors use ‘hyire’ for the cure of diarrhoea and other ailments (Vermeer, 1971). Although, most of the white clay “eye” thus sold serves geophagical purposes, some find other uses. Ewe diviners use ‘‘eye’’ in the performance of certain rituals. In addition, they rub ‘‘eye’’and other clays on exposed portions of their bodies, which practice in combination with the wearing of white cloth sets them apart from the general population. ‘Eye’ is also prescribed by witchdoctors as curative for certain illnesses, and it has become part of traditional fetish practices among the Ewe (Vemeer, 1971).

It is necessary to investigate the composition of ‘Hyire’ because it is ingested for both nutritional and medicinal reasons as any contamination might cause havoc (Vermeer, 1971).

The practice of geophagy in Ghana is predominant among the Ewe, even though it cuts across the nation. Certain sites within the Eweland environment are specially selected for the edible clays. Some of these clays are readily accepted and marketed throughout the tribal area; they are also marketed far beyond Eweland in other parts of Ghana and elsewhere in West Africa.

Additionally, geophagical clays penetrate Eweland from other source regions within Ghana, and also from more distant places such as Togo and Nigeria; these also are available in the markets of Eweland, but social constraints, cultural tradition, and probably economic factors orient the Ewe toward consumption primarily of the clays derived from their own tribal area (Vermeer, 1971).

Proof that geophagy is a firmly ensconced local behaviour is evidenced by the wide variety and volume of non-foods they mine and eat. One particular kind of clay, 'eye', mined and principally marketed and consumed in Eweland comes from the ranges of hills immediately west of the village Anfoega in the northern part of the tribal area (Vermeer, 1971). Raw clay is bought by local women at the Anfoega pithead, then processed and sold at markets to wholesalers for regional distribution.

The geophagic clays, according to Vermeer (1971), are mined from varied environmental settings. In the deltaic and coastal area of southern Eweland, sands from present beach deposits and old beach ridges are eaten. Farther inland, e.g., in the Adidome area, clay-rich B horizens of soils are mined and eaten. The most ubiquitous termitaria prove yet another very common source of edible clays; clays taken directly from the termite mounds are consumed as are mud walls of buildings constructed with such clays. All of these substances are consumed in the local areas, none entering the market system. According to Vermeer (1971), the annual production of 'eye' is estimated to be approximately 300 tons. He reported that 14 percent of Ewe men, 46 percent of Ewe women and 63 percent of the pregnant Ewe women were confirmed clay eaters, and suspected that the actual percentages may be greater still. Within Ewe culture, clay is openly purchased, openly consumed, and culturally sanctioned (Vermeer, 1971).

2.5 Quantity consumed

If hunger motivated geophagy, it would be expected that enough earth eaten would sate the appetite, i.e., to fill the geophagist's stomach. Although most reports did not quantify the amount of earth consumed, many described it qualitatively. The amount usually was small, for example: "a few morsels" (Maupetit, 1911), "size of a hazelnut" (Garnier, 1871), and "lump the size of an egg" (Whiting, 1947).

Although the incidence of geophagy is decreasing, the practice remains common in many cultures (Parry-Jones and Parry-Jones, 1992). To support this statement, Vermeer and Ferrell, 1985) cited a situation where a single Nigerian village produces 500 tons of soil yearly for consumption across West Africa. Ingestion of clay however, varies from incidental credible, ranging from 75mg per day in Amherst, USA (Stanek and Callabrese, 1995) to 650g reported in vivo in a single Gambian boy (Collinson *et al.,* 2001).

Geissler *et al.,* (1997) estimated the daily consumption to be 28g or 6 - 108g. Wiley and Katz, (1998) estimated that 30g to 50g of subsurface clay may be consumed in a day during pregnancy. Twenefour (1999), estimated that the average daily consumption of clay per respondent as 70 g ranging from 3.5 - 488 g.

Recently more rigorous biomedical studies have recorded consumption of 30 – 50g of earth per individual (Geissler *et al.*, 1977; Saathoff *et al.*, 2002; Luoba *et al.*, 2005, Young *et al.*, 2010). Abraham *et al.* (2006) reported that some Bangladeshi women may consume as much as 50 to 60g of sikor (clay) per day. According to Vermeer (2008), data from female informants in the Volta region of Ghana indicate a mean daily consumption of approximately 30g but consumption varies greatly, the range being from about a gram daily to about 300g per day. A number of women noted consumption in excess of 150g daily and such amounts do not seem unreasonably high. In Kpandu market a woman was observed eating 50g each in a space of about a quarter hour (Vermeer, 1971).

A previous study on Geophagy among school going children and pregnant women in Western Kenya by Geissler *et al,*. (1997) found out that over 70% of a sample of school going children in Nyanza province was found to consume soil at an average rate of approximately 30 mg daily.

The amounts of soils consumed need to be precisely specified; previous researchers have made calculations about intake based on amounts reported from another study, even if that study took place on a different continent, several decades in the past, or in a different age group (Hunter 1973; Smith *et al.*,1998). The amount of earth consumed varies, but quantities of 20 – 40g are typically reported. (Geissler *et al.*, 1997, and Young *et al.*, 2010).

2.6 General reasons for eating clay

The reasons given for geophagic practice ranged from simple craving to smell and texture, the soils ability to reduce the symptoms of morning sickness, hunger pangs and the belief that soils can provide some micronutrients important to health (George *et al.,* 2011).

In an oral interview with some women merchants and customers at the Kumasi Central market, it revealed that clay is consumed to appease hunger pangs, heartburns, facilitate good menstrual blood flow, good taste or smell and texture, to curb nausea, as purgative, external application on bodies of babies to make them grow plump and also for religious activities of fetish priest (Abrahams, 1997). To confirm the results of the oral interview, Twenefour (1999) posits that some clay eaters in Ghana do so because of the smell or flavour or just the feel for the clay and for some to stop diarrheoa or treat heartburns.

2.7 Cultural and Moral reasons of Geophagy

Geophagy also has cultural, traditional, and religious connotations. It suggests that it makes sense that women eat earth because of their traditional roles in some societies as potters and gardeners that bring them close to soil (Hochstein, 1968). Geissler *et al.*, (1999) adds that fecundity of the earth makes it appropriate for ingestion of soil. Gelfand, (1945) asserts that soil eating was linked to supernatural beliefs that soil has the ability to ensure fertility and a healthy pregnancy.

Traditionally 'eye' in an egg shape, in Ewe culture is ascribed to the attributes of promoting long life, health, wellbeing, and fertility. Ingestion of 'eye', therefore, imparts these qualities to the pregnant female who considers herself in a subnormal condition and prone to illness (Vermeer, 1971).

South African urban women believe that the consumption of earthy material including soils and clays enhance their beauty (Woywodt and Kiss, 2002) .

The most common reason given by males for eating clays is that it provides "pleasure" and also noted that ingestion commonly follows one principal meals of the day. (Vermeer, 1971).

In many parts of the tropics, earth eating is a widespread and open practice and is deeply ingrained in traditional culture. It is still common practice to purchase specially prepared clay tablets and other geophagic materials at the local marketplaces for consumption in rural areas of the developing world (Abrahams and Parsons, 1996).

In African societies, geophagy is a social activity that forms part of the feminine identity. It is often carried out collectively in groups of women, but hidden from men of the community (Vermeer, 1966; Abrahams and Parsons, 1996). Young *et al.*, (2008) asserted that for fear of being judged harshly geophagists conceal their behaviour.

In many parts of Central America, geophagy is clearly deeply ingrained in religious practice. The “cult” of the Black Christ has resulted in commercially traded white clay tablets available throughout Guatemala, Honduras, El Salvador, Nicaragua and Costa Rica (Hunter and Kleine, 1984). Pilgrimages to holy sites associated with the Black Christ to purchase tablets blessed by the Roman Catholic Church are made at various times throughout the year (Borhegyi, 1954).

2.8 Health Impacts of Geophagy

The best potential consequence of geophagy is the risk of ingesting soil borne infectious parasites that are concerned during pregnancy such as hookworm and Toxoplasma gonii, associated respectively with malnutrition and foetal nervous system damage (Luoba *et al.*, 2005; Abraham and Landa, 2009). Another proposed consequence of geophagy is lead poisoning, with numerous reported case studies suggesting the co-occurrence of lead poisoning and geophagy (Hackley and Katz-Jacobson, 2003; Mills, 2007). Lead exposure can lead to maternal and foetal kidney damage, encephalopathy and impaired cognitive function. Other documented health impacts include constipation, bowel obstruction, Hypokalemia, poisoning due to other toxins present in the environment and a possible exacerbation of malnutrition (Mills, 2007).

Additionally, some studies have hypothesised possible associations of maternal geophagic behaviour with negative birth outcome such as low birth weight, neural tube defects, small head circumference, premature birth, and elevated perinatal mortality; likely due to heavy metal toxicity and maternal malnutrition (Carmichael, 2003; Lopez *et al.*, 2004). According to Corbett, (2003) and Rainville, (1998) other researchers could not link specific pregnancy outcome with geophagy.

Many studies have treated geophagy as a behaviour that may provide nutrients otherwise absent in the diet (Young *et al.*, 2008). The types of soil mostly consumed tend to be high in calcium or iron (Wiley and Kartz, 1998). Studies comparing the micro-nutritional value of geophagic material and pharmaceutical supplements for pregnancy showed surprising comparability for several important nutrients including calcium, magnesium and iron (Lopez *et al.*, 2004). Although, the extent of soil absorption in the intestinal tract is unknown, it is possible that geophagists receive nutrients from the soil. Thus, there are potential benefits of geophagy that cannot be discounted and must be explored to understand the implication of the behaviour. The practice of human geophagy, particularly during pregnancy, clearly has substantial and pertinent implications for maternal and child health as well as effects on social interaction and behaviour patterns in poor, rural communities. The study of the prevalence and impacts of this behaviour is becoming increasingly important, as the growing widespread use of agrochemicals in agriculture is causing high levels of toxic chemicals in soils that may be ingested (Nicholls and Altieri, 1997). There are few good studies of geophagy. In most studies of geophagy the researchers have been out to inform the women who practice geophagy instead of being informed by the women. Another reason is that most studies have been based on western knowledge systems. Clinicians largely view geophagy as a pathological behaviour (a form of pica) calling for appropriate preventive and therapeutic intervention (Horner *et al.*, 1991). However, several studies document the medicinal value of geophagy for example, its antidiarrheal effects (Vermeer and Ferrell, 1985). Appropriate soil can detoxify toxins found as secondary compounds in plant foods by absorbing them in the gastrointestinal tract (Johns, 1990; Johns and Duquette, 1991). The other medical benefit is that some types of soil contain minerals valuable to the body. Clays consumed in most African populations have shown to contain appreciable amounts of calcium (Vermeer, 1966; Hunter, 1993).

2.9 Geophagy and Culture

Culture, environment and education affect perceptions and practices. Some of the practices accepted for pregnant women contradict bio-medical practices. By classification of western medicine as superior the lack of indigenous medical knowledge have been justified and left uncriticised. Indigenous medical practices have become silenced because they fail to be explained by western paradigms. The “silence on geophagy” among the medical personnel is also gender based. The practice is known to be in woman’s arena of knowledge. Now that clay has been proven fit to eat, it is necessary to prove that the practice is not abnormal. An analysis of why cultures and individuals eat dirt can show why geophagy is not so extraordinary. Frate (1984) declares, "Geophagy is not developed individually through biological processes but is learned". For a habit to be taught, there has to exist a motivation behind the lesson. Among several possibilities, there are primarily seven major motivations or reasons as to why people eat “dirt”.

One of the most common reasons for geophagy is religion. Many cultures believe that to eat the earth is to transfer the fertility and fruitfulness of the earth to their body. They consider clay a blessing from within almost a sort of edible prayer. In Eweland, Ghana, for example, kaolinic clay is called "the eye" and is prepared in the shape of an egg, which symbolises longevity, good health, and well being in Ewe culture (Vermeer, 1971). The egg enters the body and blesses the woman, making her as bountiful and fertile as the earth

In Guatemala, the holy clay tablets of Esquipulas show another combination of religion and geophagy. These tablets have been formed from the springs and quarries surrounding the Esquipula shrine before the arrival of Jesus Christ. "No stage of the process-mining, making, buying, blessing, or eating the holy clay tablets is unattended by “faith" (Whole Earth, 1999). Similar type of clay is sold in another part of Central America. These cakes are white and are called tierra santa. These clay items are obviously symbolically chosen and named by colour-the whiteness symbolising purity that cleanses the soul. The tierra santa is then eaten and celebrated in connection with the Black Christ Festival (Hunter, 1971).

Geophagy makes its debut at weddings as well. In India, detailed clay figurines are given as bridal gifts. As in Ewe culture, these figurines are broken and eaten during pregnancy (Ashenbach, 1987). Perhaps, too, the religious significance of eating dirt may be seen when one reflects upon Christian beliefs. When Christ died on the cross, some of his blood was shed onto the ground. Although this happened centuries ago, the symbolic value of being a part of Christ and his sufferings through eating the clay upon which His blood once flowed may still exist. Furthermore, clay is connected to Christianity through the creation of man himself and the Lord God formed man of the dust of the earth. And breathed into his nostrils the breath of life (Gen.2.7). Hence, through eating dust or clay, that from which he was created, man is able to connect with God and the life living elements which produced him.

Geophagy formerly believed to be common among communities of low social status (Halsted, 1968) but recent studies have indicated that pregnant females in affluent societies indulge in geophagic practice. It is most common in many indigenous communities especially in developing countries (Ngozi, 2008); and has also been linked to superstition (Halsted, 1968).

In Southern Africa, women and children in both urban and rural settings are engaged in geophagia. Children are more vulnerable to this habit at infancy when hand to mouth activity is high and is considered normal at that age (Abrams *et al.*, 2009).

In some parts of the world, geophagia is culturally sanctioned practice. In many parts of the developing world, earth intended for consumption is available for purchase. In Africa there are different traditions on what is good to eat during pregnancy and lactation period. (Vermeer, 1971).

These belief systems are supposed to protect the mother and the unborn baby during this period. The woman's pregnant body is seen to extend boundaries during this period. The body grows encompassing individual and social sphere. The woman is given more freedom to publicly display her emotions and needs during pregnancy (Vermeer, 1971).

