ASSOCIATION BETWEEN GEOPHAGIA AND HAEMATOLOGICAL PARAMETERS OF IRON DEFICIENCY ANAEMIA AMONGST GEOPHAGIC QWA-QWA WOMEN

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DECLARATION OF INDEPENDENT WORK

I, MANNEHENG VIOLET RAPHUTHING, hereby declare that this research project submitted to the Central University of Technology, Free State for the degree MAGISTER TECHNOLOGY: BIOMEDICAL TECHNOLOGY is my own independent work that has not been submitted before to any institution by me or any other person in fulfilment of the requirements for the attainment of a qualification.

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SIGNATURE OF STUDENT DATE

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SUMMARY

Pica is the habitual eating of non-food substances by humans and animals. It has different subgroups and these are defined by the ingested substance. Moreover, geophagia is a type of pica that refers to the consistent eating of mostly earth and earth-like substances such as clay and soil. It is observed in both sexes, all age groups and in different ethnic groups around the world. There are many reasons people give for the practice of geophagia, such as culture, hunger and health being the most prominent. Geophagic materials differ in texture, colour and taste. Soil colour classification according to the Munsell soil classification, which uses hues, values and chroma, sometimes differ with the soil colour being noticeable with the naked eye. However, geophagic clays from Qwa-Qwa are white and contain kaoline.

Geophagic materials are believed to augment mineral deficiency, especially magnesium, calcium and iron. Geophagia is practised mainly by females, especially during their child bearing years. Females are more prone to iron deficiency anaemia due to their monthly menstruation cycle. Iron deficiency is the most common cause of anaemia and is classified as hypochromic microcytic anaemia (HMA). This study focused on the health aspect of geophagia. The research question seeks to explore whether there is an association between geophagia and the haematological parameters of iron deficiency anaemia. Geophagia seems to be linked with the occurrence of anaemia, but not iron deficiency anaemia, although it is implied. It is not known if the practice of geophagia causes iron deficiency anaemia or if it is because of iron deficiency anaemia that people practise geophagia. A pilot study was done in 2007, and the results of that study prompted that this study be performed on a bigger scale.

The lack of information regarding the quantity, frequency and type of geophagic material consumed the impact of geophagia on haematological parameters and the iron status of the geophagists made it important that the primary existence of the

relationship be investigated. In addition, research to establish whether there is a relationship between geophagia and haematological parameters of iron deficiency anaemia, has not been undertaken in South Africa, especially on non-pregnant women. Geophagia seems to always be accompanied by the subject of iron deficiency anaemia and especially its prevalence in females. The bigger geophagia project was therefore an ideal opportunity to do a specific survey on geophagic women.

This was a cross-sectional study, consisting of 36 control women and 47 geophagic women, aged between 18-45 years. The participants completed a questionnaire to determine the geophagic practices, which included the colour of the clay, how frequent the clay was consumed, how much was consumed and for how long it has been consumed. Nutritional status was assessed using a food frequency questionnaire. Blood was drawn to assess the haematological and iron status of the participants.

The participants of the study were within the required age range, with no significant difference between the groups (p-value=0.7914). The most consumed colour of clay was white and white clay contains kaoline, which has the ability to absorb iron in the duodenum. The majority of the participants consumed 40 grams of clay on a daily basis, with most of the participants having done so for 5 years. Diet was ruled out as the cause of iron deficiency.

The haematological parameters indicated that the geophagic group (43%) were inclined to have hypochromic microcytic anaemia, while a small percentage of control groups (8%) had HMA; this was revealed by the red cell parameters and red cell indices. In addition, the odds ratio for the haematological results revealed that the probability of a geophagic person developing anaemia was two times greater than that of a non-geophagic person. Platelet results partially ruled out bleeding as a cause of anaemia. The median red cell distribution width indicated that the

geophagic group was inclined to have anisocytosis. The geophagic group was found to have iron deficiency (75%), whilst the control group had a small percentage with iron deficiency (22%), which was validated by the serum ferritin, serum iron and saturated transferrin (chemical analysis). The odds ratio revealed that the probability of a geophagic person being iron deficient is 3 times greater than that of a non-geophagic person. The strongest association is seen with iron study findings, because being iron deficient showed the highest odd ratio than the association with red cell morphology and even haemoglobin. Thus, participants were more iron deficient than suffering from iron deficiency anaemia.

Inflammatory and parasitic indicators proved that inflammation and infection was uncommon in both groups, and therefore did not compromise the credibility of the iron study results. Inflammatory indicators (white blood cells, erythrocyte sedimentation rate and C-reactive protein) ruled out inflammation, whilst eosinophil count showed no indication of parasitic infection for both geophagic and control groups.

To conclude, the study found that an association exists between geophagia and haematological parameters of iron deficiency anaemia amongst geophagic women in Qwa-Qwa, in that geophagic material contributes to iron deficiency anaemia.

Keywords: Pica, geophagia, iron deficiency, geophagic material, hypochromic microcytic anaemia

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LIST OF ABBREVIATIONS

CO_2:	carbon dioxide
CRP:	C-reactive protein
DMT-1:	divalent metal transporter-1
Na_2 EDTA:	disodium ethylenediaminetetra-acetic acid
Eosin Ab:	absolute eosinophil count
ESR:	erythrocyte sedimentation rate
FBC:	full blood count
Fe:	Iron
$Fe^{2+:}$	Ferrous iron
Fe^{3+}:	Ferric iron
fl:	femtolitre
g/dl:	grams per decilitre
GI:	gastrointestinal
g/l:	grams per litre
Hb:	haemoglobin
β-HCG:	beta human chorionic gonadotropin
Hct:	haematocrit
HFE:	haemachromatosis gene
HMA:	hypochromic microcytic anaemia
ICSH:	International Council for Standardization in Haematology
IDA:	iron deficiency anaemia
LQ:	lower quartile

Max:	maximum
MCV:	mean cell volume
MCH:	mean cell haemoglobin
MCHC:	mean cell haemoglobin concentration
Min:	minimum
mg:	milligram
mg/l:	milligram per litre
mm/hr:	millimetre per hour
mg/kg:	milligram per kilogram
N:	number
NTBI:	non-transferrin bound iron
O_2:	oxygen
P_{50}:	partial pressure of oxygen in the blood at which the haemoglobin is 50% saturated
PO_2:	partial pressure of oxygen in the blood
PB:	peripheral blood
pg:	picogram
PLT:	platelets
RBC:	red blood cell
Ref range:	reference range
RDW:	red cell distribution width
SD:	standard deviation
UQ:	upper quartile

µg/l:	micrograms per litre
µmol/l:	micromole per litre
WBC:	white blood cells

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Pica is referred to as the craving and compulsive eating of non-food substances for a month or more (Halsted, 1968; Louw *et al.,* 2007; Brand *et al*., 2009). Geophagia, a type of pica, is defined as a self-willing conduct by humans and animals of consuming soil, clay and earth like substances (Halsted, 1968; Hooda *et al*., 2002; Woywodt & Kiss, 2002). Geophagia has been in existence for many years and is practised all over the world (Reilly & Henry 2000; Louba *et al*., 2004; Ekosse *et al*., 2010; Momoh *et al*., 2012). However, the benefits and risks of geophagia remain vague (Woywodt & Kiss, 2002).

The reasons for the poor understanding of geophagia are: lack of awareness by researchers, consumption of different geophagic materials, the covering of geophagia by those who practise it, the judgmental nature of those who study it and research designs that are incapable of answering questions of causality (Young *et al.,* 2007). The rationale for the consumption of geophagic materials could be difficult to comprehend, but some of the reasons why people practise geophagia are as follows: hunger (Ljung *et al*., 2006), culture (Ghorbani, 2008) and health, where geophagia is believed to supplement mineral deficiency (Ghorbani, 2008).

Geophagic materials namely clay and soil differ in texture, taste and colour. Soil colour classification according to the Munsell soil classification (Cleland, 2005), which uses hues, values and chroma sometimes differ with the soil colour noticeable with the naked eye. The colour of the geophagic material is determined by its composition (Ekosse & Anyangwe, 2012). Geophagic clays from Qwa-Qwa are mainly white and white clays contain kaoline and smectite. Yellowish and reddish clays contain iron (Fe) which might be the reason why geophagic individuals consume them as a source of

Fe supplement (Ekosse *et al.*, 2010). Geophagic materials are believed to have possible benefits such as mineral supplementation, especially iron and calcium (Young *et al.,* 2007). However, according to von Garnier *et al.* (2008), kaolinite, which is a component of soil, is able to exchange and absorb cations (Fe^{2+} and Fe^{3+}) in the duodenum where iron absorption occurs. As a result iron deficiency anaemia may be associated with the ingestion of kaoline.

Geophagic materials are believed to have minerals which help with mineral deficiency (Abrahams & Parsons, 1996; Ghorbani, 2008). In addition, it is suggested that the craving could be attributed to a deficiency of nutrients or minerals such as iron, zinc and/or calcium. However, opinions on the effect of geophagy on the supplementation of minerals on geophagists are not consistent. Clinical studies showed that geophagy may alleviate, cause or contribute to mineral deficiency (Halsted, 1968; Brand *et al*., 2009; Ngole *et al.,* 2010; Starn & Udall, 2008; Momoh *et al.,* 2012). Young *et al.,* (2010) suggested that the minerals in the geophagic material are sufficient to augment iron deficiency, but are not bioavailable. von Garnier *et al.* (2008) states that kaoline in the geophagic materials has the ability to absorb and change the iron in the duodenum to a state that it is no longer bioavailable, leading to iron deficiency. Therefore, if kaoline has the ability to absorb iron from the duodenum, will a diet with sufficient iron intake make a difference?

Females are more prone to iron deficiency anaemia as a result of the iron loss suffered during the monthly menstrual cycle. Iron deficiency is the most common cause of anaemia (Hoffbrand *et al*., 2011; 26), and is classified as hypochromic microcytic anaemia (HMA) (Hillman & Ault, 2002; Hoffbrand *et al*., 2007: 23). Dietary iron absorption is normally sufficient to make up for iron loss. Iron is present in food as haem-protein complexes. Of the reasons highlighted regarding the practice of geophagia, the purpose of this study is focused on the health aspect. Whether or not there is a link between geophagia and iron deficiency is not yet established.

1.2 PROBLEM STATEMENT

Geophagy has been commonly associated with the occurrence of anaemia (Halsted, 1968; Ljung *et al.,* 2008), but not with iron deficiency, even though it is implied. Before the casualty issue between iron deficiency anaemia and geophagia can be addressed, it must be established if there is an association between these two variables. It is a known fact that Qwa-Qwa women practise geophagia and this can be attributed to geophagic materials being easily accessible. In 2007, a pilot study was conducted to investigate the relationship, and found that iron deficiency was more prominent in geophagic women in comparison with non-geophagic women (Mogongoa *et al*., 2011). Thus, a need for the study to be performed with a bigger population to confirm the findings of the pilot study was identified.

The lack of information regarding the quantity, frequency, type of geophagic material consumed; the impact of geophagia on haematological parameters and the iron status of the geophagists, made it crucial that the primary existence of the relationship be investigated. Geophagia in humans has been registered in children (Nchito *et al*., 2004) and in pregnant women (Geissler *et al*., 1998; Luoba *et al*., 2004; Ngozi, 2008; Nyaruhucha, 2009). In addition, the relationship between geophagia and haematological parameters of iron deficiency anaemia has not been investigated and defined in South Africa, especially in the case of non-pregnant women. Geophagia always seems be accompanied by the subject of iron deficiency anaemia and especially its prevalence in females, suggesting that geophagia is practised mostly by females. The bigger geophagia project was therefore an ideal opportunity to perform a specific survey on geophagic women.