In some cultures especially in productive areas like the Kilimanjaro region in Tanzania the women who have given birth get privileges of an adjusted diet to help to recover after pregnancy. They also get a culturally defined period of recovery after giving birth where they rest their body from physical work and also from marital sexual obligation. (Ekosse *et al*.,(2010)

2.10 Geophagic Clay Site Selection and Health

Geophagists typically select subsoils that are less likely to contain geohelminth eggs than earth closer to the surface, where defaecation occurs. Geophagists carefully prepare the earth they eat. Geophagic soils were heated, sifted, dried, or brushed off prior to consumption, rather than being excavated and immediately consumed. Indeed, in Indonesia, Ghana, India and Guatemala, an industry developed around geophagy that involved excavators, traders, and vendors (Vermeer, 1971)

In other places, clay preparation is handled on an individual basis, either by consumer or someone else in the household. Whether on a large or small scale, preparation of geophagic earth involves removing the impurities by crushing the earth and then sifting out sand and small stones, picking off the out crust of earth, or sometimes sieving it through cloth and baking, frying, sun drying, or smoking the earth. These preparation practices likely kill most endoparasites and other pathogens (Young *et al.,* 2007).

According to Glickman *et al.* (1999) geophagic earth should not be a vector for the transmission of parasites and other pathogens. Even though, parasitic, especially the geohelminths have sometimes been attributed to geophagy.

There is little evidence to support the transmission of hookworm by geophagy (Heymann, 2004) especially since hookworms are spread transdermally. There is conflicting evidence about whether geophagy might be a mechanism of transmission of whipworms (Trichuris) or round worms (Ascaris). None of the prepared geophagic earths from Tanzania sampled by Young *et al.*, (2007) contained live geohelminths, but in two other studies (Wong *et al.*, 1991., Geissler *et al.*, 1998) viable helminths were discovered in geophagic soils. This difference may be explained by the latter being conducted on soils eaten by children, who may have been more careless than adults about preparing the soil before consuming it (Young *et al.*, 2007).

2.11 Forms of clay Consumed

Broken bits of pottery, chunks from the earthen-walled houses, and clumps of earth found among dry beams (Young *et al.*, 2010). The eaten soils were mainly from termite mounds, clay from roadsides, yellowish soft stone from the bottoms of dried streams, and even classroom chalks, weathered stones and walls of huts were the commonly reported items ingested by geophagists (Geissler *et al.*, 1997).

2.12 The public heath importance of geophagy (pica)

2.12.1 Benefit

Clay is a well established treatment of gastrointestinal distress e.g. kaopectate. A widely used over the counter treatment for nausea, diarrhoea and vomiting, takes its name from kaolin, the clay that was formerly active ingredient (Ofoefule and Okonta, 1999).

According to Wilson (2003), most pica items are more alkaline than gastric pH and would thus function to reduce the gut acidity responsible for dyspepsia. Furthermore, the increase in pica during pregnancy is consistent with the increase in dyspepsia concomitant with many pregnancies. Frankel (1977), asserted that many geophagists attribute their pica to the reduction of heartburns or nausea.

The potential positive health effects of pica include providing micronutrients, soothing gastrointestinal distress and preventing harmful chemicals or pathogens from entering the blood stream. The ingestion of soil may help prevent asthma and aid in the development of a healthy immune system (Weiss, 2002; Callahan, 2003). Clays which are rich in aluminum compounds are considered to pass as immunologic adjuvants which might act as vaccines (Gupta, 1998).

According to Krishnamani and Mahaney (2002), monkeys that regularly eat soil have been noted to have lower parasite loads. Hunter (1993), estimated that in populations of dietary deficiencies of minerals consumption of clay may help to meet the Recommended Dietary Allowance (RDA) for some essential mineral elements. According to Grey (1841), there is an association of geophagy with exposure to toxins. For example, people in Northern Territory of Australia explained that they ate clay to "line the stomach" before eating fish they knew to be poisonous.

Chromium (Cr) is implicated in maintenance of blood sugar, prevention of atherosclerosis and control of cholesterols levels. Human studies suggest that a particular form of chromium known as Chromium picolinate, enhances insulin sensitivity, glucose removal and may improve lipid ratios in obese and type 2 diabetics. Trivalent compounds (Cr III) do not cause any serious damage to body tissue, in fact, it is an important component of a balanced human and animal diet and its deficiency is detrimental to the glucose and lipid metabolism in mammals. Cobalt is essential to humans as well as to animals (Cefalu *et al*., 2002).

2.12.2 Detrimental health conditions

The role of pica in other detrimental health conditions as explored by other investigators revealed its negative causal role as iron deficiency anaemia (IDA) and other micronutrient imbalances. Some of the detrimental health conditions include heavy metal poisonings especially lead (Shannon, 2003; Abraham *et al.*, 2006), alimentary canal damage (Ekosse *et al.*, 2010) and gestational weight gain (Ward and Kutner, 1999). Reports of lead poisoning and other toxicities in children eating contaminated soils are also available (Callaham, 2003).

Geophagia is a risk factor for the transmission of parasitic nematodes, namely *Ascaris lumbricoides* (roundworms) *Trichuris trichuria* (whipworm) *Toxocaraspp,* (hookworm) (Rogers, 1972; Bateson and Lebroy, 1978).

Soils or clays harbour billions of microbes some of which may be pathogenic and could cause disease if ingested. In addition, they may also contain toxic chemical substances which if ingested could pose a threat to human health. Hunter (1973), asserted that clay can also block the intestines, a condition that can be fatal; certain soils can combine with minerals in the diet in such a way that the minerals cannot be absorbed by the body.

Again, iron deficiencies can then cause anaemia for example zinc deficiency can produce growth retardation. There is also a geophagic syndrome marked by growth retardation, delayed maturity and liver and spleen enlargement.

It is well established that metal ions are required for many critical functions in humans. Four main groups (Na, K, Mg, and Ca) and ten transitional metals (V, Cr, Mn, Fe, Co, Cu, Zn, Ni, Mo, and Cd) are currently known or thought to be required for normal biological functions in humans.

Deficiency of some metal ions can lead to disease. Well known examples include pernicious anaemia resulting from iron deficiency, growth retardation from insufficient Zinc, and heart disease in infants due to copper deficiency. But at the same time, metal ions can also induce toxicity in humans, classical example being toxic metal poisons from mercury and lead. Even essential metal ions can be toxic when taken in excess. Geophaic persons ingest both the beneficial and the toxic elements into their bodies at the same time. Quartz particles through mastication are able to grind, crack and break dental enamel (King *et al.*, 1999). Another malignant effect of quartz particles in geophagic clays is their ability to erode gastro-intestinal lining of geophagic individuals with the possibility of perforating the Sigmoid colon (Woywodt and Kiss, 1999).

2.13 Dose- Response of chemical elements

"Poison is in everything, and nothing is without poison. The dosage makes it either a poison or a remedy". According to Montgomery (1986), in the dose response curve the positive or negative effects of trace elements are plotted as a function of the dosage. For example:

- Lead and Mercury have no beneficial or harmful effect in low concentration but will be toxic in high concentration and fatal in very high doses.
- The element calcium is needed to avoid deficiency. Benefits only increase up to a point after which no particular benefit or harm is derived from consuming higher levels. Calcium is hard to overdose.
- Copper and Molybdenum in small amounts is necessary for optimum health but too much can be toxic or even lethal/ fatal.

2.13.1 Recommended Dietary Allowances for some determined Elements.

Elemental quantification of the samples using XRF show evidently that clays selected for this study could be sources of both essential (Fe, Mn, Cr and Zn) and toxic metals, Pb and As. The various roles these elements play regard human health are discussed as follows;

Iron (Fe) metal plays a vital role as an essential component of haemoglobin for human beings. It facilitates the oxidation of carbohydrates, protein and fat to control body weight, which is a very important factor in diabetes. The dietary limit of it in food is 10 to 60 mg/day (Kaplan *et al*., 1993).

Recommended daily requirement for elements such as Fe was 8mg by WHO/FAO (WHO, 1992). However, The U S food and Drug Adminstration defines an intake up to 45mg of iron per day as safe but severe overdose occurs when the amout is 50-100 times more than the RDA (Sharon, 2014).

Low amount of iron causes gastrointestinal infection, nose bleeding and myocardial infarction (Hunt, 1994). In other words, iron is very important in many physiological processes such as formation and transportation of haemoglobin (Kaplan *et al.*, 1996). Iron deficiency is the most common cause of anaemia worldwide (Brand *et al.*, 2009; Ovon Garnier and Stunitz e tal., 2008).

One of the reasons for iron deficiency anaemia is the result of an imbalance between the absorption of iron and an excess loss of iron because of bleeding. This phenomenon occurs more often in women of child bearing age due to menstrual blood loss and pregnancy. Functional iron in the human body is in the form of haem iron, which is found in haemoglobin and it is responsible for the transportation of oxygen. Storage iron is present in macrophages as haemosiderin and ferritin; both forms are available for the production of newly formed erythroblasts. Another source of iron is present in muscle as myoglobin and most cells in the body have iron containing enzymes (Hoffbrand *et al*., 2006).

In a healthy individual, iron is recycled, therefore the actual loss of iron is 1mg per day through the excretion of urine, faeces, nails, hair and skin; and absorption from a well-balanced diet is also 1mg. When senile red cells are broken down the released haem iron binds with transferrin for transportation to macrophages for erythroblasts production in the bone marrow (Hoffbrand *et al*., 2006).

Iron toxicity results when the amount of circulating iron exceeds the amount of transferrin available to bind it, but the body is able to vigorously regulate its iron uptake. Thus, iron toxicity from ingestion is usually the result of extraordinary circumstances like iron tablet over-consumption (Andrews, 1999). The human body has a limited capacity to remove excessive iron; body iron normally eliminated through the stool, urine and exfloliation of epidermal cells (Kaufmann, 1992).

Vanadium is naturally occurring element. It is widely distributed in the earth's crust at an average concentration of approximately 100 mg/kg. Vanadium is found in 65 different minerals. Vanadium is a micronutrient that the body needs but in low doses. High doses of vanadium have been tested as aid to controlling blood sugar levels in diabetic people like chromium a trace mineral. The exact dosage for vanadium is not known but estimate range from 10mcg - 30mcg daily (Harland and Harden-Williams, 1994). In general, vanadium is non toxic after oral administration because of its poor absorption from the gastrointestinal tract, but it may become toxic after parenteral administration.

Chronium compounds are found in the environment due to erosion of chromium-containing rocks and can be distributed by volcanic eruptions. The concentrations range in soils is between 1 and 300 mg/kg (Kotas, 2000).The daily intake of Chromium has been recommended for adults by US National Academy of Sciences to be 50 to 200 mcg (Watson, 1993). In a similar vein, the National Academy of Sciences has established a safe and adequate daily intake for Cr (III) in adults of 50 - 200 mcg/day. On the average, adults in the United States take in an estimated 60-80 micrograms of Cr (III) per day in food.

The health effects of chromium are primarily related to the valence state of the metal at the time of exposure. Trivalent Cr (III) and hexavalent Cr (VI) compounds are thought to be the most biologically significant. The toxic action of chromium is confined to the hexavalent compound Cr (VI), which is a highly toxic and carcinogenic and generally is considered 1,000 times more toxic than Cr (III) and may cause death to humans and animals if ingested in large doses (Cr III) is an essential dietary mineral in low doses (Dayan and Paine, 2001). The toxic effects of chromium (Cr) intake is skin rash, nose irritations, bleeding, stomach upset, kidney and liver damage, nasal itch and lungs cancer; chromium deficiency is characterized by disturbance in glucose, lipids and protein metabolism (McGrath and Smith, 1990).

Health experts suggest that, the daily B_{12} RDA is 2.4 mcg for adults and adolescences. Although it is essential to have sufficient amount of cobalt intake, it can be a disadvantage when this mineral is taken in excessively high levels. The total daily intake of cobalt is variable and may be as much as 1 mg (Alloway and Ayres, 1993). Having high amount of cobalt can decrease the fertility in men and affect the heart. When taken in a long periods of time, cobalt can cause over-production of red blood cells, and damage the heart muscles as well as the thyroid gland (WHO, 1995).

According to Montgomery, (1996), aluminum; even without considering pharmaceutical sources, the typical daily dietary intake of aluminum varies widely, from 3 to 100 mg (Callahan, 2003; Mahan, 2007; and Willhite, 2012). The normal dietary intake of aluminium was also reported to be between 3-20 mg/day of which only about 15 mcg is usually absorbed and then eliminated through the kidneys (Willwhite, 2012).

Significant sources of aluminum include baked goods prepared with chemical leavening agents (i.e.baking powder), processed cheese, grains, vegetables, herbs and tea. Aluminum toxicity apparently is not a concern for healthy individuals. Cooking foods in aluminum cookware does not lead to detrimental intakes of aluminum. Ingestion of high dietary aluminum most likely does not cause Alzheimer's disease Encarta, (2009) but may exacerbate this disease. Moreover, high intakes of aluminum through such sources as buffered analgesics and antacids by susceptible individuals (i.e., those with impaired kidney function including the elderly and low-birth-weight infants) may lead to pathological changes.

Silica is necessary for collagen formation and development and has become the basis for these physiological effects. Collagen is the tough fibrous material that holds us together. Many aging problems are a direct results of body's inability to maintain adequate collagen. As we age silica levels decline and without adequate tissue levels of silica, we manifest symptons of ageing such as joint disease, weakened digestion and wrinked skin etc.

An average person ingest 20 - 60 mg of silica per day in their diets. Results from people who supplement with silica at 375 mg per day supports silica effectiveness and reinforce the fact that 20 – 60mg per day is not adequate. (Lemmo, 1998).

Magnesium ions regulate over 300 biochemical reactions in the body through their role as enzyme co-factors. They also play a vital role in the reactions that generate and use ATP, the fundamental unit of energy within the body's cells. Magnesium is a macro-mineral, which, unlike trace minerals, is needed by the body in large amounts. Calcium, sodium, and potassium are also macro-minerals. WHO/FAO's maximum level of magnesium is 420mg. Magnesium has a daily requirement of 300 – 400 mg/day (Judy, 2003).