1.3 THE AIM OF THE STUDY

The aim of the study is to determine whether there was an association between geophagia and haematological parameters of iron deficiency anaemia amongst geophagic women in Qwa–Qwa.

1.4 OBJECTIVES

- To determine the geophagic practices (age of participants, clay colour, frequency of consumption, quantity of clay consumed and nutritional statuses of participants)
- To determine the effect of geophagia on haematological and chemical findings of iron deficiency anaemia.
- To eliminate the influence of inflammatory indicators on the chemical findings of iron deficiency anaemia
- To eliminate the influence of parasitic infection as the cause for iron deficiency anaemia.

1.5 DISSERTATION STRUCTURE

Chapter 2 provides full information in relation to geophagia, iron deficiency anaemia and the link between the two, to help foster a better understanding and interpretation of the study. In Chapter 3 the materials and methods used in the study are explained. The summarised results in a form of tables, graphs and figures are presented in Chapter 4, the discussion of the results is captured in Chapter 5 and Chapter 6 provides the meaning of the findings and recommendations. The references used in this dissertation are contained in Chapter 7.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Pica is not only referred to as the craving and compulsive eating of non-food substances but also the consumption of specific foodstuffs (Louw *et al.*, 2007). Pica involves the consumption of dozens of food and non-food substances, including, hair, paint chips, butter, lettuce, peanuts, ice, paper, cigarette butts and ashes (Reilly & Hendry, 2000; Young *et al*., 2007). Pica in humans has many different subgroups, each defined by the substance that is ingested. Some of the most commonly described types of pica are eating earth, soil or clay (geophagia), ice (pagophagia) and starch (amylophagia) (Bick *et al*., 1993). Pica has been observed in different ethnic groups worldwide, in both primitive and modernised cultures, in both sexes, and in all age groups (Louw *et al.,* 2007).

In 1968 Halsted stated that geophagia is seen as a medical problem, on the other hand it is proven to be a medicament and thought to have been beneficial for 2000 years. The deficiency of trace elements has been paired with clay (Halsted, 1968). Ellis & Schnoes (2006) links severe cases of pica to cases of nutritional deficiency that include iron deficiency. In some countries or even ethnic groups geophagia in women is accepted and passed down generations. It is believed that geophagia is beneficial to women, especially during child their bearing age as a source of iron. Geophagia is missed by doctors and not commonly reported, but when reported about 50% of patients are frequently associated with severe iron deficiency (Bick *et al.,* 1993).

2.2 GEOPHAGIA

Geophagia originates from the Greek words geo-(earth) and phag-(eat) (Thompson, 1995). Geophagia is consuming soil and earth-like substances such as clay and chalk or the uncontrollable consumption of large quantities of earth, soil and clay (Halsted, 1968; Woywodt & Kiss, 2002). Geophagia in some literature is said to be performed especially to augment a scanty or mineral-deficient diet or as part of a cultural tradition (Webster, 2009). Consumption of soil is a common behaviour in children as a means of exploring their surroundings (Ljung *et al.*, 2006). Geophagy is often associated with women, most commonly pregnant women as they have an increased requirement for iron (Nyaruhucha, 2009).

2.2.1 Histological background

The first description of geophagia in Greek literature was during 460-377 BC, credited by Hippocrates (Woywodt & Kiss, 2002). Geophagia has been reported to have been in existence from as early as the fourth century (Ekosse *et al*., 2010). A Roman writer Cornelius Celsus 14-37AD used skin colour as a diagnostic sign: saying that people with bad skin colour and not suffering from jaundice, are geophagic. Moreover, Woywodt & Kiss (2002) used Cornelius' saying as a point that supports the link between geophagia and anaemia. Veryser in Utrecht in 1694 described pica as a mental, mind and stomach disorder (Woywodt & Kiss, 2002). Geophagia was described and written about in many European, African, Asian, North and South American countries in the 18^{th} and 19^{th} century (Woywodt & Kiss, 2002). Many different papers on geophagia in African countries (South Africa, Nigeria, Tanzania, Kenya, Zambia), Asia and in the Americas are published on geophagia (Ellis & Schnoes, 2006).

2.2.2 Epidemiology of geophagia

Abrahams *et al*. (2005) stated that geophagia is a human practice that has been in existence for a long time. From its origins in Africa, geophagia was undertaken by humans and spread across the globe due to slavery, although apparently there are regions such as Japan, Korea, Madagascar and South America where the practice is limited or has not been recorded (Abrahams *et al.*, 2005). Clark (2001) suggested that the earliest evidence on the practice of geophagia was found at the prehistoric site in the local basin in the Kalambo River valley above the famous falls on the border between Tanzania and Zambia. The practice of geophagia occurs worldwide and it is found in Africa, Asia, the Americas, Europe and the Far East. Ellis & Schnoes (2006) states that geophagia is a widespread practice in western Kenya, southern Africa and India. Again it is reported in Australia, Canada, Israel, Uganda, Wales, Turkey and Jamaica. In some countries, like Uganda for example, soil is available for purchase for the purpose of consumption.

Soil ingestion is usually associated with mineral deficiency, bacterial and parasitic infections. Geophagic material might be contaminated with geohelminths parasites: *Ascaris*, *Trichuris* and hookworms (Ghorbani, 2008). Mineral deficiency is the historical and intuitive basis for geophagia as it is believed that geophagia supplements mineral deficiency, especially iron, calcium, magnesium and zinc (Brand *et al.,* 2009). Throughout the world geophagia is practised by all people despite their age, colour, sex, social and economic status (Vermeer & Frate, 1979; Abrahams & Parsons, 1996)

Reports on geophagia indicate a high prevalence of geophagy among certain human groups (Thomson, 1997; Nchito *et al*., 2004). Geophagia is widely described among pre-pubescent children of both genders, and predominantly in post-pubescent females. In South Africa, especially in the eastern Free State, the consumption of clay is common. This is enhanced by mining sites being near residential areas and clay being sold in the market area. Geophagic materials differ

from region to region; even those that practise geophagia prefer to consume a specific type of clay.

2.2.3 Soil classification

Soil colour classification may differ between the description given by people practising geophagia and the scientific classification. In human geophagia, most studies classify soil colour according to the naked eye description of the people practising geophagia and the researchers. Thus it was of great value to this study to perform a scientific soil colour classification to check if the soil colour we see with the naked eye differs from the scientific soil colour classification. For the scientific soil colour classification the Munsell soil colour classification was used. The reason for choosing this soil colour classification is because it is the only one that classifies soil by colour while other soil classifications use texture, profile and structure (Tarau *et al*., 2012). Munsell colour system is straightforward, dependable, flexible and efficient. It organises colour in such a way that it is easy to identify a specific colour.

2.2.3.1 *Munsell classification*

The Munsell colour system is a colour space that specifies colours based on three colour dimensions: hue (principal colours), value (lightness), and chroma (colour purity).

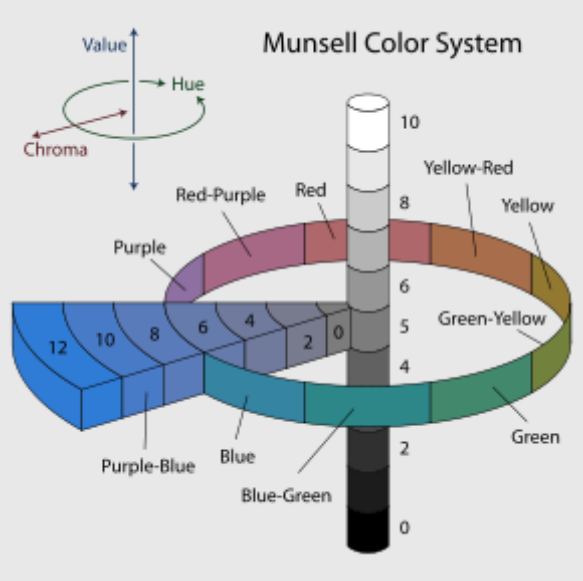

Figure 2.1: The Munsell colour system showing: a circle of hues at value 5 and chroma 6; the neutral values ranges from 0 to 10; and the chroma of purple-blue (5PB) at value 5 (Cleland, 2005).

Hue (refers to the colour of the soil)

Munsell separated the horizontal circles into five key colours (hues): red, blue, purple, green and yellow, along with five intermediate hues halfway between adjacent principle hues for example yellow red - YR (Cleland, 2005). Cleland (2005) added that each of these 10 steps is then broken into 10 sub-steps, so that 100 hues are given integer values (although in practice, colour charts conventionally specify 40 hues rather than 100, in increments of 2.5). Two colours of equal value and chroma, on opposite sides of a hue circle, are complementary colours, and mix additively to the neutral grey of the same value (Cleland, 2005).

Value (refers to lightness of colour)

Value varies vertically along the colour solid, from black (value 0) at the bottom, to white (value 10) at the top. Neutral grey colour lies along the vertical axis between black and white (Cleland, 2005).

Chroma (refers to purity of the colour)

Chroma, measured radially from the centre of each slice, represents the purity of a colour, with lower chroma being less pure (Cleland, 2005). Note that there is no intrinsic upper limit to chroma. Different areas of the colour space have different maximal chroma coordinates (Cleland, 2005). For instance light yellow colours have considerably more potential chroma than light purples, due to the nature of the eye and the physics of colour stimuli (Cleland, 2005). This led to a wide range of possible chroma levels, up to the high 30's, for some hue–value combinations (though it is difficult or impossible to make physical objects in colours of such high chroma, and they cannot be reproduced on current computer displays). Vivid soil colours are in the range of approximately 8 (Cleland, 2005).

Illustration of colour specificity and its meaning:

5P 5/10

Listing of the three numbers for hue, value and chroma is a way of fully specifying colour. For instance, a fairly saturated purple of medium lightness would be 5P 5/10 with 5P meaning the colour in the middle of the purple hue band, 5/ meaning medium lightness, and a chroma of 10 (Cleland, 2005). This study makes use of the key colours (hues): red, blue, purple, green and yellow (Cleland, 2005). These colours will be presented in the results chapter.

2.2.3.2 *Types of clay consumed*

Geophagic materials are found in all sorts of shapes and sizes, and these differ from region to region and from country to country. Preference of choice in clays include colour and texture (Ekosse *et al.*, 2010). Some prefer the clay a bit powdery, while some others prefer the clay in rock form. These clays even differ in taste; some are tasteless while some are sour (Reilly & Henry, 2000). The clays differ in appearance as shown in Figure 2.2. In a country there can be more than one type of clay consumed by the inhabitants, depending on the different ethnic groups and availability. The geophagic materials are picked or mined from carefully selected

areas, for example termite moulds, pits, riverbanks or house walls (Reilly & Henry, 2000).