The estimated safe and adequate intake for copper is 1.5 - 3.0 mg/day. Excessive ingestion of Cu could lead to severe mucosal irritation and corrosion; capillary, hepatic and renal damages; and gastrointestinal and neural disturbances (WHO, 1993). Cases of copper toxicity are rare but may occur. Intake of supplements exceeding 3mg copper/day for a protracted period of time may be cause for concern (Judy, 2003).

Zinc concentration in the soil depends largely on the parent marerial. In general zinc is found associated with sulfide of other metals, including lead, copper, cadmium and iron (McDowell, 1992). The common range of zinc total concentration in normal soil is between 10 and 300 mg/kg (Hassett and Banwart, 1992). Zinc (Zn) is an essential trace element which plays an important role in various cell processes, including normal growth, brain development, behavioural response, bone formation and wound healing (Hunt, 1994). Zinc is generally considered to be non-toxic, but can cause vomiting, dehydration, electrolyte imbalance, abdominal pain, nausea, dizziness, lack of muscular coordination, and renal failure (WHO, 1993). Although it may be essential for human health, an intake of 5mg/day is considered toxic, and may lead to gastrointestinal disturbances, icteric discoloration nausea and dermatitis (Alloway and Ayres, 1993) confirms finding by WHO, 1993 but nine years afterwards, researchers as Hooda *et al*. (2002) reported the recommended daily allowance (RDA) of Zn to be 10mg. Judy, (2003) a year later Judy reported the maximum recommended daily requirement of zinc to be 15 mg/kg. This depicts how science is dynamic. Based on the maximum daily reguirement by Judy (2003), Zinc concentrations the in the clays was within the RDA range and hence did not pose any health threat.

WHO (1993) reported that Cadmium has long biological half-life in the body and accumulate with age. High levels above 60 mcg/day is known to affect renal, skeletal and respiratory systems and causes itai-itai disease.

Nickel compounds released to the environment will adsorb to sediments or soil particles and become immobile as a result. Nickle uptake will be high when people eat large quantities of vegetables from polluted soils. Humans may be exposed to nickel by breathing air, drinking water and eating food. High nickel concentrations on sandy soils can damage plants. For humans, high uptake of nickel will result in respiratory failure, prostrate cancer, lung cancer, nose cancer (Lenntech, 2001). Nickel can be carcinogenic, and may cause dermatitis, eczema, vertigo and dyspnea to exposed human population (WHO, 1993).

According to Montgomery (1986), the dose responses indicate that calcium is needed to avoid deficiency. Benefits only increase to a point after which no particular benefit or harm is derived from consuming higher levels. Calcium is hard to overdose. However, the maximum dose requirement is 1000 -1500 mg/kg (Kaplan *et al.*, 1996).

Manganese is a humble trace element that is found in very small quantities in the human body, but it plays a large role in body processes. According to the University of Maryland Medical Center (2007), it is used in such diverse mechanisms as the creation of connective tissue, bone creation, blood-clotting factor creation, sex hormone creation, fat metabolism, calcium absorption and blood sugar regulation. It is also a component in a specific type of antioxidant known as superoxide dismutase. This enzyme has been studied for its role in the fight against aging, heart disease and cancer.

The adequate intake level for manganese for adults is 2.3 mg per day for men, 1.8 mg per day for women, 2 mg per day for pregnant women and 2.6 mg per day for breastfeeding women. Manganese is an essential nutrient, and eating a small amount of it each day is important to stay healthy. Manganese is present in many foods, including grains and cereals, and is found in high concentrations in many foods, such as tea. The amount of manganese in typical western diets is (about 1–10 mg manganese per day) appears to be enough to meet daily needs. Human diets with too little manganese can lead to slowed blood clotting, skin problems, changes in hair color, lowered cholesterol levels, and other alterations in metabolism. In animals, eating too little manganese can interfere with normal growth, bone formation, and reproduction (ATSDR, 2008).

Potassium is both a mineral and an electrolyte, which means it can conduct electrical impulses in the body. Another common electrolyte is sodium. Sodium and potassium work together in the body via a process known as the "sodium-potassium pump." This exchange of sodium and potassium in human cells ushers in ATP, which is an energy source for the body. The body relies on a balance of potassium to keep it functioning normally. Too little or excess potassium can cause health conditions that affect the heart and brain. Because the body requires balanced potassium levels to perform, an excess or deficiency can cause symptoms. Potassium deficiency, known as hypokalemia, can cause the heart to beat irregularly, constipation, fatigue, muscle damage, muscle weakness and muscle paralysis. Excess potassium, called hyperkalemia, can also affect human heart, causing heart beat to slow. It also leads to muscle weakness and tingling in hands and feet. According to Pamploma-Rogers, (2008) the recommended amount of potassium for most adults is 4,700 mg. Athletes, might require more. Fortunately, potassium is easily obtainable through a variety of dietary sources.

Sodium helps regulate the amount of water in the cells and the overall fluid balance in the body. As blood volume increases, the kidneys increase the excretion of sodium, which reduces fluid levels. The electrolyte assists with maintaining normal blood pressure.

The body needs small amounts of dietary sodium to work properly. Many processed foods contain sodium which the body only needs 200 mg of the electrolyte each day.The average American eats approximately 3,400 mg of sodium each day (Dietary Guidelines for Americans, 2010). Hyponatremia occurs when sodium levels are low. Low-sodium diets, increased sweating, vomiting, diarrhoea, kidney disease, medications, increased age and heart failure can all cause decreased sodium levels. A rapid decrease in sodium causes severe symptoms and can be fatal. As sodium levels drop, symptoms such as headache, nausea, restlessness, fatigue, confusion and a decreased level of consciousness can occur which eventually develops to coma or death. Treatments for minor hyponatremia include altering the amount of salt a person eats and the amount of water she drinks. More severe hyponatremia may require medications and intravenous fluid (MFMER,2011).

On the other hand, hypernatremia is having too much sodium in the blood which is typically a result of dehydration. Conditions such as vomiting, diarrhea, kidney dysfunction and the use of diuretics can cause dehydration. The main symptom of hypernatremia is severe thirst, because the body tries to dilute the sodium concentration. Without treatment, confusion, seizures and death may occur (Adams, 2011).

Molybdenum is an essential trace element for human, animals and plant health. Molybdenum in human is to act as a catalysts for enzymes and help to facilitate the breaking down of certain amino acids in the body. The Recommended Dietary allowances generally is 45mcg/day. However, pregnant women can take up 50 mcg/day. Typical American diet contains molybdenum levels well above the RDA up to 1,500mcg which could lead to exessecive excretion of copper but research shows no negative effect of excessive molybdenum intake (National Academy of Science,1989).

Rubidium is a minor element required in small doses. It is relatively non toxic element and has not shown toxicological concern from the nutritional point of view. Rubidium deficiency apparently depresses growth and life expectancy in growths. No specific side effects are found associated with rubidium but in rare cases. Side effects like blistering skin, discloration, dryness are observed. The daily recommended requirement of rubidium is 1 to 5mg. (Elson, 1984).

Barium in nature occurs in many different forms of compound. The two forms of barium compounds are barium sulfate and barium carbonate, which are often found in nature as underground ore deposits (ATSDR, 2007). Barium has no biological role in human health (CDCP, 2003) but barium chloride ($Bacl_{2.}$ $2H_2O$) has stimulant action on heart and other muscles and raises blood pressure. The daily RDA of Barium is 0.02 mg (CDCP, 2003).

Lead (Pb) occurs naturally in the environment and has many industrial uses. Everyone is exposed to low levels of lead through food, drinking water, air, household dust, soil, and some consumer products. However, exposure to even small amounts of lead may be harmful to your health. Lead induces various toxic effects in humans at low doses than previously thought with typical symptoms, such as colic, anaemia, headache, convulsions and chronic nephritis of the kidneys, brain damage and central nervous system disorders. WHO (1998) prescribed maximum limit of lead to be 0.01ppm In a study by Samali *et al,.* (2012) on herbal tea found the maximum limit as 10 mcg/g, while the dietary intake limit of it per week as 3 mg/week.

Exposure to lead may have subtle effects on the intellectual development of infants and children. Infants and toddlers are particularly vulnerable to the harmful effects of lead because they are undergoing a period of rapid development; furthermore, their growing bodies absorb lead more easily and excrete lead less efficiently than adults. In addition, infants and young children are more likely to ingest lead because of their natural habit of putting objects into their mouths. (Young *et al.*, 2011).

Arsenic occurs naturally in soils as a result of the weathering of the parent rock (O'Neill, 1995). Although it occurs in igneous rocks, the greatest concentrations tend to be found in argillaceous sedimentary rocks (e.g. shales and mudstones) and in heavily sulphidic mineralised areas (O'Neill, 1995; Kabata-Pendias and Mukherjee, 2007). Arsenic is classified by U.S. Environmental Protection Agency (EPA) and other organizations as carcinogen (Maryland Department of the Environment, Health and Mental Hygiene (2007) . The EPA has set a limit of 0.01 parts per million (ppm) for arsenic in drinking water. Levels of arsenic in soil from 5 ppm up to 20 ppm are generally viewed as safe, even if contact with arsenic at these levels continues for many years. If the level of arsenic in soil is within this range, the potential for any health effect is very small (Environmental Health Information, 2007). Long term exposure to arsenic has been shown to increase the risk of cancer of the skin, lungs, urinary bladder and possibly kidney, liver and prostrate. However, the liver has the ability to convert absorbed arsenic to less hazardous forms and the kidneys then remove it in the urine (Vermeer, 1971).

2.14. Bioavailability of geophagic elements

Bioavailability may be defined as the proportion of an ingested nutrient that is absorbed and either utilised in a metabolic pathway or sequestered in body stores (Benito and Miller, 1998). In other words bioavailability is the fraction of the chemical that can be absorbed by the body through the gastrointestinal system, the pulmonary system and the skin (DEFRA and Environment Agency, 2002c). It can be expressed as the ratio of an absorbed dose to an administered dose:

$$Bioavailability\ = \frac{Absorbed\ dosed}{Administed\ dosed} \times 100$$

The majority of the analyses of pica materials measure total mineral concentrations but do not attempt to assess bioavailability. This approach almost certainly over-estimates the content of bioavailable nutrients and is analogous to an agronomist assessing the ability of a soil to grow crops on the basis of the total nutrient mineral content of the soil without considering what is available for uptake by the crop (Wilson, 2003). Soils with high cation exchange capacity (largely negative surfaces) can bind and adsorb nutrient cations to their surfaces, decreasing their availability for absorption into the blood stream (Hooda *et al*., 2004). In simulated human digestion, Hooda *et al*., (2004) also reported that decrease absorption of zinc, iron and copper in solution due to the presence of soil. As food enters the stomach, the pH of the stomach contents (digesta) rises than the pH of the ingested food. This stimulates acid secretion, causing the pH of the digesta to gradually decline. As the pH decreases, many nutrients, e.g. Fe, become more soluble. This increase in solubility favours subsequent absorption in the small intestine. The absorption of iron and possibly other nutrients is impaired in people with low gastric acid secretion (Schubert, 2007) presumably because the release of nutrients from foods into soluble forms is reduced. Solubility in the duodenum is a key factor affecting iron absorption. Because iron is much more soluble at low pH than at neutral pH, it follows that iron absorption will be impaired by consumption of substances that may buffer stomach acid and thereby prevent the pH from going as low as it otherwise would. For example, calcium carbonate, a widely used antacid, depresses iron absorption in rats. For these reasons, it is important to measure the pH and buffering capacity of pica substances (Prather and Miller, 1992).

In a study, Geisseler *et al.* (1998) determined bioavailability of iron and zinc in clay extraction by the simulation condition of the human tract using the equivalent pH of the stomach and the temperature as maintained at a constant of 37°C body temperature.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

Clay Samples: Samples of dry clay lumps (non-salted) were obtained from three different local markets within the Kumasi Metropolis in the Ashanti Region of Ghana, namely Central market, Asafo and Fante Newtown markets. These samples have been reported to come from a single source Anfoega, a town in the Volta Region of Ghana popularly known for mining clay soil as a major commercial activity.

3.2 Method

A total of 100 pieces (lumps) weighing between 25 to 35g were purchased from different wholesalers and retailers at each market into sterile polythene bags. The bags were carefully sealed and then transported to the laboratory for processing into fine powder suitable for laboratory analysis.

Samples from the markets were first sun dried by spreading them evenly on clean sheets of polythene materials for three days and kept in a dry and dust free place at a room temperature to extract moisture and avoid extraneous contamination. There was no pre-treatment other than sun drying and simple crushing and grinding of the geophagic samples into powder using crucible mortar and pestle and sieved with a 180 micron pore size filter under hygienic laboratory condition to obtain the desired powder. The lumps of clay were wholly ground to obtain homogenised particle sizes of 180 microns. The method of coning and quartering as explained by Radojevic and Bashkin, (1999) was used to obtain representative samples. For example, the powdered sample was mixed thoroughly for about 15minutes in a 1500 ml beaker with a spatula and later poured the sample onto a clean plastic tray in a cone shape.

The cone was flattened and formed a circular layer of soil. This was then divided into four quarters using a clean spatula. Samples were taken from the two opposite quarters and the remaining clay discarded. The two retained parts were heaped into a cone and quartered repeatedly until six soil samples of 70g, were obtained and suitable for chemical and mineralogical analysis.

3.2 Analytical Methods

3.2.1 XRF Analysis

Samples of clay labeled 1,2,3,4, and 5 were sent to Geological Survey Department for X-ray fluorescence (XRF) analysis. Samples were measured into sub samples of 4g and 0.9g of wax was added to the sub sample. The sub sample of 4.9g was suitable specimen for laboratory analysis.

After obtaining the suitable laboratory sample, it was then poured into a laboratory cup where small plastic balls were added. The sample was put into a homogenizer and vibrated at a frequency of 15 Hz for 3minutes. After the vibration for homogenization, the small balls were removed from the sample cups before being transferred into the compressor to compress sample into a pellet now suitable for the XRF waves to pass through to aid in elemental detection.

The pellets were fixed into cups in the XRF chamber and closed to avoid the spread of waves. The XRF was connected to a PC where the elements were detected based on the energy levels of the waves which appeared in peaks. The wax added to the clay had binding ability hence it binds clay into pellets. Besides, the balls were used to homogenise the loose clay particles whilst liquid nitrogen which is a cooling agent was fixed into a chamber in the XRF machine to cool down its temperature. The analysis was done to detect major and minor elements in the clay samples.