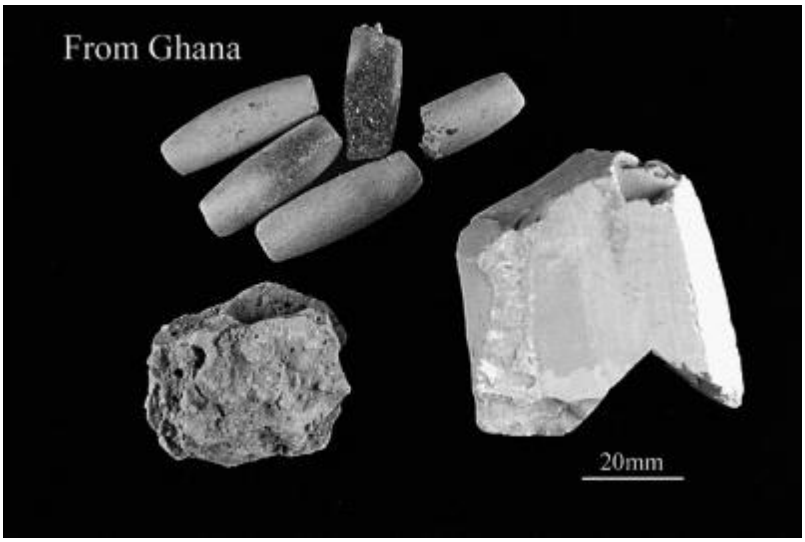

FIGURE 2.2: Soil samples taken from the markets in Ghana (Reilly & Henry, 2000) & Qwa-Qwa

2.2.3.3 Chemical composition of geophagic material

Geophagic materials consumed contain clay minerals, which are secondary minerals derived from chemical alterations of mostly feldspars and micas (Ekosse *et al*., 2010). Ngole *et al*. (2010) found that the colour of the soils infers the presence of iron (Fe) and other related cations, which may not be bioavailable in the gastrointestinal (GI) tract. Kikouama *et al*. (2009) stated that white clays contain kaolin and smectite, with yellowish clays containing goethite. White/khaki soft

geophagic clays preferred by most of the respondents of the study done in the Limpopo and Free State, South Africa are dominated by kaolinite and/or smectite (Ekosse *et al*., 2010). Moreover yellowish and reddish clays contain Fe, which might be the reason for their consumption. Kaolinite has the ability to change iron to a state which the body cannot utilize, at the same time absorbing the iron in the duodenum. This deprives the body of iron, thus leading to iron deficiency anaemia (Kikouama *et al*., 2009).

2.2.4 Reasons for geophagia

The different types of clays consumed are influenced by different properties, such as texture, colour, smell, flavour and the reasons for consumption. For example, white clay is commercially sold as a remedy for diarrhoea (Ljung *et al.,* 2006). Those that practise geophagia have different reasons for the practice, reasons which vary from cultural, socio-economic, religious, the geophagic materials' texture, taste and colour, while some believe it to have health benefits.

2.2.4.1 *Cultural explanation*

The reference to soil as dirt makes geophagia unacceptable in some cultures (Nchito, 2004; Ghorbani, 2008). On the contrary some look at it as a gift from the ancestors and consumption is seen as a sign of fertility (Ghorbani, 2008). It is suggested that the origin of geophagy may be based on the fertility of the earth, thus women eat soil before, during and after pregnancy (Abrahams *et al.,* 1995). In Malawi, geophagic practice is seen as a sign of pregnancy in women (Ghorbani, 2008).

2.2.4.2 *Religious explanation*

Geophagia is a common practice in black women in the southern parts of the United States. Geophagia is commercially exploited in United States-Mexican border towns, where cakes of clay imprinted with impressions of Christ are sold for children to suck on (Bick *et al.,* 1993). In Guatemala clay briquettes with cathedral designs on them are sold to pregnant mothers (Bartas & Ekman, 2001).

2.2.4.3 *Social and economic explanation*

In developing countries, earth is mostly consumed due to poverty (Ghorbani, 2008). Some people consume earth because they have nothing else to eat, thus earth makes them full and is used as a food detoxifier during famine (Ljung *et al.*, 2006). In a study done in Tanzania, it was found that geophagia is mostly practised by females, despite their social economic status, thus hunger is rules out as the core cause of geophagia (Young *et al*., 2010). In Southern Africa, especially in Lesotho, Swaziland and South Africa, especially the Eastern Free State, vendors sell soil as a means of earning an income.

FIGURE 2.3: Vendors selling clay for money in Qwa-Qwa

2.2.4.4 *Health explanation*

Among the different reasons people give for practising geophagia, the health reason is very prominent. The main reasons for human geophagic behaviour are: the detoxification of noxious substances, alleviation of gastrointestinal upset, such as diarrhoea, supplementation of mineral nutrients and the alleviation of excessive acidity in the digestive tract (Tateo & Summa, 2006). Most people that practise geophagia claim that it is because earth or clay gives them the minerals that they need (Abrahams *et al.*, 2005). Minerals claimed to be found in soil are calcium, iron

and magnesium (Abrahams *et al.*, 2005). This is the reason why most people who consume earth or clay are females, especially during child bearing age, pregnancy and lactating women. In general, females during the child bearing years are often diagnosed with iron deficiency anaemia.

A negative aspect is that dirt or earth eating can cause infections such as parasitic infections (Ellis & Schnoes, 2006). Some literature views it as a consequence of poverty and famine; on the other hand some researchers state that there is a link between geophagia and iron deficiency anaemia (Bartas & Ekman, 2001). It is still unclear, however, whether anaemia prompts geophagia (to compensate for iron deficiency) or whether geophagia is the cause of anaemia (Nchito *et al.*, 2004). It is conventionally assumed that geophagia may help supplement mineral nutrients in individuals with a limited intake of trace elements such as iron (Hooda *et al*., 2002). This hypothesis is based on the bulk nutritional composition of geophagic material and the assumption that these nutrients are potentially available for absorption in the body (Hooda *et al.,* 2002). However, geophagic materials contain kaoline which is believed to instigate iron deficiency as it absorbs iron in the duodenum, thus leading to iron deficiency (von Garnier *et al*., 2008). Geophagia is associated with iron deficiency anaemia. Barton *et al.* (2010) stated that where there is geophagia there is iron deficiency anaemia.

2.3 IRON DEFICIENCY ANAEMIA

Anaemia is defined as the lowering of the haemoglobin concentration below the established normal levels (Hoffbrand *et al*., 2007: 20). It can be due to increased destruction or decreased production of red blood cells (Besa *et al*., 1992; Nabili, 2011). Anaemia is dependent on the haemoglobin concentration; taking in consideration the age, sex and altitude of residence (Lombard, 2009). Anaemia, whether due to iron deficiency or not, is primarily quantitative. Furthermore, anaemia is a clinical rather than diagnostic sign (Bick *et al.,* 1993: 258). A shortage of iron leads to a limited production of haemoglobin, which affects the production of red blood cells over time (Frantz, 2007). Iron deficiency is the most common cause of anaemia worldwide. Moreover, iron deficiency can develop independent of anaemia (Hoffbrand *et al*., 2007: 28). Iron deficiency is associated with geophagia and may be under-diagnosed (von Garnier *et al.,* 2008). Geophagia is a good indicator for iron deficiency, yet often goes unrecognised (Louw *et al.*, 2007).

2.3.1 Dietary iron and distribution of body iron

Iron is present in food as ferric hydroxides, ferric-protein and haem-protein complexes. The absorption of iron differs from food to food, although meat is a better source of iron than vegetables (Hoffbrand *et al.,* 2007: 31). Only 5-10% of iron is absorbed from a normal Western diet, which contains 3-5mg of iron (Harmening, 1992: 81; Hoffbrand *et al.*, 2007: 31; Rodak *et al.,* 2007: 117). In an adult the concentration of iron is 50mg/kg in males and 40mg/kg in females, of blood containing about 2000mg of iron (Hoffbrand *et al.*, 2011: 26). The remaining iron is contained in the storage proteins ferritin and haemosiderin, found mainly in the reticuloendothelial cells of the liver, spleen and bone marrow (Hoffbrand *et al.*, 2011: 26; Rodak *et al.,* 2007: 117). Myoglobin accounts for 4-5% of body iron (Hoffbrand *et al.,* 2011: 27).

2.3.2 Proteins important in iron storage and transport

Transport and storage of iron is mostly mediated by proteins: haemoglobin, transferrin, ferritin and transferrin receptors.

- Haemoglobin

  The main function of red blood cells (RBC) is to carry oxygen (O_2) to tissues and return carbon dioxide (CO_2) to the lungs from the tissues. This is due to the haemoglobin (Hb) that can be found inside the RBC. Iron is a crucial element in Hb synthesis (Hoffbrand *et al.,* 2007: 17; Rodak *et al.*, 2007: 106-108). Haemoglobin contains four haem groups linked to four globin chains and can bind to four oxygen molecules. Iron is important for haem synthesis as it links together the haem structure.

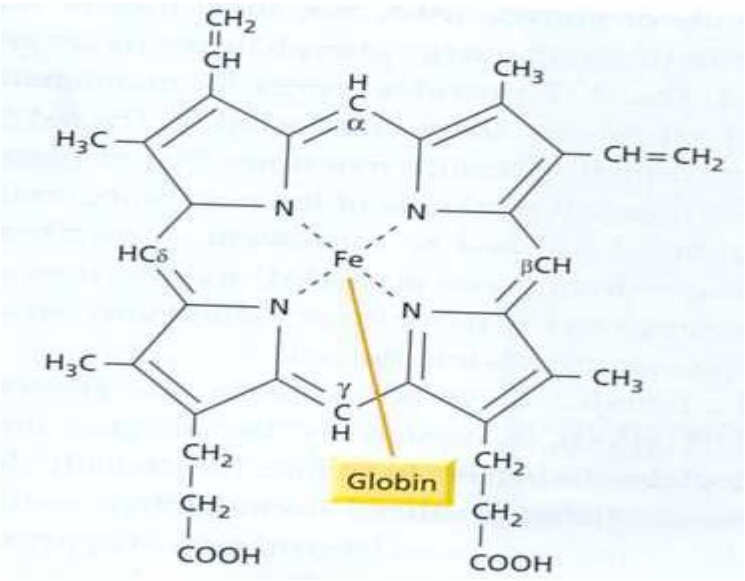

Figure 2.4: The structure of haem (Hoffbrand *et al.,* 2007: 17).

- Transferrin

  Transferrin is predominantly synthesised by the liver, synthesis being inversely related to iron stores. It can contain up to two atoms of iron. It delivers iron to tissues that have transferrin receptors, especially erythroblasts in the bone marrow, which incorporate the iron into haemoglobin (Hoffbrand *et al.,* 2007: 28).

- Ferritin

Ferritin is a primary iron storage protein and provides a reserve of iron. It is a water-soluble protein-iron complex. It contains up to 20% of its weight as iron (Hoffbrand *et al.,* 2007: 27).

- Transferrin receptors

Transferrin receptors sense the degree of saturation of transferrin, and also transports iron into tissue (Hoffbrand *et al.,* 2007: 31).

2.3.3 Iron absorption

Absorption of iron occurs in the duodenum and depends not only on the amount of iron in the diet, but also the bioavailability of iron as well as the body's needs for iron (Hoffbrand *et al.,* 2011: 33). Organic iron is partly absorbed as haem and partly broken down in the gut to inorganic iron (Hoffbrand *et al.,* 2007: 32). Non-haem iron is released from food as Fe^{3+} and reducing agents (cytochromes b_1) reduce iron to Fe^{2+}. Iron is transferred from the lumen of the gut across the enterocyte microvilli by divalent metal transporter-1 (DMT-1) (Hoffbrand *et al.,* 2007: 32; Rodak *et al.*, 2007: 117). In the duodenal enterocyte, dietary iron is reduced to the ferrous state by duodenal ferric reductase (Dcytb), transported into the cell by the divalent metal transporter-1 (DMT-1), and released by way of ferroportin into the circulation (Morgan & Oates, 2002).

The movement of iron from the cells into the plasma is regulated by ferroportin at the basolateral surface (Figure 2.5). Hepatocytes take up iron from the circulation, either as free iron or transferrin-bound iron (through transferrin receptor 1 and transferrin receptor 2) (Morgan & Oates, 2002). Transferrin receptor 2 may serve as a sensor of circulating transferrin-bound iron, thereby influencing the expression of the iron regulatory hormone hepcidin. The hepcidin response is also modulated by human hemochromatosis gene (HFE) and hemojuvelin. Hepcidin is secreted into the circulation, where it down-regulates the ferroportin-mediated release of iron from enterocytes, macrophages, and

hepatocytes (Morgan & Oates, 2002). However, in iron deficiency less iron is delivered to the crypt cells, which results in increased expressions of DMT-1 (Hoffbrand *et al.,* 2009: 77).

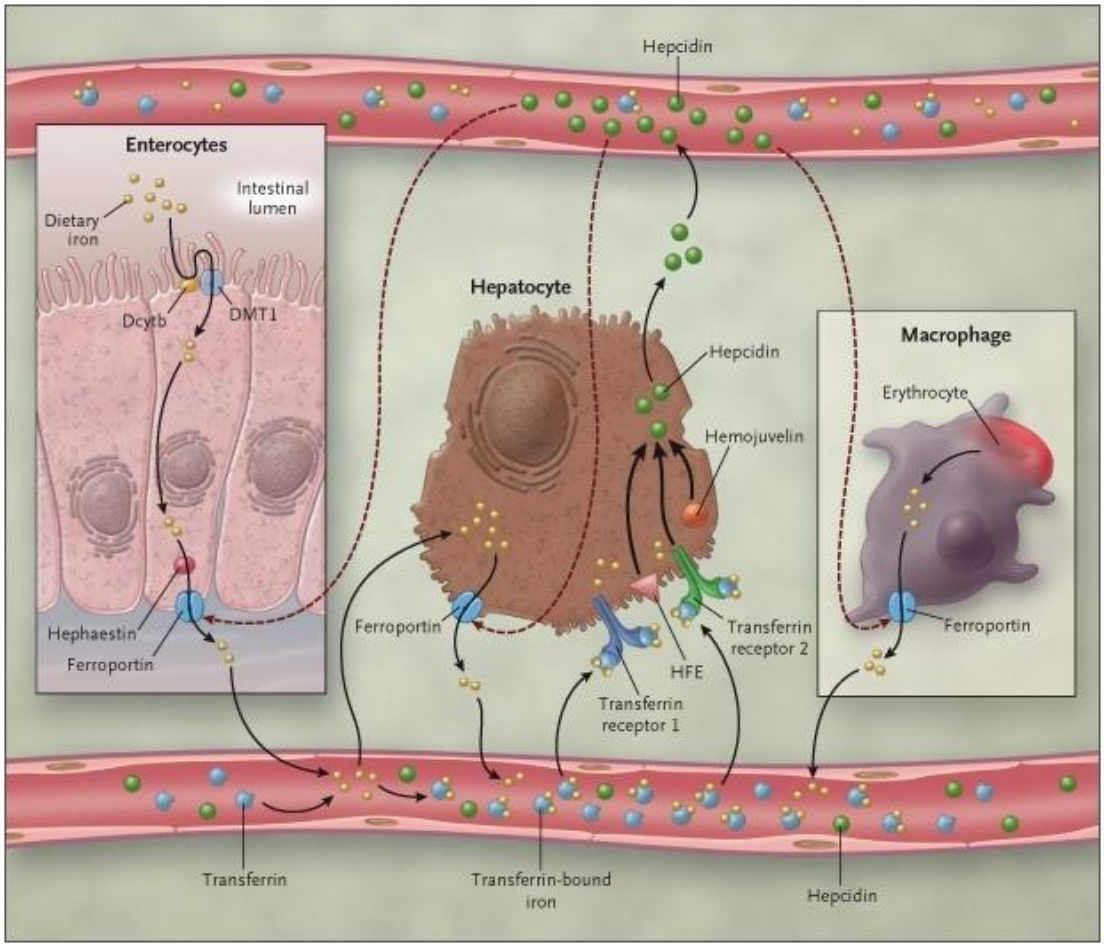

Figure 2.5: Regulation of iron absorption (Fleming & Bacon, 2005)

2.3.4 Iron uptake and haem synthesis

Most of the transferrin iron (85%) normally enters developing red blood cells for incorporation into haemoglobin (Hoffbrand *et al.,* 2011: 34; Rodak *et al.*, 2007: 119). Transferrin receptors concentration is inversely proportional to the amount of iron in the cell (Hoffbrand *et al.,* 2011: 34-35). Iron is released from the

endosome and transported into the mitochondria by mitoferrin or enters ferritin (Rodak *et al.*, 2007: 117; Hoffbrand *et al.,* 2011: 35). Haem synthesis occurs largely in the mitochondria by a series of chemical reactions. The mitochondria are the main sites of protoporphyrin synthesis, iron is supplied from circulating transferrin, globulin chains are synthesised on ribosomes (Figure 2.6). Ultimately protoporphyrin combines with iron in the ferrous state to form haem, each molecule of which combines with a globulin chain (Hoffbrand *et al.,* 2007: 16).

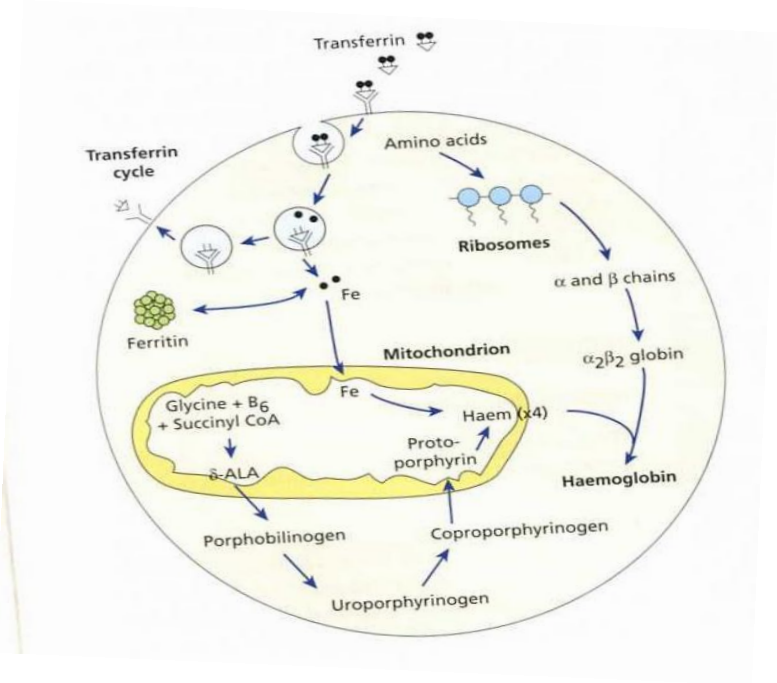

Figure 2.6: Haemoglobin synthesis in a developing red cell (Hoffbrand *et al.,* 2007: 16).

2.3.5 Normal red blood cell breakdown

The breakdown of red blood cells frees haem from red cells and liberates iron for circulation through transferrin. Iron coupled with transferrin is used in the synthesis of haem. As demonstrated in the Figure 2.7 below (Hoffbrand *et al.,* 2011: 74).

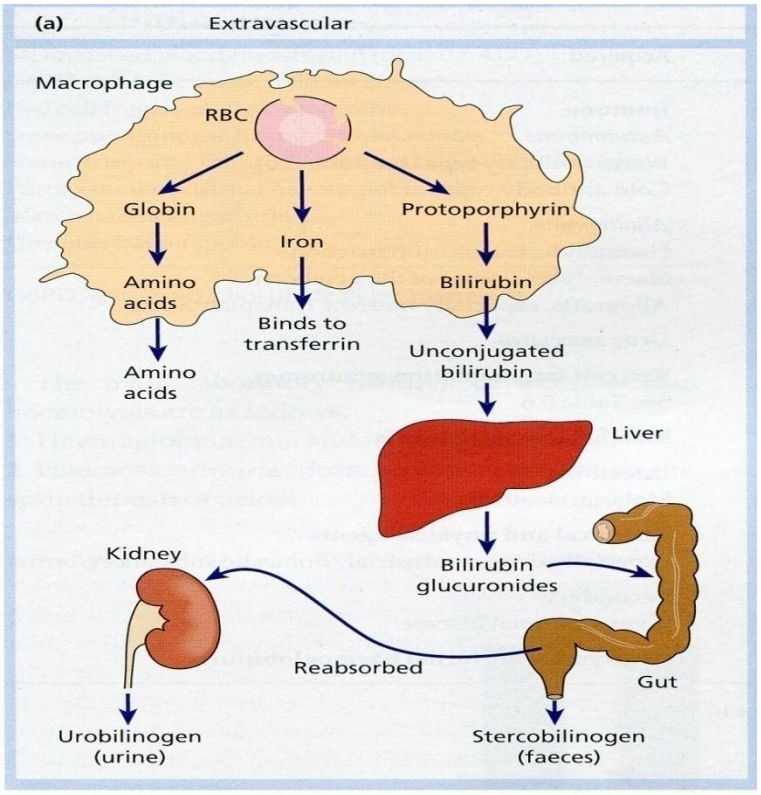

Figure 2.7: Normal red blood cell breakdown (Hoffbrand *et al*., 2007: 59)

2.3.6 Causes of iron deficiency anaemia

Iron deficiency is caused when the rate of loss or use of iron exceeds its rate of uptake and assimilation (Lombard, 2009). Iron deficiency causes microcytic (small red cells), hypochromic (pale red cells) red blood cells, which are measured by measured by the red cell indices mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) respectively. Hypochromic microcytic anaemia can be caused by a defect in the haemoglobin synthesis, such as a lack of iron or decreased iron release from macrophages, failure of protoporphyrin or globin chain synthesis as shown in Figure 2.8 (Hoffbrand *et al*., 2007: 28).

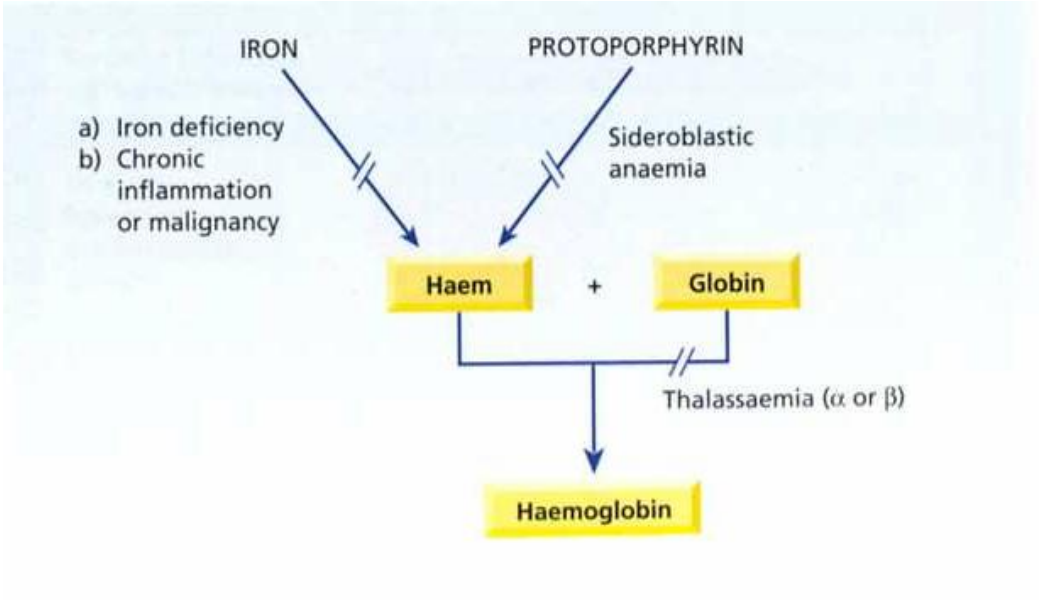

Figure 2.8: The causes of hypochromic microcytic anaemia (Hoffbrand *et al*., 2007: 28)

Although there are many reasons for the development of iron deficiency, the four most common ones are malnutrition, malabsorption, chronic blood loss and an increase in iron demand.