3.2.2 Mineralogical analysis

X-ray diffraction analysis was done using X'pert High Score Philips Analytical made by Technical Corporation of Germanay. At theta/^{0}2A500 and 40kw, 40mA, the material was irradiated and the various peaks were shown graphically. However, relative intensities were used against the positions of the angle at (^{0}2 theta) to identify the various minerals present in the material**.**

The mineralogical composition was investigated to determine the various mineral phases in the clay samples

3.2.3 Biochemical analysis

3.2.3.1 Procedure

Twenty-four female rats were used for the experiment. They were purchased from Small Animals Unit of the Department of Animal Science (KNUST) and kept in the animal house in the Pharmacology Department of KNUST. The temperature of the room was maintained at 37^{o}C with adequate lighting cycle for 24 hours.

The animals were cared for according to the international regulations governing the use and care of laboratory animals. They were housed in metal cages and maintained on standard feeds from Gafco at Tema, Ghana. Drinking water was allowed *ad libitum*.The animals were allowed to acclimatise for two days. Each animal was weighed prior to commencement of the experiment and on the 28th day of treatment to determine their growth.

At the pharmacology laboratory, the animals were divided into 4 groups of six animals each. Group 1 served as the control group. Groups 2, 3, and 4 were the test or experimental groups.

Powdered clay sample was prepared in stock solution of 40g of powder dissolved in1000ml of distilled water into suspension and was stirred continuously prior to administration. Each millitre of suspension contained 40mg/ml. The clay was administered as a suspension in varied doses per body weights as follows; 0.2ml to Group 2 , 0.6ml to Group 3 and 1.0ml to Group 4 orally via gavage (using 1ml syringe) once daily to the rats.The Control Group 1 was fed only on feed and distilled water *ad libitum*.The doses were calculated based on the average weight of the rats. This was done for a period of 28 days.

The animals were humanely sacrificed under chloroform anesthesia and blood samples were drawn from each rat after severing the jugular vein with sterile laboratory blades.Two millilitres (2ml) blood samples were collected into Ethylenediamine tetraacetic acid (EDTA) tubes and sent to Kwame Nkrumah University of Science and Technology (KNUST) hospital for full blood count and biochemical analysis. Some dissected organs like the liver, kidney, stomach and uterus were preserved in 10% formalin and sent to Komfo Anokye Teaching Hospital (KATH) for histology analysis to determine the effects of clay in the internal organs

3.2.3.2 Weekly body weight

The body weight of rats was assessed using a sensitive balance during the acclimatisation period before commencement of dosing and on the day of sacrifice.

3.2.4. Relative organ weight

Some important organs namely, liver, kidney, stomach, hearts, lungs, pleen, ovaries, and uterus were carefully dissected out and weighed in grams (absolute organ weight). The relative organ weight of each animal was then calculated as follows**:**

$$Relative\ organ\ weight = \frac{Absolute\ organ\ weight\ (g)}{Absolute\ body\ weight\ (g)} \times 100$$

3.2.5 Statistical analysis

Data for the biochemical assays were expressed as mean± standard error of mean (SEM). Statistical analysis was performed by one way ANOVA using Neuman Keuls post hoc test to ascertain differences between treatment groups. All analysis was performed using GraphPad Instat 3 (version 1.1, 2007).

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Introduction

This chapter shows the results and discussions on chemical, mineralogical and biochemical properties of geophagic clay samples under investigation.

The results are presented in two sections, A and B. Section "A" represents geological results and Section "B" represents bio-chemical results. The geological results include those generated from XRF and XRD whilst biochemical results were analysed using graphpad.(software)

4. 2 Chemical and mineralogical composition of the clays.

The chemical and mineralogical composition of clay samples are presented in tables 4.1 and 4.2 respectively.

4.2. 1 Major Oxides

Tables 4.1 and 4.2 show major and minor elemental composition of the clays. The chemical composition of the clays varied. The dominant oxides consisting of SiO_2, Al_2O_3, and sum more than 70% Wt. iron and potassium

Twenty five (25) minor elements were determined and presented in Table 4. 2. The dominant elements detected were V, Cr, Co, Rb, Zr, and Ba. Minor elements in the table vary in concentrations. Some of the elements detected are toxic when ingested above acceptable levels however, lead (Pb) eaten in smaller quantities would accumulate and cause defects to the organs (WHO, 1995). According to Ekossee, (2010) not all minor elements detected are of biological significance from either a nutritional or toxicity point of view. Among the elements, heavy metals such as Cr, As, Cd, Mn, Zn, and Ni may be toxic when ingested above the recommended dietary level.

The results in Table 4.1 indicate that, the five geophagic samples contained more silica ranging from 56% to 59% higher than any other oxide.

Table 4.1 Major oxide present in the clay

Major elements as oxides (wt%)	**Sample Numbers**				
	1	**2**	**3**	**4**	**5**
S_iO_2	56	58	59	56	58
TiO_2	0.69	0.71	0.72	0.70	0.72
Al_2O_3	16.51	16.70	17.34	16.83	17.22
Fe_2O_3	4.33	4.40	4.47	4.15	4.27
SO_3	0.62	0.61	0.66	0.61	0.6
MgO	2.23	2.38	2.28	2.15	2.18
CaO	0.06	0.06	0.07	0.06	0.06
Na_2O	1.05	1.07	0.99	0.82	1.06
K_2O	3.75	3.84	3.93	3.78	3.87
P_2O_5	0.15	0.16	0.17	0.16	0.16
L.O.I	14.50	12.50	10.50	14.50	12.50
TOTAL	**100.13**	**100.45**	**100.22**	**100.52**	**100.80**

Source: Analyses by Geological survey Department, Accra.

The concentrations of alumina and ferric oxides were also comparatively high. Concentrations of potassium and magnesium recorded third highest as indicated in Table 4.1. However, oxides of tin, sulphur, calcium, and phosphorous recorded less than 1%wt whilst Sodium oxide recorded a little above 1 %wt in the samples 1, 2,and 5 but little nearer to 1%wt in samples 3 and 4.

Loss of ingintion (L.O.I.) is the mass of water, organic matter and other substances which were removed from the soil after heating at 900^0C, expressed as a percentage of the ignite mass (Radojevic and Baskin,1999).

$$LOI = \frac{weight\ before - \ weight\ after}{weight\ before} \times 100$$

From table 4.2, high lost on ignition was observed in samples 1 and 4 followed by sample 2 and 5 with sample 3 producing the lowest LOI..

Table 4.2: Minor elements measured in ppm but figures in parenthesis are in (mg/g)

Element (ppm)	**Sample Numbers**				
	1	**2**	**3**	**4**	**5**
V	224 (0.2)	212(0.2)	226(0.2)	200(0.2)	221(0.2)
Cr	115 (0.1)	117(0.1)	125(0.1)	121(0.1)	124(0.1)
Co	110 (0.1)	104(0.1)	96(0.1)	69(0.1)	70(0.1)
Ni	12 (0.01)	7(0.01)	5(0.01)	5(0.01)	7(0.01)
Cu	21(0.02)	41(0.04)	32(0.03)	31(0.03)	32(0.03)
Zn	27(0.03)	78(0.08)	34(0.03)	30(0.03)	31(0.03)
Ga	14 (0.01)	22(0.02)	19(0.02)	18(0.02)	19(0.02)
As	6 (0.006)	7(0.007)	9(0.009)	8(0.008)	9 (0.009)
Rb	106 (0.1)	113(0.1)	115(0.1)	111(0.1)	112(0.1)
Sr	57(0.1)	60(0.1)	61.6(0.1)	56(0.1)	60(0.1)
Y	27(0.03)	26(0.03)	26(0.03)	25(0.03)	26(0.03)
Zr	263(0.3)	268(0.3)	273(0.3)	266(0.3)	273(0.3)
Nb	29 (0.03)	31(0.03)	31(0.03)	30(0.03)	31(0.03)
Mo	1(0.001)	1(0.001)	1(0.001)	1(0.001)	1(0.001)
Ab	2(0.002)	2(0.002)	2(0.002)	2(0.002)	2(0.002)
Cs	5(0.005)	4 (0.004)	2(0.002)	2(0.002)	6(0.006)
Ba	785(0.8)	841(0.8)	850(0.9)	780(0.8)	913(0.9)
La	36(0.04)	36(0.04)	43(0.04)	41(0.04)	46(0.05)
Ce	77(0.1)	69(0.1)	82(0.1)	74(0.1)	81(0.1)
Ta	19(0.02)	12(0.01)	10(0.01)	9(0.01))	8(0.01)
W	10 (0.01)	10 (0.01)	6 (0.01	5(0.01)	4(0.004)
Pb	25(0.03)	28(0.03)	26(0.03)	26 (0.03)	24(0.02)
Bi	3(0.003)	3(0.003)	3(0.003	3(0.003)	2(0.002)
Th	17(0.02)	16 (0.02)	16(0.2)	14(0.1)	15(0.2)
U	8(0.008)	5(0.005)	2(0.002)	2(0.002)	4(0.004)

Source: Geological Survey Department, Accra.

Table 4.2 indicates the minor elements obtained from the XRF analysis of the clays under investigation. The amount of lead (Pb) obtained was 0.03mg/g in four samples and 0.02mg/g in the fifth sample. This value exceeded levels recommended by WHO/FAO of 0.01ppm (WHO, 1995; Gichumbi *et al*., 2011).

Recent scientific studies on lead show that adverse health effects are occurring at lower levels of exposure to lead (Pb) than previously thought and induces various toxic effects in humans at low doses with typical symptons such as colic, anaemia, headache, convulsions, chronic nephritis of the kidneys, brain damage and central nervous system disorders (WHO,1998). The high level of lead detected in clay could be the cause of kidney necrosis as was found in the histology studies.

Arsenic (As) level obtained was 0.006 mg/g as against natural levels of arsenic in soil of 10mg/kg as was detected in a study by Callabrese *et al.*, (1990). However, the RDA for asernic in food is 0.00l mg/g (Callabrese *et al*, 1990) and is generally viewed as safe even if arsenic at this level continue for many years, (Environmental health Information, 2007). Arsenic in the samples was higher when compared with arsernic concentration in food.

Vanadium concentrations in each of the five samples was 0.2mg/g higher than the general RDA values of 0.03mg/g per day. Although vanadium is an essential nutrient, its functional role in humans has not been established. However, increases in abortion rates and decreased milk production have been observed in vanadium-deprived goats (Harland and Harden Williams, 1994). Even though diabetic patients may need higher doses to normalize blood glucose and lipid levels, improve insulin sensitivity, prevent or reverse secondary complications such as cardiomyopathy, cataract development, and impaired antioxidants status, an average healthy human requires vanadium in small doses since high doses could cause ill health to consumers as evidenced to be highly toxic (Harland and Harden-Williams, 1994).

The natural concentration range of chromium in soil is between 1 and 300mg/kg. Chromium concentrations in the five samples was 0.1mg/g as compared with the RDA concentration value of 0.2mg/g. The clay supply low chromium to consumers when compared with the

RDA of 0.2mg/g. Chromium is selectively accumulated in liver and kidney. Established safe and adequate daily intake of Cr (*111*) in adults is reported to be 50 mcg - 200mcg (0.05mg/g - 0.2mg/g) per day (National Academy of Science, 1989). However, Watson (1993) reports that adults in United States take in an estimated average of 60 mcg – 80mcg Cr (*111*) per day in food which therefore, imply low dietary supply but found within the universal adult RDA range of 50 – 200 mcg detected in food sources. Chromium Cr (*111*) does not apparently pose a health threat by virtue of its nature.

In the analysed clay samples cobalt concentrations was 0.1mg/g, and was lower than the RDA values of 0.35mg/g as quoted by Young *et al*. (2008) and asserted in literature that these values in soil markedly improve health. Daily intake of cobalt is variable and may be as much as 1mg (Lenntech, 2001). Cobalt in small amounts is essential to many living organisms including humans. Cobalt helps maintain good levels of calcium in the body. Too much cobalt can decrease fertility in men and affect the heart. Cobalt is needed in small quantities for physiological activities, values detected are not likely to pose a health threat (Lenntech,2010).

Nickel is a trace mineral required by the body in small quantities. Concentration values of nickel in the five samples was 0.01mg/g lower than the RDA value of 0.2mg/g. According to Center for Disease Control and Prevention (2003), Americans consume about 170mcg(0.2 mg/g) of nickel each day in food. Nickel plays a role in the circulation of some proteins and can contribute to production of hormones, lipids and cell membranes (NAS, 1989). Also, used in the body to breakdown glucose for energy (Dayan and Paine, 2001). Nickel concentrations detected in the samples was lower than the dietary allowance asserted by Center for Disease Control and prevention (2003) for Americans.

High nickel concentrations on sandy soils can damage plants. For humans, high uptake of nickel will result in respiratory failure, prostrate cancer, lung cancer, nose cancer (Lenntech, 2001). Nickel can be carcinogenic, and may cause dermatitis, eczema, vertigo and dyspnea exposed to human population (WHO, 1993).