Malnutrition: Nutritional deficiency due to insufficient consumption of iron to meet the body's daily requirement (Hoffbrand *et al*., 2011: 20). This could be due to poverty, hunger and socio-economic status.

Malabsorption is faulty or incomplete iron absorption. For example, poor iron absorption in gluten-induced enteropathy causes iron deficiency (Hoffbrand *et al*., 2011: 40). As some of the geophagic material contains kaolinite, which changes and absorbs iron in the duodenum, it leads to iron deficiency.

Chronic blood loss occurs commonly due to heavy menstruation and chronic haemorrhages, such as females who experience excessive bleeding, which in turn will lead to an increased iron demand by the body (Turgeon, 1993: 99; Lombard, 2009; Hoffbrand *et al*., 2011: 39-40).

Increased iron demand occurs mostly in pregnant woman, infants, adolescents and menstruating women. They have an increased iron demand, and are thus at risk the risk of developing anaemia. An example is a menstruating woman that loses a lot of blood, which results in a decreased iron and increased demand.

2.3.7 Symptoms and signs of iron deficiency anaemia

Symptoms of chronic anaemia are weakness, tiredness, tachycardia, possibly chest pains and shortness of breath (Harmering, 1992; Lombard, 2009).

As iron deficiency anaemia develops, a patient may develop specific symptoms and signs.

- The tongue may appear smooth, shiny and inflamed (glossitis).
- The mouth may appear eroded, swollen, and tender at the corners.
- Pica, craving for strange foods such as clay (geophagia) and ice (phagophagia) may develop (Harmering, 1992; Lombard, 2009).
- Decreased appetite (Frantz, 2007).

The presence or absence of signs and symptoms can be considered under four major headings, namely: speed of onset, severity, age and haemoglobin oxygen dissociation curve adjustment.

Speed of onset: rapidly developing anaemia causes more symptoms than anaemia that develops slowly, because there is less time for the cardiovascular system to adjust.

Severity: mild anaemia often produces no signs and symptoms, but symptoms are usually present when the haemoglobin is below the normal reference range.

Age: the elderly body does not tolerate anaemia as well as young people do, because of the effect a lack of oxygen has on the organs when normal cardiovascular compensation is impaired.

Haemoglobin oxygen dissociation curve: in general, anaemia is associated with a rise of 2,3-diphosphoglycerate in red cells, and a shift in the dissociation curve to the right, so that oxygen is given up more readily to tissues. When there is a shortage of oxygen, the β chains are apart, allowing entry of the metabolite 2,3-diphosphoglycerate, resulting in a lower affinity of the molecule for O_2 and causing the sigmoid form of the haemoglobin O_2 dissociation curve (Figure 2.9). An increase

in O_2 affinity causes the curve to move to the left (P_{50} falls), and a decrease to O_2 affinity causes the curve to move to the right (P_{50} rise) (Hoffbrand *et al.,* 2007: 17).

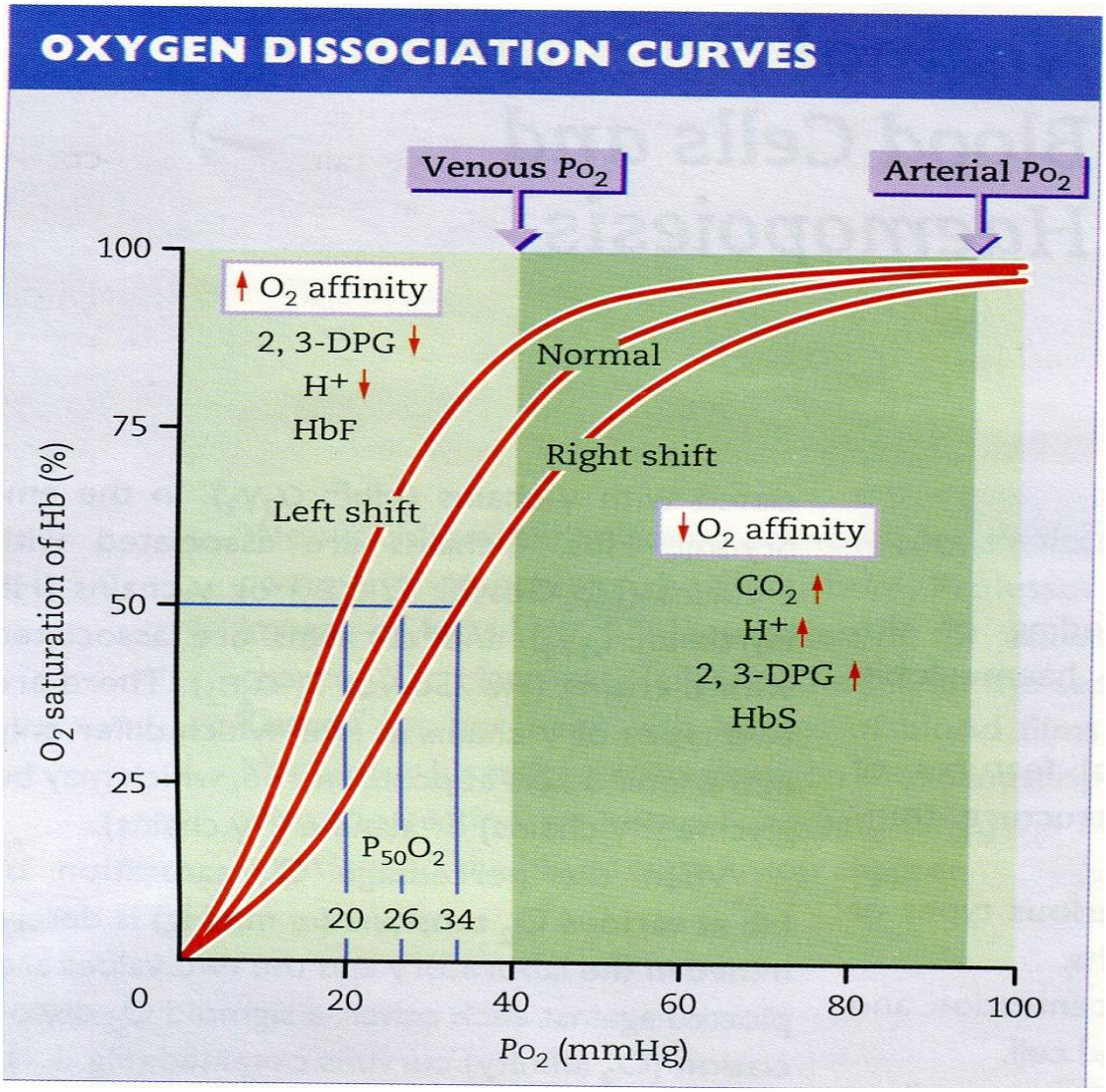

Figure 2.9: The haemoglobin oxygen dissociation curve (Hughes-Jones *et al.,* 2004: 2)

2.3.8 Stages in the development of iron deficiency anaemia

von Garnier *et al.* (2008) states that ingestion of geophagic materials which contain or is composed of kaolinite, cause the exchange and absorption of cations (for example, Fe^{2+} and Fe^{3+}) in the duodenum, where iron absorption occurs. Geophagia may cause iron deficiency anaemia because of impaired iron absorption (Starn & Udall, 2008). Extreme stages of iron deficiency can lead to iron

deficiency anaemia. When the iron stores in the body are depleted and red blood cells are microcytic and hypochromic, this is called iron deficiency anaemia (Lombard, 2009). Geophagia is not always linked to iron deficiency anaemia, but also with anaemia. Nchito *et al.* (2004) found an association with iron by measuring the ferritin levels, although the haemoglobin did not correlate this finding. Thus, one can become iron deficient without having iron deficiency anaemia.

STAGE 1 (LOW INTAKE OF IRON)

Iron intake is lower than the loss in this stage, and continuation leads to the iron stores depletion. Due to the decrease of iron in the stores, the body compensates by increasing iron absorption in the diet, thus increasing the iron binding capacity of the cells (Lombard, 2009). Lichtin (2008) states that this stage is characterised by lowered bone marrow iron stores, while the haemoglobin (Hb) and serum iron stay normal. The serum ferritin levels, however, fall to <20ng/mg. The compensatory increase in iron absorption causes an increase in iron binding capacity.

STAGE 2 (DEPLETION OF STORES)

The availability of iron in the stores for the formation of red blood cells is low (Lombard, 2009), and erythropoiesis is impaired. Although the transferrin level is increased, the serum iron level and transferrin saturation decreases. Erythropoiesis is impaired when serum iron falls to <50µg/dL (<9µmol/L) and transferrin saturation to <16%. The serum transferrin receptor level rises (>8.5mg/L) (Lichtin, 2008).

STAGE 3 (MORPHOLOGY OF RED CELLS IS NORMAL)

The morphology of red cells looks normal, even though anaemia has developed, making it normochromic normocytic anaemia (Lichtin, 2008; Lombard, 2009). The conversion of protoporphyrin to haem stops, and thus the haemoglobin synthesis is impaired (Besa *et al.*, 1992).

STAGE 4 (MORPHOLOGICAL CHANGE OF RED CELLS)

Morphology of the red blood cells has changed to microcytic and hypochromic (Lombard, 2009). The change of the red cells occurs because of the reduction in mean cell haemoglobin (MCH) and the reduced mean cell volume (MCV). The reason for this is because of the continued iron stores depletion (Besa *et al.*, 1992).

STAGE 5 (IRON DEFICIENCY ANAEMIA)

Prolonged iron depletion causes a decrease in intra-cellular levels of iron dependent enzymes, thus causing the tissue to change (Besa *et al.*, 1992). This causes the symptoms and signs of iron deficiency to become evident. Iron deficiency has affected the tissues (Lombard, 2009).

2.4 LABORATORY DIAGNOSIS OF IRON DEFICIENCY ANAEMIA

A laboratory diagnosis of IDA is two-tailed: haematological analysis, which is useful in detecting anaemia, and biochemical analysis, which confirms iron deficiency.

2.4.1 Haematological analysis

Haematological analysis is undertaken to check for the presence of anaemia. Studies done on geophagic women mostly link geophagia with anaemia, but never really classify the anaemia into hypochromic microcytic anaemia or even link it to iron deficiency anaemia (Abrahams *et al*., 2005; Louw *et al*., 2007; Ghorbani, 2008). Iron deficiency anaemia is classified as hypochromic microcytic. With iron deficiency anaemia it is important to perform the full blood count, with emphasis on the red blood cell count (RBC), haemoglobin (Hb), mean cell haemoglobin (MCH), mean cell volume (MCV) and morphology of the blood smears (Turgeon, 1993: 101). Haematological analysis can be divided into sub-headings, red cell parameters, red cell indices and other counts. The analysis of red blood cell parameters and indices are the first phase in the diagnosis of iron deficiency anaemia.

2.4.1.1 *Red cell parameters*

Typically iron deficiency anaemia will exhibit the following characteristics in the laboratory (Turgeon, 1993:102): the haemoglobin will be reduced, as iron is a building block for haemoglobin, thus a decrease in iron causes a decrease in haemoglobin, which in turn affects the haematocrit (Hct) (packed cell volume). In addition, a decrease in haemoglobin signifies anaemia. While the red blood cell may initially be normal, it will decrease as the iron deficiency continues.

2.4.1.2 *Red cell indices and red cell distribution width*

In the case of iron deficiency anaemia red cell indices demonstrate a decrease in mean corpuscular volume (MCV), thus indicating that the cells are microcytic. Mean corpuscular haemoglobin (MCH) and the mean corpuscular haemoglobin concentration (MCHC) are also decreased in iron deficiency anaemia, which indicates the haemoglobin concentration in and colour of a cell – thus the hypochromia of the anaemia. The red cell distribution width (RDW) may increase, showing a variation in the cell size (Sultana *et al.,* 2011).

2.4.1.3 *Peripheral blood smears*

Examination of blood smear may show a hypochromic, microcytic red cell, confirming the decreased MCV and MCH. This type of anaemia may initially be normochromic normocytic, or may present with anisocytosis (different sizes of red blood cells) and hypochromia; all manifestations of the anaemia will exhibit both the hypochromic and microcytic red blood cells (Turgeon, 1993: 101). Other morphological findings are occasional target cells and pencil-shaped poikilocytes, with a low reticulocyte count.

2.4.1.4 *Other blood counts and inflammatory indicators*

Other tests performed to investigate bleeding and parasitic infestation and inflammation include platelets, eosinophil count, erythrocyte sedimentation rate (ESR) and C - reactive protein (CRP). Platelet count is done to partially rule out

bleeding, as bleeding is a loss of blood resulting in iron deficiency. White cell count and eosinophil count are done to partially rule out inflammation and infections. All the three are expected to be normal, while reticulocytes maybe decreased or normal (Briggs & Bain, 2011). The erythrocyte sedimentation rate and C-reactive protein (CRP) are used to rule out inflammation and malignant diseases, which cause anaemia of chronic disorders (Hoffbrand *et al*., 2007: 320).

2.4.2 Chemical analysis

Chemical analysis is utilised to classify the kind of anaemia that is dealt with, as many disorders can lead to hypochromic microcytic anaemia (HMA). Clinical chemistry analysis is done to assess the iron status (Bermejo & Garcia-Lopez, 2009). The analyses include serum iron, total iron binding capacity, transferrin saturation and serum ferritin (Bermejo & Garcia-Lopez, 2009).

2.4.2.1 *Serum iron*

Serum iron gives an estimate of iron being carried in the blood, by measuring the iron bound to transferrin, but does not give an accurate iron concentration in the body's cells (Worwood & May, 2011). In iron deficiency anaemia serum iron is reduced. (Hillman & Ault, 2002: 54-55).

2.4.2.2 *Ferritin*

Ferritin is a protein in the body that binds to iron; most of the iron stored in the body is bound to ferritin. The amount of ferritin in the body shows how much iron is stored in the body. A decrease in ferritin levels often indicates that an iron deficiency is present (Hillman & Ault, 2002: 54-55). Ferritin levels are affected by infection and inflammation, thus it is important to rule them out.

2.4.2.3 Transferrin

Transferrin is a plasma protein that transports iron through the blood. The blood transferrin level is tested for diverse reasons: to determine the cause of anaemia, to examine iron metabolism (for example, in iron deficiency anaemia) and to determine

the iron-carrying capacity of the blood (Worwood & May, 2011). Low transferrin can impair the haemoglobin production (since iron is needed in haemoglobin production), and so doing lead to anaemia. Low transferrin can occur due to poor production of transferrin by the liver or excessive loss of transferrin (Worwood & May, 2011). Many conditions, including infection and malignancy, can depress transferrin levels. The transferrin is abnormally high in iron deficiency anaemia.

2.4.2.4 *Transferrin saturation*

Transferrin saturation reflects the ratio of serum iron to the total iron-binding capacity (Worwood & May, 2011). This value indicates how much of the transferrin that is available to bind serum iron, is actually bound to iron (e.g. 40% means that 40% of free iron is being carried by transferrin). When the capacity of transferrin is exceeded, non-transferrin-bound iron (NTBI) is produced (Figure 2.10). Less than 10% of transferrin saturation is indicative of iron deficiency anaemia (Hillman & Ault., 2002: 23)

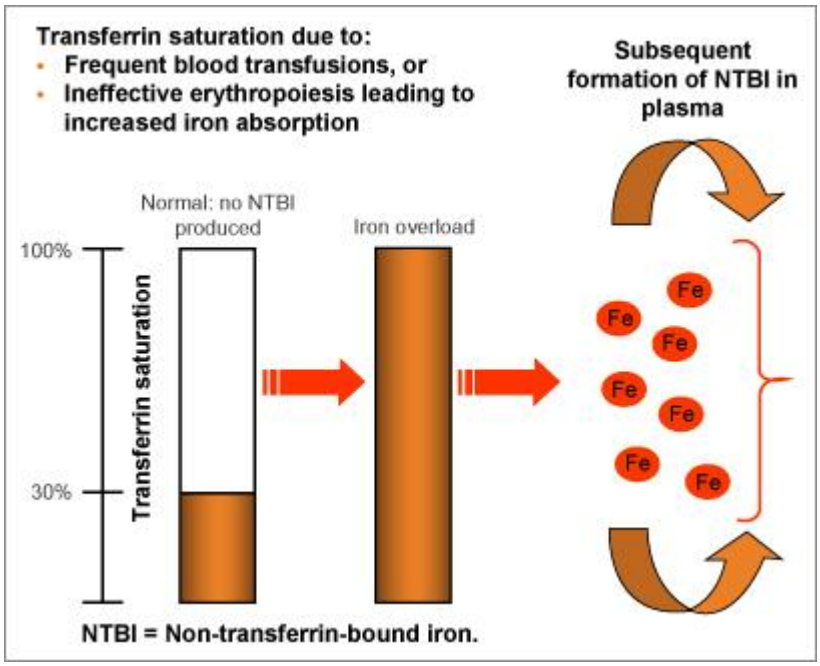

Figure 2.10: Formation of NTBI at higher transferrin saturation levels. http://www.ironhealthalliance.com/diagnostics/transferrin-saturation-measurement.jsp [Accessed 19 October, 2013])

The assessment of transferrin saturation levels has received some interest in recent years. Further evaluation of these techniques is required to validate the methodology and to describe the relationship with patient prognosis.

CHAPTER 3

MATERIALS AND METHODS

3.1 INTRODUCTION

Qwa-Qwa is located in the eastern Free State, about 380 kilometres north-east of Bloemfontein. Several trips were made during the recruitment phase of this study to identify voluntary participants, with two trips made for the collection of blood specimens. Due to the distance between Bloemfontein and Qwa-Qwa accommodation had to be acquired. Permission was requested from the guesthouse owners to establish a temporary laboratory for some of the haematological analysis and blood separation at the accommodation sites. Laboratory analyses were performed in accordance with pathology laboratory requirements, and all medical waste was returned to the Central University of Technology, Free State (CUT) to be discarded according to the medical waste requirements.

3.1.1 Ethical consideration

This study was submitted to and approved by the Ethics Committee of the University of the Free State, reference number ETOVS 104/08. The study was conducted according to “International Good Clinical Practice Guidelines” and adhered to the contents of the Declaration of Helsinki (WMA General Assembly). Each participant was given an identification number to ensure patient confidentiality. In addition, during statistical analysis and data capturing no names were used rather the allocated participant number.

3.1.2 Study design

This was a cross-sectional case control study, consisting of a control and geophagic group. The participants were randomly recruited from sub-urban areas of Qwa-Qwa. The participants were from the same household or living under the same

environmental conditions. The recruitment was done by a field worker by means of a questionnaire months in advance, however, some participants were recruited during blood collection. Blood samples were collected on two different visits to Qwa-Qwa.

3.1.3 Study population

Months before the initial blood collection, the field worker made several trips to Qwa-Qwa informing and recruiting participants around the area for the project. Two groups of female volunteers residing permanently in Qwa-Qwa were recruited to participate in this study.

3.1.4 Sample size

A total of 86 female participants complied with the selection criteria and blood samples were obtained from them. The first group consisted of 37 females who did not practice geophagia as a result representing the control group while the second group consisted of 49 females who practiced geophagia thus representing the geophagic group.

The inclusion criteria for the control group were as follows:

- Female not practising geophagia
- Females between the ages of 18-45 years
- Female residents who resides permanently of Qwa-Qwa

The inclusion criteria for the geophagic group were as follows:

- Female practising geophagia (duration for soil consumption was at least a month)
- Females between the ages of 18-45 years
- Female permanent residents who resides permanently in Qwa-Qwa

The exclusion criteria for both the geophagic and control groups were:

- Non-permanent residents of Qwa-Qwa
- Lactating women
- Pregnant females (beta human chrorionic gonadotropin (βHCG) was performed on all samples to rule out pregnancy)

3.1.5 Participants identification

Recruitment questionnaires (Appendix A) were completed by participants, with assistance from the fieldworker, in their language of preference. These questionnaires obtained information about the type of soil consumed, the period of consumption, quantity consumed as well as the colour of soil.

3.1.6 Participants informed consent

After identification of participants; the scientific importance, expectations and risks of the research were explained to the participants in their language of preference by the field worked (Appendix B – research information document). Once the field worker had finished explaining, the participants were given the opportunity to reiterate to the field worker what they understood about the study, thus giving the field worker an indication whether the participants completely understood their involvement. Moreover, the participants were given an opportunity to ask questions. From there on, the field worker handed the participants the consent form (Appendix C) for the participant to read through and ask questions, then give their consent if they agreed.

3.2 MATERIALS

At the offset of the project, a number of workshops were held by members of a geophagic research panel, during which a questionnaire was compiled for the recruitment of subjects. This questionnaire was used by the fieldworker to identify subjects for the geophagic and control groups. The researcher only became involved once the collection of blood specimens started. Seeing that this section of the project

involved the haematological results, those apparatus routinely used in the haematology laboratory are listed in the next section.

3.2.1 Apparatus / Instruments / Consumables

The apparatus used during the procedures were in good working condition and calibrated to give accurate results. Table 3.1 lists the apparatus, tests performed and the supplier, while Table 3.2 lists the control and reagents used to calibrate and operate the instruments.

TABLE 3.1: Instruments used during the procedures

Instruments	Test performed	Supplier
ABX Pentra 60	Full blood count	The Scientific Group
Primo Star light microscope	Peripheral blood smear morphology	Zeiss
HEMA-TEK 2000 slide stainer	Peripheral blood smear	Siemens

TABLE 3.2: Controls and consumables used during the procedures

Controls/ reagents	Catalogue No	Supplier
ABX control L	2062207	The Scientific Group
ABX control H	2062208	The Scientific Group
ABX control N	2062203	The Scientific Group
ABX Diluent	0901020	The Scientific Group
ABX Eosinofix	0206010	The Scientific Group
ABX Basolyse	0906003	The Scientific Group
ABX Alphalyse	0906013	The Scientific Group
ABX Cleaner	0903010	The Scientific Group
HEMATEK Stain pack	4405	Siemens
Sediplast ESR tubes	10191	Lasec

3.3 METHODS

Seeing that this study was part of a bigger research project, some of the laboratory analyses were performed by the researcher and some by colleagues involved in the bigger project. Chemical analysis, specifically iron studies and C-reactive protein

(CRP), were performed by Ms van Wyk at the Chemical Pathology Laboratory, National Health Laboratory Service – Universitas Tertiary Hospital, Bloemfontein. Nutritional analyses were performed by Ms van Onselen, soil classification according to Munsell soil classification was done by Ms Smith. The researcher administered the questionnaire to the participants, helped in the collection of the samples, prepared samples for chemical analysis, performed full blood counts and erythrocyte sedimentation rate on all the samples collected.