Table 4.3: Recommendaed Dietary Daily Allowances of minor and major elements and quantities in clay studies

Element	RDA mg/g	Levels in Clay mg/g	Benefits	Detrimental effects (hazards)	Reference
V	0.03	0.2	High dose control sugar levels in diabetic people.	Non toxic deficiency	Harland and Williams (1994)
Cr	0.20	0.1	Moves blood sugar into cells to be used as energy. Reduces blood cholesterol and triglycerides level. Cr picolinate is well absorbed than other forms and plays part in cholesterol lowering benefits	Cr (VI) very toxic; Low Cr levels may cause high cholesterol andincrease risk of coronary diseases.	(Watson, (1993),Dayan and Paine, (2001).
Co	0.35	0.1	Formation or red blood cells, prevention of pernicious anaemia, proper growth etc.	Decrease fertility in men, over production of red blood cells and damaged to heart and thyroid glands,anaemia and retarded growth.	NAS,(1989), Elson,(1984)
Ni	0.2	0.01	Iron absorption, Treating weak bones (osteoporosis), Prevent anaemia	Skin rashes, anemia, high intake leads to prostrate cancer, lung cancer and nose cancer	Lenntech, (2001).
Cu	3.0	0.04	Proper growth, prevent premature ageing, utilization of iron, proper enzymatic reactions etc.	Severe mucosal irritation, and corrosion, hepatic and renal damages. Low resistance to infections, osteoporosis, anaemia etc.	Judy, (2003)
Zn	15	0.08	Normal growth, brain development, wound healing etc.	Causes vomiting, dehydration, electrolyte imbalance, nausea and dermatitis.	Hunt, (1994). Committee on RDA (1989).
As	0.01	0.006	Physiological role not clearly defined	Cancer of the liver,lung,urinary bladder and possibly the kidneys and prostrate etc.	EPA//EH1., (2007)
Mo	0.05	0.002	Act as catalysts for enzymes. Facilitate breaking down of certain amino acids in the body	Causes are rare, however, signs may include defects in uric acid production.RDA above 1500mcg (1.5mg) could lead excess excretion of copper.	National Academy of Science, (1989)
Pb	0.01	0.03	No biological benefits is known	anaemia, headache, convulsions and chronic nephritis of kidneys, low intellectual development of children, brain damage and central nervous disorders.	WHO, (1998)
Ba	0.02	0.9	No biological role.	($Bacl_2.2H_2O$) has stimulant action on heart and other muscles, raises blood pressure etc.	National Academy of Science (1989), Centre for disease control and prevention, (2003).
Rb	5	0.1	Ability to alter the function and replications of cancer cells.Enyme synthesis and ensure iron in blood and adequate absorption regulation. Good agent to treat cancer.	No specific side effects are found associating with Rb but in rare cases side effects like blistering skin, discoloration and dryness are observed.	Elson (1984)

Fe_2O_3	12	43	Essential of haemoglobin.	Anaemia, myocardial infarction etc.	Kaplan etal, (1996) Pamplona-Rogers, (2008).
MnO	2.3	ND	Blood clottting factor regulation. Fat metablosim. Bone creation and sex hormone creation. Anti- aging.	Slowed blood clotting. Skin problems, changes in hair colour, lowered cholesterol etc.	ATSDR (2008)
MgO	400	24	Reactions that generate and use ATP needed in large quantities.required by body in large quantities.	Deficieny is related to it enzymatic reactions and regulatory neurotransmitters manisfestation and personality changes occur	(Judy, 2003; National Academy of science 1989).
SiO_2	30	560	Strengthting if connective tissues and bones, taking care of nails, hair and skin. Prevention of atherosclerosis, insomnia, skin disorders and Tuberculosis.Inceases vit D and calcium in diet	Poor development of bones, thinning of the hair, brittleness of nails, formation of wrinkles and general ageing of skin	Lemmo, (1998)
Al_2O_3	100	168	Use as antacids and laxatives	High doses exacerbate Alzheimers disease. Low birth weight of infants. Reduced absorption of cholesterol	Willhitte (2012)
K_2O	2000	38	Conduct impulses in the body. Exchange of sodium and potassium ushers in ATP	Excess potassium affect the heart and brain. Hypokalemia causes heart to beat irregularly. Constipation,fatigue, muscle paralysis.	Pamplona-Rogers (2008)
Na_2O	3400	11	Maintenance of normal blood pressure. Regulates the amount of water in the cells.	Low sodium levels (Hyponatremia) increases sweating, vomiting, diarrhoea, kidney disease and heart failure	Dietary Guidelines for Americans (2010)
CaO	1300	0.6	For vascular constraction and vasodilation, muscle function, nerve transportation, intracellar signaling and hormonal secretions. 99% of calcium stored in bones and teeth where it supports their structure and function	Low calcium leads to poor absorption and hence bone breakdoen. Normal aging process eg. Post menopausal women due to decreases amount of estrogen. Hypercalcemia and calciuria etc.	Pamplona-Rogers, (2008).

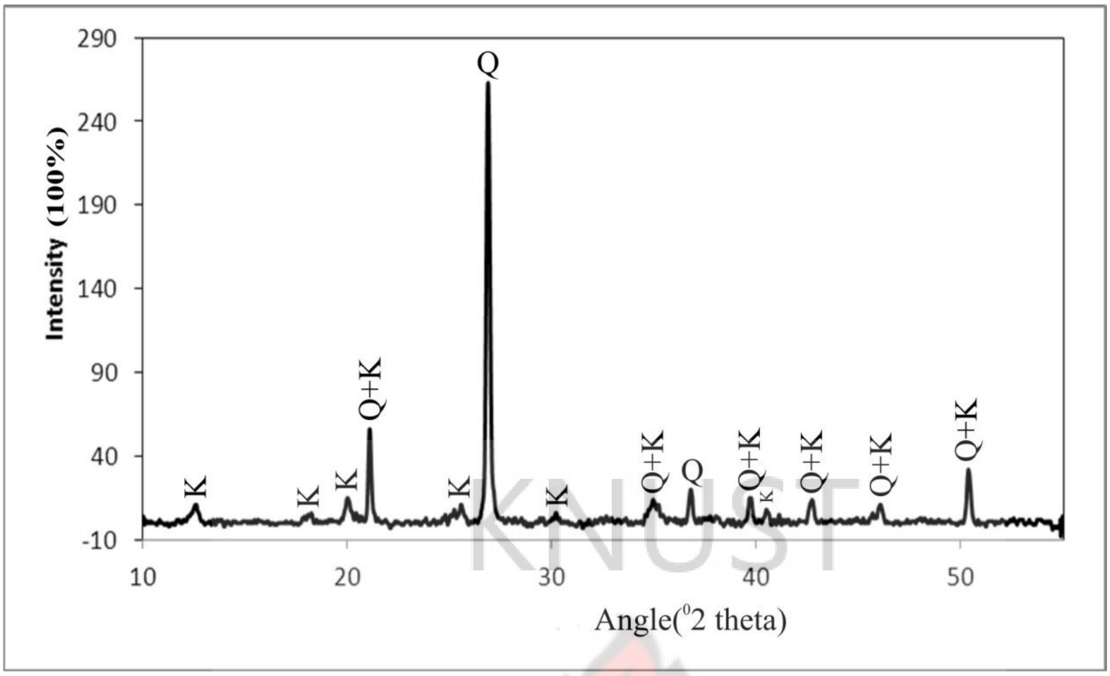

Figure 1: Representative X-ray powder diffractogram of geophagic clay sample from Kumasi markets (note: K = kaolinite, Q = quartz).

4. 3 Clay minerals analysis

Figure 1 presents peaks of minerals found in the clay sample after analysis.The chemical compositions of the geophagic clay samples were dominated by SiO_2 and Al_2O_3 which were related to quartz and kaolinite as in a study on mineral analysis conducted by (Ekosse *et al*., 2011). In a similar study, Deer, *et al.* (1992) identified major mineral Quartz (SiO_2) and Kaolinite (Al_2Si_2O $(OH)_4$ as the dominant minerals.The presence of kaolin minerals agreed with the protection hypothesis (Young *et al.*, 2008), given that kaolin minerals have long been used in pharmaceutical formulations to treat the causes and the symptoms of gastrointestinal distress (Gonzalez *et al*., 2004).

4.3 Biochemical Studies

Table 4.4: Indicates treatment schedule in the experimental groups

Group	Dosage (g)	Duration (Days)
Control	1ml of distilled water only	28
(0.1)	0.2ml of suspension (1g/10mlof clay)	28
(0.3)	0.6 ml of suspension (1g/10ml of clay	28
(1.0)	1ml of suspension (1g/10ml of clay	28

In Table 4.4, represents the toxicity treatment regimen. The control group was fed *ad libitum* on 1ml of distilled water and feed for the period of 28 days. The treatment groups were fed on the clay substance once daily at varied doses of 0.2ml, 0.6ml and 1ml (1g/10ml of clay) in dissolved suspension for 28 days each.

4.3.1 Absolute Average Weekly weight gains

Results in Table 4.5 indicate that from Day 0 to Day 28, there were variable changes in absolute body weight of rats in all groups. The Control group gained marginal weight of 39.89 (139.26g – 99.37g) through out the duration of treatment whereas rats fed on 1.0 g/kg weight was observed (99.14– 169.10 g) to have a negative growth of -69.96g.

Table 4.5 Absolute weights of rats from Day 0 to Day 28 treated with varied doses of geophagic clay suspension.

Treatment	Average weight (g)		Change in weight (g)
(g/kg/b.wt)	Day 0	Day 28	
Control	99.37±2.35	139.26±3.76	39.89
0.1g	69.50±1.49	229.25±4.49*	159.75
0.3g	107.57±3.16	142.43±6.32	34.86
1.0g	169.10±5.53	99.14±3.84**	-69.96

All mean values that contain superscript stars ** are significantly different ($P < 0.05$) as compared to the control.

Surprisingly, the Group labelled 0.1g/kg gained the highest weight (229.25 – 69.50g) 159.75g far above the Control, 0.3g/kg and 1.0g/kg.The change in the body weights is significant. At smaller doses there was weight gain but as the dose increases to 0.3g/kg and 1.0g/kg, weight decreases and finally turned to have negative effect on weight. In a similar study (Agbede, 2003) reports that, chickens' growth rate decreases with increase of fiber in diet. Agbede assigned a reason as poor utilization of the diet. The negative growth could be attributed to the heavy metals such as lead and arsenic detected in the clay which far exceeded the WHO/FAO limit of 0.01 ppm. In a similar study, Rafique *et al*., (2008) confirm that animals having continuous exposure to heavy metals usually loose weight.

4.3.2 Weight of organs of rats after 28 days of treatment

The values of liver, kidney, stomach, heart, lungs, spleen, ovaries and uterus obtained for study groups; Control, 0.1g/kg.bwt, 0.3g/kg/bwt, 1.0g/kg/bwt, showed no significant differences (P> 0.05) among post treatment means as shown in Table 4.6

Table 4.6: Weight of organs of rats after 28 days treatment

Organs	**Dosage**			
	Control	**0.1g**	**0.3.g**	**1.0g**
Liver	5.75±0.25	5.29±0.29	6.01±0.11	5.91±0.35
Kidney	1.03±0.04	1.05±0.03	1.13±0.02	1.08±0.04
Stomach	1.32±0.49	1.28±0.10	1.49±0.04	1.26±0.09
Heart	1.26±0.89	0.72±0.04	0.82±0.02	0.72±0.05
Lungs	2.36±0.33	2.45±0.28	1.56±0.22	1.88±0.28
Spleen	0.65±0.13	0.73±0.67	0.84±0.04	0.58±0.07
Ovaries	0.13±0.02	0.11±0.01	0.36±0.22	0.13±0.01
Uterus	0.54±0.14	0.36±0.31	0.53±0.06	0.51±0.11

The differences in mean values among treatment groups and between the Controls are not significantly different (P> 0.05)

Study conducted by Moore and Dalley, (1999) indicated that an increase in organ-body ratio is an indication of inflammation while a decrease may be due to cell constriction. The non significant differences in the organ-body weight ratio observed in the treated animals compared to the control implied that clay did not cause any cellular constriction and or inflammation (Schmidt *et al.*, 2007). This findings compared favourably well with the findings of Amresh *et al*. (2008) in which the administration of Cissampelos pareira did not produce any effect on organ - body weight ratio investigated. Comparing results of Table 4.6 on relative organ weight with the literature stated, there is no doubt that findings from the clay fed to the rats showed no significant difference and therefore, imply that the clay had no effect on organ weight after the treatment.

4.3.3 Effects of geophagic clay on the haematology of rats

Table 4.7: Effects of geophagic clay on the Haematology of rats after 28 days treatment

Parameters	Dosage Control	0.1g	0.3.g	1.0g
RBC	7.14±0.43	6.99±0.19	6.83±0.16	7.36±0.08
HB	13.92±0.60	13.58±0.38	13.12±0.33	13.80±0.33
HCT	45.02±1.76	43.50±1.61	43.98±1.75	44.16±1.03
MCV	63.48±1.61	62.25±1.60	64.32±2.00	60.02±0.89
MCH	19.62±0.46	19.43±0.29	19.22±0.46	18.76±0.33
MCHC	30.90±0.38	31.30±0.50	29.90±0.56	31.26±0.23
WBC	8.260±1.40	7.867±0.652	8.38±0.86	8.82±1.17
LYMP	62.63±2.14	79.38±3.95**	76.96±3.56**	84.28±0.65**
NEUT	37.38±2.14	22.65±3.81**	26.03±2.49	15.73±0.65**
RDW-CV	14.73±0.74	12.78±0.36**	16.40±0.57	14.25±0.67

All mean values that contain superscript stars ** within each row are significantly different ($P < 0.05$) as compared to the control.

Table 4.7 shows the results of haematological examination. The mean values for Red blood cells (RBC), Haemoglobin (HB), Haematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular Haemoglobin (MCH), Mean corpuscular haemoglobin concentration MCHC) and White Blood Cells (WBC) obtained for study groups (0.1g, 0.3 and 1.0g) showed no significant differences from Control group. Also, there were no significant differences between the study groups. In the same Table 4.7, The mean values of Lymphocytes and Neutrophils were statistically significant at ($P < 0.05$) when treatment groups were compared with Control. However, WBC values in both control and treatment groups was not significantly different.The findings therefore, require further scientific enquiries. The elevation of the lymphocytes and Neutrophils might imply that clay have been contaminated physically or biologically since the material was not cultured for microbial contaminants before administration.The lymphocytes and Neutrophils are white blood cells that defend the body against viral and bacteria invasion. There exist a relationship between lymphocytes and Neutrophils, their mean values usually adds up to 100%. This means that when one increases the other reduces to make up the balance.The reduction in neutrophils concentrations suggest that clay may posses some anti-neutrophil activity which confirms a .study by (Garg *et al.,*1997) when he carried out a toxicity study in rats fed on nature cure bitters.

The mean values of Red blood cell distribution width-Concentration volume (RDW-CV) of treatment groups (0.3g and 1.0g) showed no significant difference ($P > 0.05$) as compared with the Control. However, the treatment 0.1g, indicated a significant difference ($P<0.05$) between control and other treatment groups. Low values in the group is not dose dependent but could be attributed to haemolysis or probably a reaction of the EDTA tube that contained the blood. The reduction in RDW-CV did not influence the blood biochemistry since other indices were normal.

In conclusion, the significant differences between treatment groups and the control in some haematological parameters were trivial in other words, not causal at (P< 0.05).

4.3.4 Liver function test

The summary of the liver fuction test is presented in Table 4.8.