3.3.1 Specimen collection

For haematological analyses, 4.0ml disodium ethylene diaminetetra-acetic acid (Na_2EDTA) blood was collected for full blood counts, erythrocyte sedimentation rate (ESR), and peripheral blood smears. For iron studies and other biochemical analyses, 7.5ml clotted blood was collected. Phlebotomy was performed by qualified medical technologists who are experienced in phlebotomy, following the standard phlebotomy procedure as routinely used at the haematology section of the Central University Technology, Free State. During the first trip a total of 53 blood samples were collected, while a total of 33 was collected during the second trip. Making the total number of participants 86, but only 83 participants were used as 3 were found to be pregnant.

3.3.2 Specimen preparation

Within four hours of phlebotomy, clotted blood specimens were centrifuged, separated and aliquoted into specimen tubes, which were stored at -20^{o}C. The serum was defrosted once and used for C-reactive protein (CRP) and chemical analysis.

3.3.3 Haematological analysis

Haematological analyses were performed on the EDTA (purple top tube) specimen within four hours of sample collection, and these were performed in the Qwa-Qwa area in a mini-laboratory set on site. Tests performed were full blood counts, blood smears preparation and erythrocyte sedimentation rate.

3.3.3.1 Full blood counts

The full blood counts were performed on the ABX Pentra 60, using EDTA anti-coagulated blood specimens within four hours of collection. The ABX Pentra 60 uses current impedance changes, spectrophotometry, a double hydrodynamic sequential system coupled with cytochemistry and measurements of transmitted light, to measure the different parameters of the full blood count. The spectrophotometric technique is used for Hb detection. Hct is measured as a function of the numeric integration of MCV. MCH and MCHC are calculated from RBC, Hb and Hct.

3.3.3.2 The peripheral blood smears

Even though the differential count of the WBC is obtained on the ABX Pentra 60, these results were confirmed by doing the differential count on the blood smear. The morphological abnormalities of all the blood cells were also confirmed by evaluating the peripheral blood smear. The smears were prepared in the temporary laboratory using the EDTA blood specimen according to a method described by Bain & Lewis (2011). The slides were correctly labelled, air dried and fixed in methanol. Within four days the slides were stained using the HEMA-TEK 2000 SLIDE STAINER and the differential counts were counted using 3.7 oil immersion lens, with a total magnification of 1000x, at the haematology laboratory of the Central University of Technology, Free State. Afterwards data was recorded and statistically analysed.

3.3.3.3 Erythrocyte sedimentation rate

ESR was performed within four hours of blood collection. Na_2EDTA venous blood was diluted to a ratio of 4 volumes of blood to 1 volume of sodium citrate, and self-aspirated into a Sediplast disposable tube. The Sediplast disposable tube stood upright on a stable and non-vibrating surface for an hour. After an hour had elapsed, the distance between the top of the plasma meniscus and the top of the packed red blood cells were read. The results were expressed as mm/hour. The method used was the adapted closed system Westergren method using Sediplast disposable tubes

(Osel-Bimpong & Burthem, 2011). The Westergren method is recommended by the International Council for Standardization in Haematology (ICSH).

3.4 STATISTICAL ANALYSIS

Data was captured by the researcher on a data form and then processed in Microsoft Office Excel®. Further analyses of the questionnaires and data were performed by a statistician using SAS Version 9.1.3. Descriptive statistics, namely frequency and percentage, were calculated for categorical data. Deviations of the means, standards and percentiles were calculated for numerical data. Data from the control group was compared to the study group and appropriate p-values and/or confidence intervals were calculated. However, results of each participant were compared with the normal ranges for the parameter, and then mean and standard deviations were calculated for each group. Data was presented in a form of tables and graphs. If p-value was >0.05, then there was no significant difference between the mean or median values of the control group and the geophagic group. In addition, if the p-value was <0.05, then there was a significant difference between the mean or median values of the control group and the geophagic group existed.

The significant difference between the geophagic and control group signifies that the data of both groups was different, but it does not estimate the amount of difference. Therefore, to quantify the degree of difference and to answer the question in layman's terms, the relative risk ratios were calculated for haematological and chemical analyses that are important for the diagnosis of iron deficiency anaemia. The data was converted into categorical data by indicating how many participants had decreased values in each group. Thereafter the categorical data was used to calculate the risk ratios. The relative risk ratios were calculated by means of Chi square calculations, using Epi-info software™ version 3.5.3. The risk ratio is deemed significant if the range, which represents the population estimate, does not include one. The 95% confidence interval was utilised for the calculations.

CHAPTER 4

RESULTS

4.1. INTRODUCTION

The aim of this study was to establish whether there was a relationship between the practice of geophagia and haematological parameters of iron deficiency anaemia amongst geophagic women in Qwa-Qwa, where the practice of geophagia is a common phenomenon. The geophagic and control groups consisted of people living under the same environmental conditions, as well as people who were in their child bearing years, namely between 18-45 years. A total of 86 samples were collected, of which 36 samples were used as a control group as 1 sample was excluded because the participant was found to be pregnant. Forty-seven (47) samples were used as the geophagic group, seeing that 2 samples were also excluded, as the participants were also found to be pregnant.

In this chapter, the comparison between the ages of the geophagic and control groups is presented; followed by the clay consumption habits of the geophagic group presented under geophagic practices. The red cell parameters, red cell indices, red cell distribution width (RDW) and platelets are summarised under haematological analysis, while the iron studies results are tabulated under chemical analysis. In conclusion, inflammatory and parasitic indicator results will be presented.

4.2. GEOPHAGIC PRACTICES

Data used in this section was obtained from questionnaires that were completed by the interviewer during the recruitment stages, as well as from other researchers who were part of the bigger project. It encompasses the age of the participants, type of clay consumed according to the participants, type of soils consumed according to Munsell soil classification identified by Theron & Smith (2011), frequency, quantity of consumption and nutritional status performed by van Onselen & Walsh (2011).

4.2.1. Age of participants

Age was an important factor, as criteria specified that the women should be between 18-45 years, the major child bearing age group. All statistical data concerning the age of both the geophagic and control groups were similar, as depicted in Table 4.1. In addition, there was no significant difference between the geophagic and control groups (p = 0.7914). Moreover, the geophagic group's results indicated that almost 75% (confirmed by the UQ = 30 years) of the geophagic population was 30 years and younger.

Table 4.1: Ages of participants for both control and geophagic groups

Age	N	LQ	Median	UQ	Minimum	Maximum	p-value
Control	36	20.0yrs	**23.5yrs**	31.0yrs	18.0yrs	44.0yrs	0.7914
Geophagic	47	20.0yrs	**23.0yrs**	30.0yrs	18.0yrs	43.0yrs	

N= number of participants, LQ= lower quartile, UQ= upper quartile

4.2.2. Type of clay consumed according to participants

The type, quantity and frequency of clay consumption were important because it gave an indication of the type of clay preferred, the amount consumed and the period of consumption, respectively. Figure 4.1 gives the colour of the clay consumed according to the 47 participants' description.

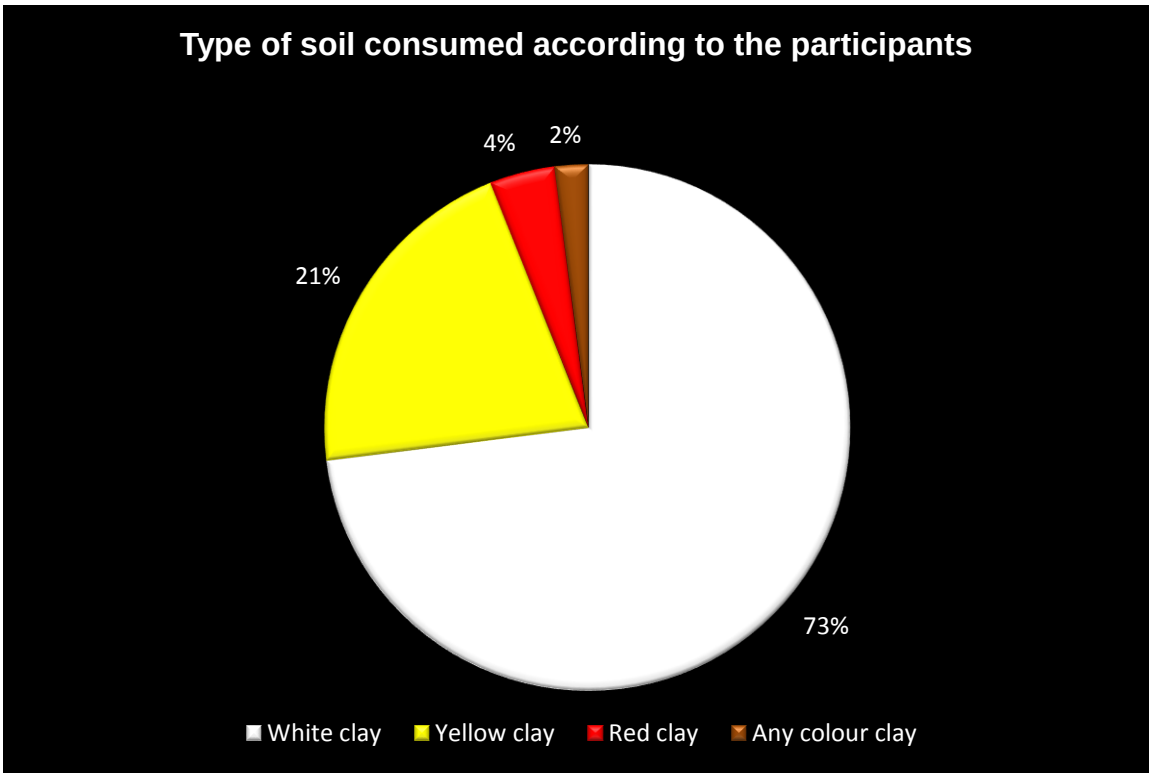

Figure 4.1: Type of clay consumed

Almost three quarters (73%) of the participants consumed white clay, followed by yellow clay with 21%. Two percent (2%) of the geophagic population stated that they consumed any colour clay, while 4% indicated that they consumed red clay.

4.2.3 Type of clay consumed according to the Munsell classification

Theron & Smith (2011) collected 12 clay samples from vendors and 11 clay samples from mining sites in Qwa-Qwa. The author performed soil classification using Munsell soil classification and found the following: the dominant soil colours from the mining sites and vendors were grey and white, while brown, green and red were in the minority (Figures 4.2 and 4.3).