Table 4.8: Effects of intake of geophagic clay on liver functions tests

Liver function test	**Dosage**			
	Control	**0.1g**	**0.3.g**	**1.0g**
Albumin	36.58±1.61	40.80±1.21	40.66±3.83	43.22±4.49
Globulin	53.63±2.01	42.19±4.17	44.52±1.81	41.10±3.68
Protein	90.21±3.62	82.99±5.38	85.18±5.64	84.32±8.17
ALT	71.33±5.30	77.62±3.93	68.10±4.60	79.80±1.52
ALP	42.0±43.01	36.30±50.44	53.8±103.8	802.7±28.07**
AST	21.10±21.71	25.40±11.62	22.1±6.88	181.7±7.00
GGT	5.18±0.16	5.52±0.28	6.26±0.39	4.700±0.43
TBIL	2.66±0.32	7.33±2.09	3.12±0.21	3.400±0.54
DBIL	1.58±0.14	3.95±1.15	2.12±0.15	2.160±0.51
INDBIL	1.08±0.22	3.24±0.89*	0.98±0.13	1.220±0.13

All mean values that contain superscript star(s) ** within each row are significantly different (P < 0.05) as compared with the control.

In table 4.8, the synthetic ability of the liver was well preserved as shown by the liver plasma, Albumin, Globulin and Total Protein. Albumin forms part of the total concentration of the blood serum protein and both are manufactured in the liver. (Mayne, 1996). The plasma enzymes, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Gamma Glutamyl transferase (GGT) are makers of hepatocellur damage. Aminotransferases are sensitive but relatively nonspecific indicators of liver cell injury. Bilirubin is a major product that results from the breakdown and destruction of old red blood cells. It is an important product with diagnostic values that is removed from the body by the liver (Chowdburry *et al*.,1989). The conjugating ability of the liver was intact as revealed by Total and Conjugated bilirubin levels. The absence of significant effects on these parameters in the study samples may be an indication that the normal functioning of the liver was not affected, comparing to the study of constipated rats treated with Aloe ferox extracts (Rahman *et al*., 2001). The serum Akaline phosphatase (ALP) and indirect bilirubin (IDBIL) at doses 1.0g/kg and 0.1g/kg were significantly different from all treated groups and controls at P value of (0.05) respectively. High elevation could lead to extrahepatic cholestasis when both serum bilirubin and ALP are increased. Increased ALP is extensively used as a tumor marker and also present in bone injury, pregnancy, or skeletal growth, testicles, liver, kidney, intestines. The rise in indirect bilirubin (INDBIL) at dose 0.1g/kg could be either haemolysis, obstruction during bile transport or artifact. The rise in ALP at highest dose does not indicate dose dependent and does not present liver problem since all other parameters were intact. Direct Bilirubin (DBIL) obtained for study groups 0.1g, 0.3g and 1.0g showed no significant difference ($P>0.05$) from control and among the study groups. Aspartate aminotransferase (AST) concentrations were consistently higher than Alanine aminotransferase (ALT) levels which are expected since body cells contain more AST than ALT (Mayne, 1996). Usually, about 80% of AST is found in the mitochondria whereas ALT is purely cytosolic enzyme. Therefore, AST appears higher in concentration in a number of tissues (liver, kidney, heart and pancreas) and is released slowly in comparison to ALT. But since ALT is localized primarily in cytosol of hepatocytes, this enzyme is considered a more sensitive marker of hepatocellular damage than AST and within limits can provide quantitative assessment of the degree of damage sustained by the liver (Al-Mamary *et al.*, 2002). There was no possible toxity found in the liver of the rats after feeding with the clay as stated by (Rahman *et al.,* 2001). This further confirmed the significant elevation in Alanine aminophosphatase (ALP) level at 1.0g/kg could be an indication of bone disease and not liver since GGT and ALP are enzymes of the bile ducts and in situations where GGT is normal and ALP is elevated, it then means that there might be a disease relating to bones. However, rise in plasma ALP is usually a characteristic finding in liver cholestasis, (Kaneko, 1989) that could be due to damage to structural integrity of the liver which is reflected by increase in the activity of ALP in the serum probably as a result of leakage from altered cell membrane structure (Yakubu *et al.,* 2003).

Liver histology results indicate that the liver at all dose levels in all treatment groups showed conditions of necrosis and chronic hepatitis, which was attributed to heavy metals detected in the XRF analysis of clay.

4.3.5 Effects of geophagic clay on lipid profile

The summary of the effects of geophagic clay on lipid profile is presented in Table 4.9.

Table 4.9: Effects of geophagic clay on lipid profile

Treatment g/kg/b.wt	Cholesterol	Triglycerides	HDL	VLDL	Coron .Risk
Control	2.22±0.15	0.75±0.19	0.83±0.05	0.34±0.09	3.14±0.77
0.1	4.11±0.79	2.65±0.66	0.72±0.12	1.19±0.29	5.76±3.34
0.3	3.02±0.42	1.67±0.50	0.78±0.09	0.77±0.23	5.22±1.08
1.0	3.70±0.39	3.75±0.75*	0.94±0.07	1.32±0.37	4.95±0.48

All values are expressed as mean ± SEM. *($P < 0.05$.) significantly different from control.

There was a dose dependent increase in serum cholesterol concentration, this elevation was significant at only 0.1g/kg as against the control and other treatment groups. Treatment doses 0.3 and 1.0g/kg were also higher than the control, however, the difference was insignificant using Neumann-Keuls post hoc test. Similarly, triglycerides levels were apparently increased at all dose levels tested and this effect was remarkable at 1.0g/kg. This was significantly different from control and among treated groups. HDL levels decreased in concentrations at 0.1 and 0.3g/kg but increased at dose 1.0g/kg however, the increase at dose 1.0g/kg was not significantly important.

Mean values for VLDL concentrations increased at 0.1 and 1.0g/kg with reduction occurring at 0.3g/ kg. Values of the coronary risk increased at all the dose levels tested although this increases did not appear to be dose dependent.

Mean values for treatment groups as against the control showed normal cholesterol level. There was no significant difference among treatment groups and control at significant level ($P<0.05$). The three forms of cholesterol; High density lipoproteins (HDL), Low density lipoproteins and Very low density lipoprotein showed no significant difference when mean values were compared with the Control group. VLDL cholesterol is a predisposing factor for coronary risk. In the study, the cholesterol levels were normal and hence did not influence the risk of coronary disease. Mean values of treatment groups as against the control showed no significant difference. However, mean values for triglycerides in Table 4.10 indicated elevation in the group fed on high dose of 1.0g whereas the other two treatment groups as compared to the control showed no significant difference. A high triglyceride level may be present in artherosclerosis, hypothyroidism, liver disease, pancreatitis, myocardial infarction, metabolic disorders, toxemia, and nephrotic syndrome (Stamler, 1986). Even though, there was elevation in triglycerides, that did not show any sign of coronary risk. This therefore, implies that the clay eaten for 28 days did not have any negative effect on the lipid profile.

4.3.6 Effects of geophagic clay on kidney function

The results of the kidney function is presented in Table 4.10.

Table 4.10: Effects of intake of geophagic clay on kidney function test

Treatment g/kg	Creatinine	Urea	Uric acid
Control	86.94±20.06	5.83±1.05	575.8±41.89
0.1g	68.07±9.27	6.985±0.76	665.0±73.33
0.3g	65.12±3.62	6.916±0.40	690.4±34.56
1.0g	38.02±2.238*	4.683±0.44	718.8±11.46

*$P < 0.05$ significantly different

Renal function indices such as serum electrolytes, urea, creatinine and uric acid can be employed to assess the functional capacity of the kidney (Sunmonu and oloyede, 2006). Urea is the end product of protein metabolism and its concentration is influenced by the rate of excretion. Creatinine is the waste product of muscle metabolism while uric acid is the major catabolic product of purine metabolism (Tietz, 1986). The serum levels of these metabolites have been used as important indices for the evaluation of toxic effects of chemicals on the kidney (Davis and Bredt,1994).

There was a dose dependent reduction of creatinine concentration in all treatment dose levels as compared to control and very significant at dose 1.0g at ($P < 0.05$). As the dose increases the levels of excretion of creatinine reduces leading to a corresponding increase in uric acid at the highest dose.The increases and reductions in urea and uric acid concentrations were not significantly different. Creatinine metabolism takes place in the muscles and low muscle mass results in low creatinine levels. Furthermore, as muscle protein breaks down, amino acids return to the amino acid pool. Some is channeled to purine/pyrimidine metabolism resulting in increased uric acid as observed in the table.

The reduction in urea and increase in uric acid values at the highest dose are not statistically significant. Uric acid is normally excreted through the urine. High levels are noted in gout, infections, kidney disease, alcoholism, high protein diets, and with toxemia in pregnancy. The reduction in creatinine excretion at the highest dose and urea concentrations in the rats indicate that intake of the clay substance may affect the functioning of the kidney and indicates poor clearance of the kidney as compared with control and other treatment groups and therefore means that the clay could be nephrotoxic.However, serum creatinine and BUN are insensitive indices of glomerullar filtration rate. There could be about 50 to 70% decrease in Glomerullar Filtration Rate (GFR) before serum values of Blood Urea Nitrogen (BUN) can develop.(Casarett and Duoll,2010). Low levels are sometimes seen in kidney damage, protein starvation, liver disease, or pregnancy.

This disagrees with the findings of Kaneko, (1989) in his toxicity study in rats fed on nature cure bitter, found out that reduced levels of urea, uric acid and BUN probably indicate that Nature Cure Bitters did not interfere with the renal capacity to excrete these metabolites and was also attributed to good clearance levels of kidneys (Oduola *et al.,* 2007).

Inspite of these findings, histology results of the kidney indicated that at all dose levels the kidney suffer some forms of impairment. Common conditions were moderate to severe tubular necrosis and chronic inflammations due to heavy metals present in the clay, especially lead.

4.3.7 Relative organ weights of rats on clay for 28 days

Relative organ weights of female Sprague Dawley rats is summarized in Table 4.11.

Table 4.11: Relative organ weights of rats fed on clay for 28 days

Organs of rat g/kg	Control	0.1g	0.3g	1.0g
Liver	3.24±0.19	2.57±0.16	3.24±0.23	3.60±1.17
Kidney	0.58±0.02	0.51±0.03	0.61±0.04	0.65±0.07
Ovaries	0.07±0.00	0.05±0.00	0.18±0.10	0.08±0.01
Stomach	0.63±0.14	0.62±0.00	0.80±0.06	0.79±0.09
Heart	0.63±0.14	0.6±0.06	0.80±0.06	0.79±0.09
Lungs	1.33±0.19	1.2±0.13	0.82±0.10	1.05±0.17
Uterus	0.29±0.07	0.18±0.015	0.28±0.03	0.35±0.05
Spleen	0.36±0.07	0.35±0.04	0.46±0.04	0.37±0.04

All mean values are not significantly different at $P>0.05$.

There was no significant changes in the relative weights of the liver, kidney, stomach, heart, lungs, spleen, ovaries and uterus of treated rats in relation to control groups. There is no significant difference when control is compared with treatment groups and among groups at ($P>0.05$).

Figure 2: Relative liver weight

In figure 2, relative liver weights of rats have been compared against the doses administered during the treatment period. Weight change in treated rats is dose dependent. As doses increase there is a corresponding weight gain when treatment groups are compared among groups and with the control. This therefore, suggests that the clay may contain some substances that have the potentials for weight gain. In the treatment groups, the weight gained can be expressed over the amount of substance administered during the feeding period.

The decline in relative weight of animals fed on 0.1g could be attributed to change of environment and possibly because of the lowest dose administered to the group.

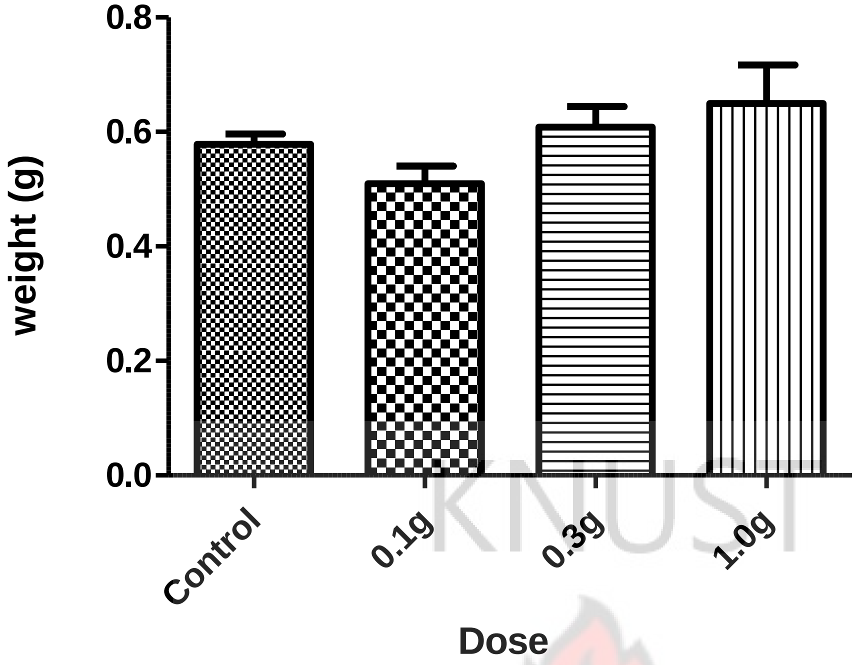

Figure 3: Relative kidney Weight

Data in figure 3: The relative kidney weights are compared among treatment groups and control group. The weights of the treatment groups increased in order of dose administration. The higher the dose the more the weight gain. The approximated mean weight value for control group is 0.6g whereas the treatment groups had mean weights of 0.5g, 0.6g and 0.7g respectively. The increase in weights therefore, imply that the clay contain some weight gaining factors. According to Moore and Dalley, (1999) an increase in organ-body weight ratio is an indication of inflammation while a decrease may be due to constriction.

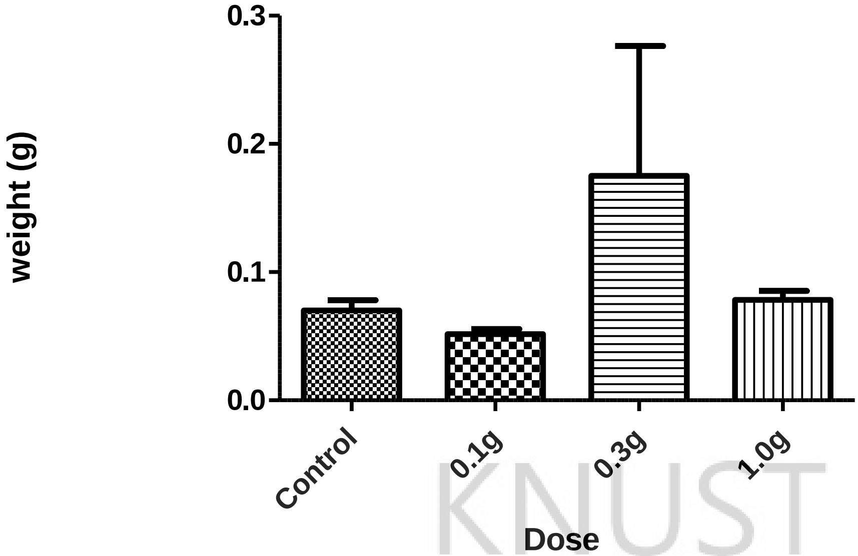

Figure 4: Relative weight of Ovaries

Figure 4, shows relative weights of ovaries compared against the doses administered. The animals fed on 0.1g indicated a decrease in weight as compared with the Control and among treatments. The mean weight values for the control is 0.07g, for the treatment groups 0.1g (0.05), 0.3g (0.2), and 1.0g (0.08) respectively.The treatment group of 0.3g has an abnormal mean value of 0.2g as against other groups and control.The long standard deviation bar indicate that the mean values are well dispersed from the original mean. This deviation could be attributed to in accurate computation of values since all mean values centred around 1g/kg. The implication is that ovaries could over produce ova and secrete abnormal estrogen and progesterone which are enzyemes responsible for reproduction.

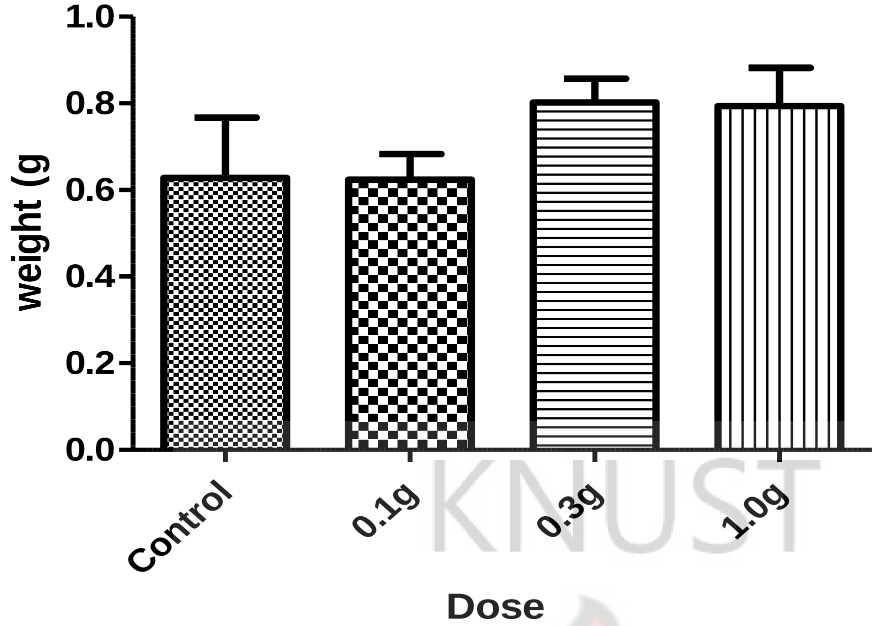

Figure 5: Relative Weight of Stomach

In figure 5, the Control and treatment group 0.1g showed almost equal weights of 0.6g. Rats fed 0.3g and 1.0g respectively gained weight of 0.8g. Weight gain could be attributed to the doses administered since low doses induce low weight and high doses induce high weigts as indicated in the figure 5.The stomach serves as a conduit, storage organ that churns and temporary stores ingested substance.The functionability of the stomach may contribute to the variation in relative weights.

The histology report on the stomach indicated that there were increased proliferation of the mucuous glands with increased dosage and may suggest reaction (adaptation to the luminal effect of the clay).

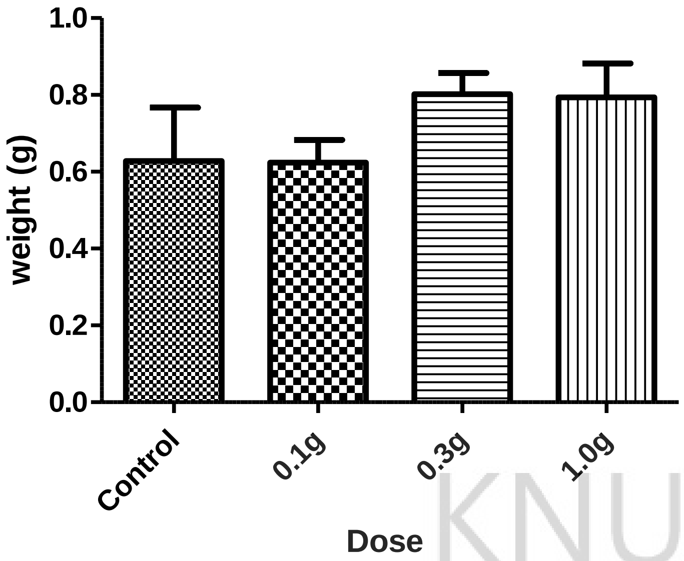

Figure 6: Relative Weight of Heart

In figure 6, the weights of the heart are lower in Control and treatment group fed 0.1g. Whereas the 0.3 and 1.0g groups showed appreciable increase in weight. This means that higher doses enhance weight gain.

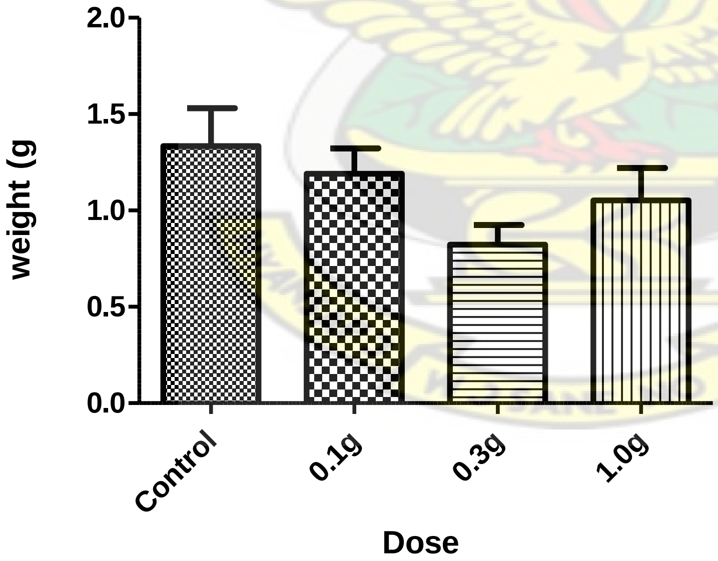

Figure 7: Relative Weight of Lungs

In figure 7, weight in treatment groups declined and was more pronounced in the group fed on 0.3g of the clay. This therefore, indicated that the decline might be the cause of other factors and not dose related since other groups fed on 0.1g and 1.0g of clay showed weight gains. The group fed on 0.1g showed a remarkable weight gain as compared with the control group. However, this group often obtains weight decline.

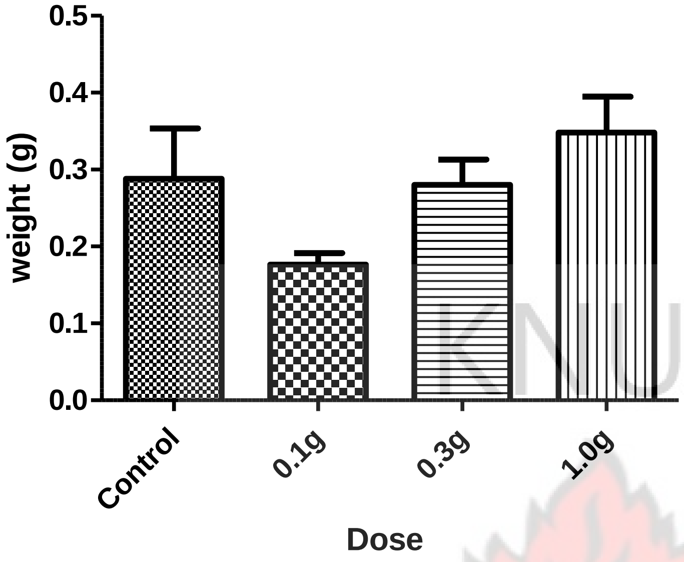

Figure 8: Relative Weight of uterus

In figure 8, weights of uterus compared with the control group were significant at P values 0.05. The Control gain a mean weight of 0.3g whilst the treatment groups fed 0.1g gain mean weight of 0.2g, 0.3g gained mean weight of 0.3g, and 1.0g gained mean weight of 0.4g respectively. There was a decline in weight on the group fed on 0.1g as compared with the control and treatments. The changes in weight among treatments is dose depended. The doses and weights increase according to the quantity of doses administered. Notwithstanding, the 0.3g gained the same mean weight as control and 1.0g groups gained higher weights than the control. The weight gain in the uterus indicated that the clays contain substances that enhances weight gain.

The histology studies comfirmed that the weight gains are as a result of (acute endometritis) inflammations that may be related to cyclical changes of the rats and not the effect of the clay as stated by Moore and Dalley (1999).

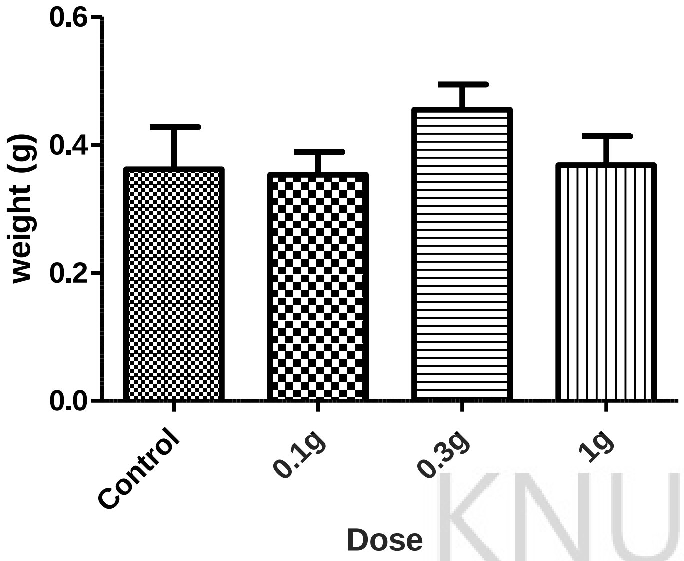

Figure 9: Relative Weight of Spleen

In figure 9, mean values for control and tested groups 0.1g and 1.0g had approximated values of 0.4g each whereas dose 0.3g attained a mean value of 0.5g/kg.This is not dose depended. The rise in dose 0.3g/kg might be an external influence and not the dose per se.

4.4.7 Histology results **of geophagic clay on the stomach, liver, kidney and uterus of female Sprague Dawley rats.**

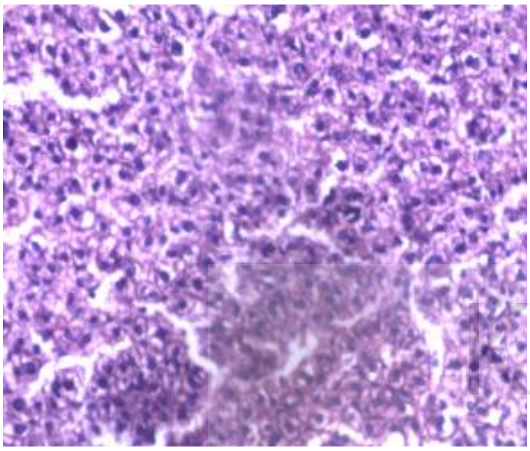

Plate 1: Liver biopsy from the control slide shows well preserved architecture with focal peri-portal.

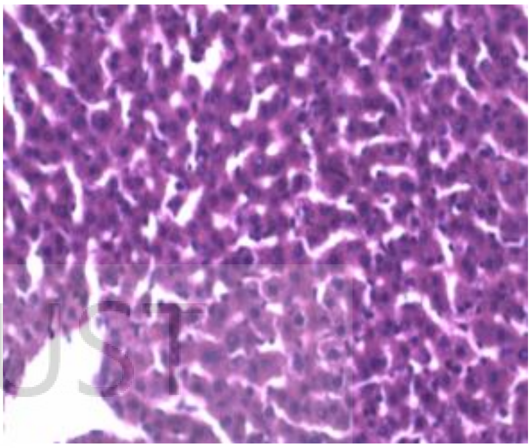

Plate 2: Liver biopsy from the 0.1g slide shows mild centric zonal necrosis and chronic hepatitis

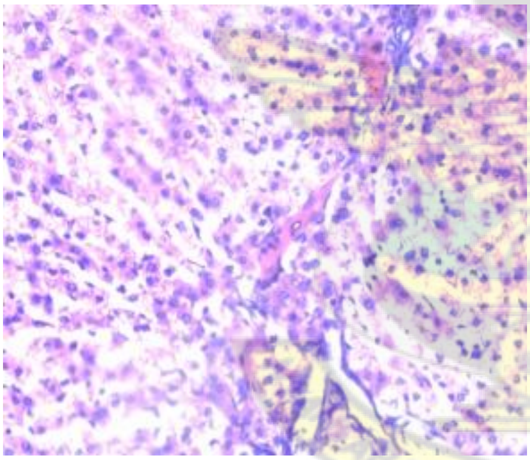

Plate 3: Liver biopsy from the 0.3g slide shows marked centric zonal necrosis and chronic hepatitis

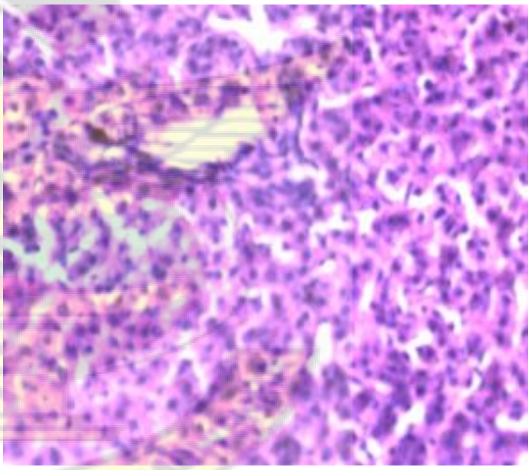

Plate 4: Liver biopsy from 1.0g similarly shows centric zonal necrosis, fatty change (micro vesicular) and chronic hepatitis.

Conclusion: Consistent of heavy metal poisoning

.

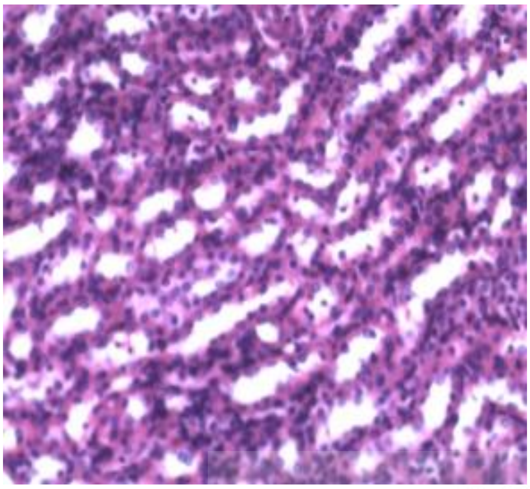

Plate 5: Section of kidney biopsy from the control slide shows that the cortical and medullary regions are fairly well preserved.

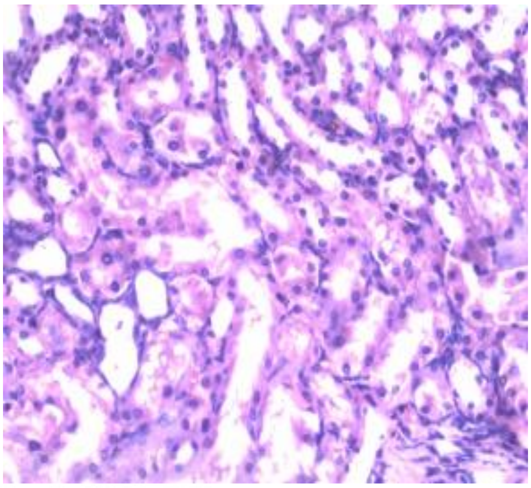

Plate 6: Section of kidney biopsy from the slide labeled 0.1g of clay shows similar features as in the control slide, but in addition, shows cortical tubular necrosis.

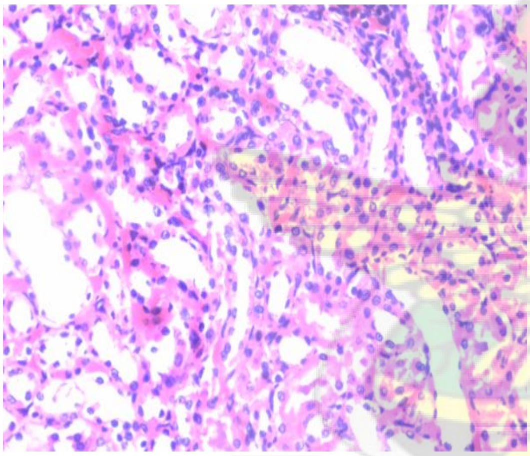

Plate 7: Section of kidney biosy from the slide labeled 0.3g shows moderate to severe tubular necrosis.

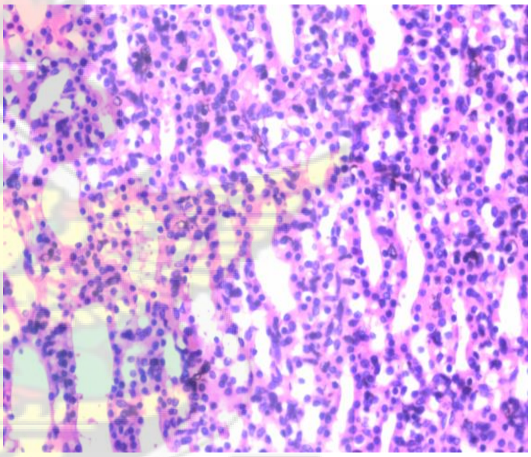

Plate 8: Section of kidney biopsy from the slide labeled 1.0g shows marked tubular necrosis with sloughing of the cuboidal epithelial lining and intraluminal accumulation of amorphous pink secretion.

Conclusion: The histologic features are consistent with heavy metal poisoning.

Comment. Because of the small sample size, (just a case) it would be difficult to make a specific diagnosis.

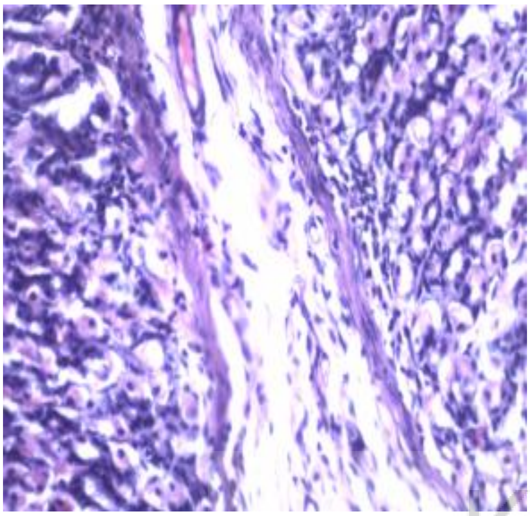

Plate 9: Section of gastric biopsy from the control slide shows normal antral (mucous glands), body and cardio-oesophageal type mucosa.

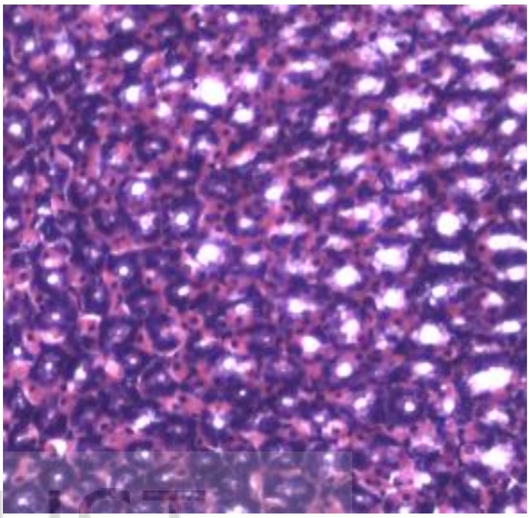

Plate10: Stomach biopsy from slide 0.1g shows moderate increase in the number of antral type mucous glands.

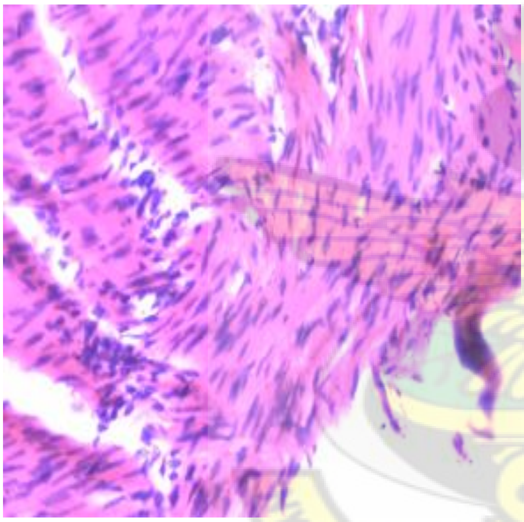

Plate 11: Section of stomach biopsy from the slide labeled 0.3g of clay shows an infarcted tissue not appropriate for diagnosis.

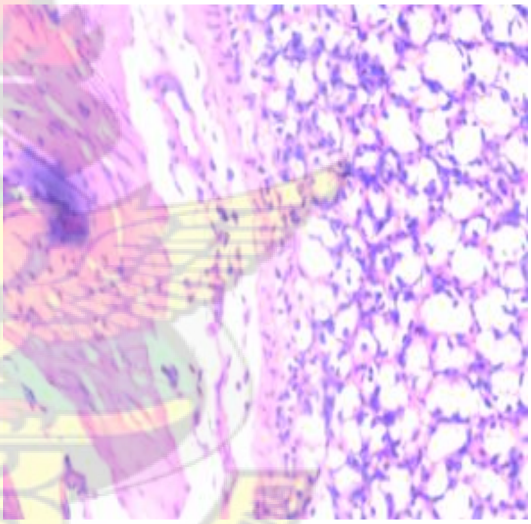

Plate 12: Section from slide labeled 1.0g of clay shows marked proliferation of the mucous glands, involving the antrum, body and the cardiac parts of the stomach.

Conclusion: The increased proliferation of the mucous glands with increased dosage may suggest reaction (adaptation) to the luminal effect of the clay**.**

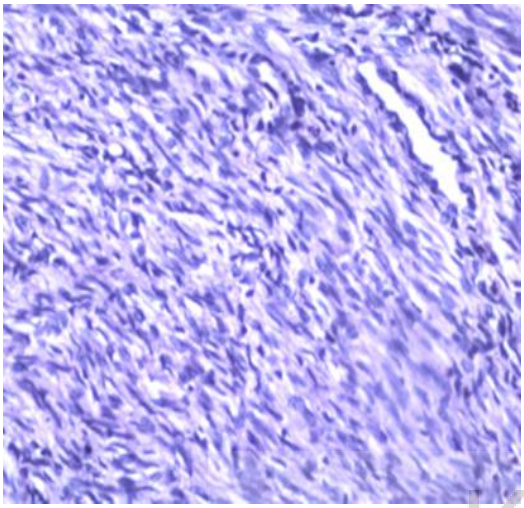

Plate 13: Section of slide of rat uterus of the control group

Plate 14: Section of slide of rat uterus fed on 0.1g of clay

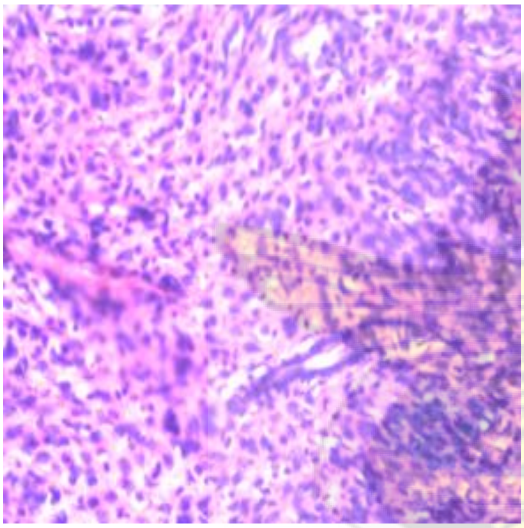

Plate 15: Section of slide of rat uterus fed on 0.3g of clay.

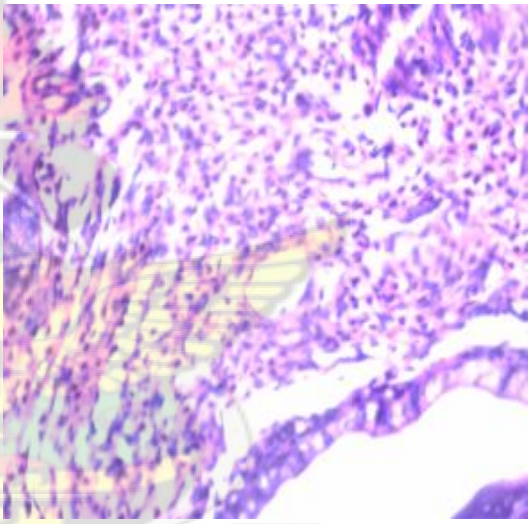

Plate 16: Section of slide of rat uterus fed on1.0g of clay

Sections from the control and the test slides all show infiltration of the endometrial stroma by acute inflammatory cells (neutrophils). There is stromal oedema and heamorrhage. These changes are of acute inflammation and may be related to the cyclical changes of the rats and not the effect of clay.

The uterus was studied because females are associated with clay eating which could have negative effects on the reproductive system.

In conclusion, it is clear in the histology results that concentrations of clay elements had no effects on the uterus but effects were pronounced in the stomach, liver and kidney. The physiological changes could be due to heavy metal present above the recommended daily allowance.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The chemical composition of the clays studied were varied. The dominant oxides comprised of SiO_2, Al_2O_2, which constitutes more than 70% of all elements present in the clays. Fe_2O_3 and K_2O were also high in concentration. Of the 25 minor elements determined, seven of them namely; V, Cr, Co, Rb, Zr,Ba, and Pb were the dominant ones. Mineralogically, Kaolinite and quartz dominated in the clays. Geophagists who consume these clays may benefit from their nutritional and medicinal values. However, the presence of heavy metals such as Ba, As, V and Pb above RDAs may be a cause for health concern. Not withstanding, clay supply more iron than the body requires.

The studies also revealed that rats which were fed on lower dose of 0.1 g/kg out-performed their counterparts in the other treatments in terms of weight gain. All the haematogical parameters and lipid profile were statistically similar. However, liver function test and kidney function test was affected and could be concluded that clay elements in high concentrations did affect the functioning of body organs as shown in the histology results. Some of the elements in the clay was found to be above the RDA level and could correlate with the histology results but had no clear pathological effect on the biochemical results.This therefore, depicts a good correlation between chemical elements and histological results which are statistically significant.

In general, clay could provide nutritional elements especially as indicated in the study and could also be harmful because lead (Pb) in the study was higher than RDA.

Nothwithstanding, eating the substance for a longer time may be harmful to human health.

5.2 Recommendations

Considering the fact that geophagic practice cannot be eliminated, edible clays should be refined and standardised for consumption.

Educate geophagic individuals on health risks and benefits as well as preparation, storage and use of geophagic clays should be encouragingly intensified.

Further experiments should be conducted to validate the findings in this work :-

1. Subjecting geophagic clays to treatment to avoid contamination (physical and biological)
2. Using pregnant female rats to mimic the human situation.
3. Measure Serum minerals to find what is absorbed as against what is given that may have pathological consequences.
4. Experiment to look at the benefits of geophagia on the immune system is recommended.
5. Examine haematologically Howell Jolly bodies from thin blood films.

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