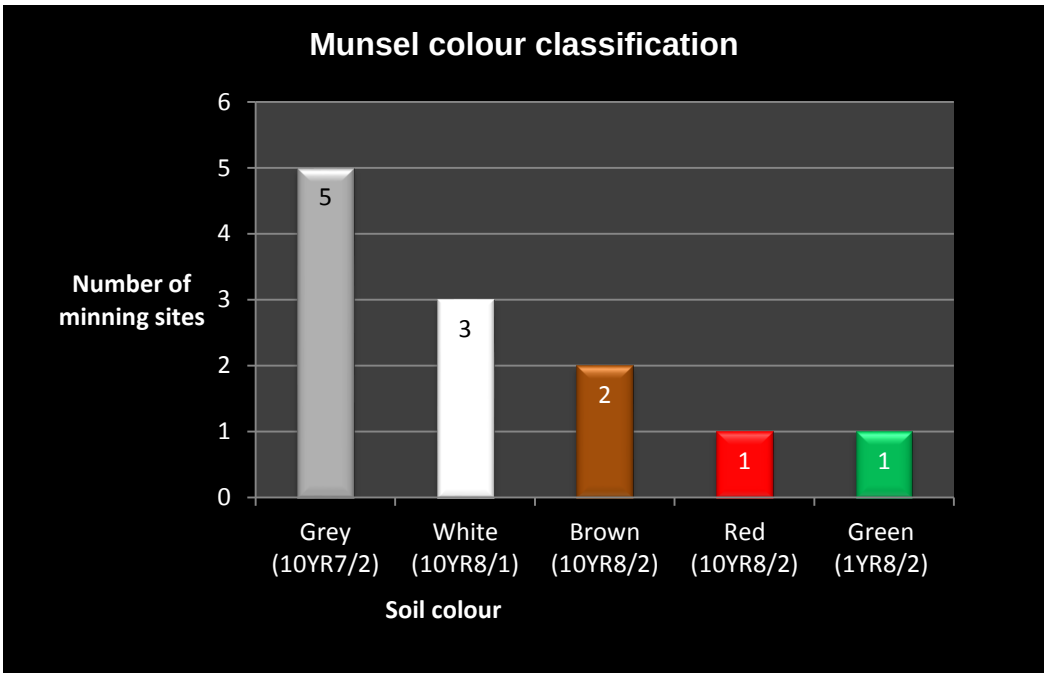

Figure 4.2: Clay colour classification of clay samples from mining sites in Qwa-Qwa

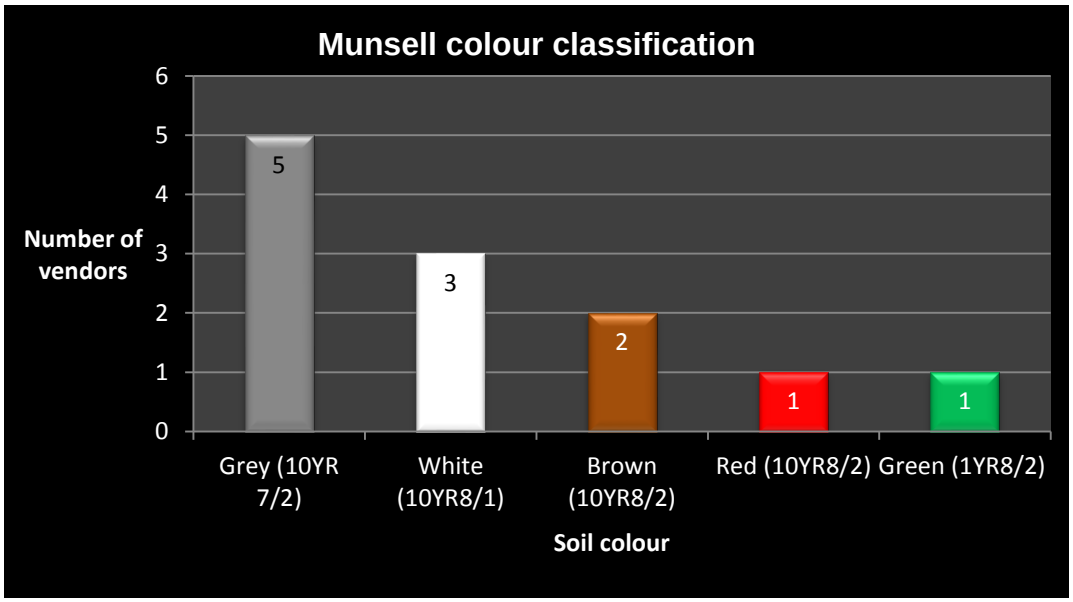

Figure 4.3: Clay colour classification of clay samples from vendors in Qwa-Qwa

4.2.4 Frequency and quantity of clay consumption

The frequency of clay consumed is presented below (Figure 4.4), followed by tabulated results of the quantity consumed by each individual.

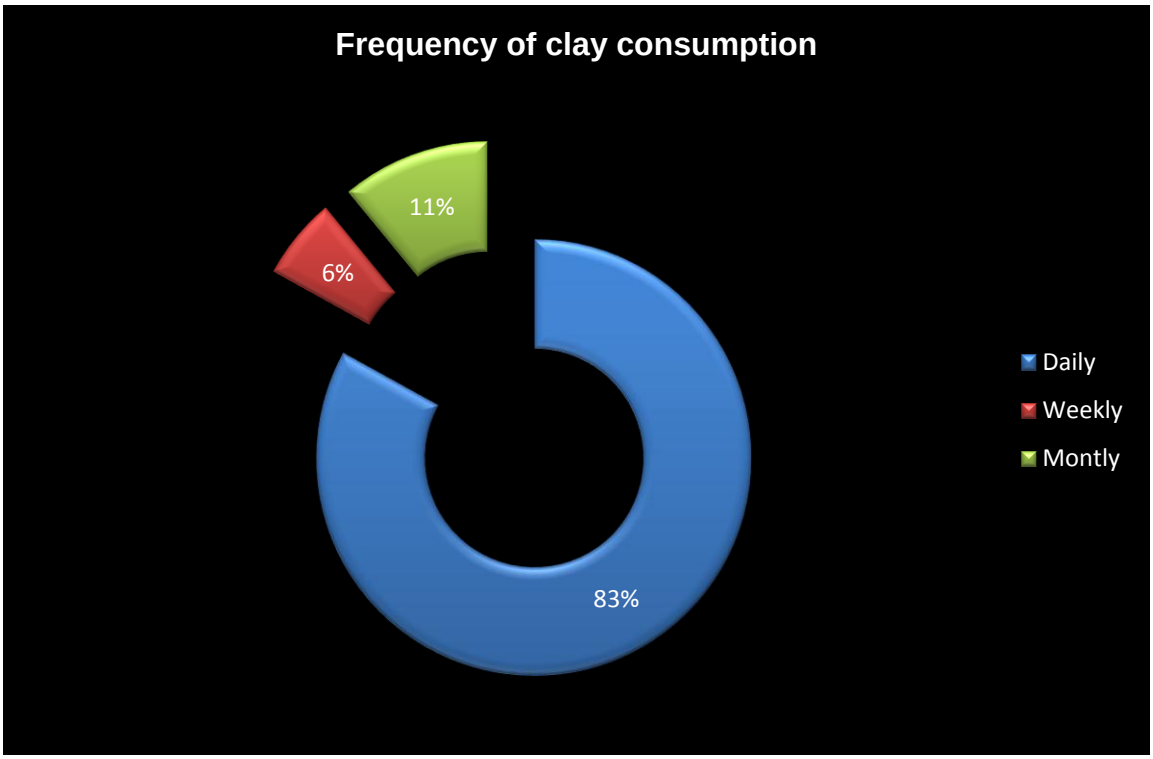

Figure 4.4: The frequency of clay consumption in the geophagic group

The geophagic practices raised a lot of concern. From Figure 4.4 it was noted that the majority of the participants (82.9%) consumed clay daily, with almost seven percent (6.38%) weekly and almost eleven percent (10.6%) monthly.

Table 4.2: Years of clay consumption and the quantity

Variants	N	LQ	Median	UQ	Minimum	Maximum
Quantity of soil consumed	47	20g	**40g**	80g	5g	120g
Years of consumption	47	3yrs	**5yrs**	7yrs	1yrs	24yrs

N= Number of participants, LQ= Lower quartile, UQ= Upper quartile, yrs= years

The geophagia research group weighed 5bags of clay and determined the average weight to be equivalent to 20grams. This bag was used as an example to obtain the estimated amount of soil consumed by participants. What caused great concern was that the median amount of clay the participants consumed was 40grams, with a

maximum daily consumption of 120 grams. The numbers of years participants have been consuming clay were also high, as depicted in Table 4.2. The median number of years was 5years, with 24years being the maximum number of years.

4.2.5 Nutritional status

van Onselen & Walsh (2011) assessed the nutritional status of the geophagic and control groups with the aid of a food frequency questionnaire. These investigations were undertaken to eliminate nutrition as a possible cause of iron deficiency. Both the geophagic and control groups had an intake of kilojoules that did not differ significantly, with the protein, carbohydrates and fat intake of both groups being within the recommended ranges as presented in Table 4.3. This was also supported by the mean body mass index of both geophagic (25.6kg/m^2) and control groups (25.1kg/m^2), which were similar. Both groups' means were on the upper limit of the recommended range of 18.5 – 24.9kg/m^2; in addition both groups had a standard deviation of 5.3kg/m^2.

Table 4.3: Mean kilojoule intake of both groups measured by the food frequency questionnaire

	Geophagic group	Control group	Recommended intake
Kilojoules intake per day	10324.31 kj	10763.94kj	
Protein	12.48%	13.62%	10 – 35%
Carbohydrates	54.06%	54.40%	45 – 65%
Fat	33.14%	31.53%	25 – 35%

The calculated mean calcium intake of both groups was lower than the normal recommended daily intake, while the mean sodium for both groups was higher. The magnesium's mean was decreased for the geophagic group, but within range for the control group. However, the calculated mean iron and phosphate intake for both groups were within range, although the geophagic group's iron intake was slightly lower than that of the control group. There was no significant difference between the

groups indicating that the participants were consuming a similar diet, as presented in Table 4.3 and Table 4.4.

Table 4.4: Nutrient intake of both groups as measured by the food frequency questionnaire

	Geophagic group Mean ± SD	Control group Mean ± SD	Recommended daily intake
Sodium / mg	2438.1 ± 917.5	2500.9 ± 101.4	1000 – 2300
Calcium / mg	502.0 ± 216.3	589.1 ± 250.2	1000 – 2500
Magnesium / mg	287.5 ± 93.9	314.7 ± 76.8	310 – 1100
Iron / mg	11.6 ± 3.3	13.5 ± 4.6	12 -18
Phosphate / mg	1057.1 ±320.5	1209.0 ± 330.9	700 – 2500

4.3 HAEMATOLOGICAL ANALYSES

The haematological analysis section consisted of red blood cell parameters and indices. The red cell parameters consisted of a red blood cell count, haemoglobin concentration and haematocrit. In addition, a platelet count was also included in this section. The red cell indices, however, consisted of mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration and red cell distribution width. All haematological analyses were performed using the ABX Pentra 60 and the normal; high and low controls were also run. The normal reference ranges were obtained from Dacie & Lewis Practical Haematology (Bates & Lewis, 2011: 14-15).

4.3.1 Red blood cell parameters and platelet count

The mean RBC count for both geophagic and control groups were within the normal reference range (3.8-4.8x10^{12}/l), as depleted in Table 4.5. However, the geophagic group's results were lower than that of the control group, a difference that was statistically significant (p=0.0114), though not clinically significant (Table 4.5).

Table 4.5: Red blood cell parameters and platelets of geophagic and control groups

Variable	N	Mean	SD	Min	Max	p-value	Ref range	Units
RBC								
Control	36	**4.37**	0.37	3.35	5.21	**0.0114**	3.8 - 4.8	10^{12}/l
Geophagic	47	**4.17**	0.34	3.43	4.93			
Hb								
Control	36	**13.24**	1.17	10.10	15.90	**<0.0001**	12 – 15	g/dl
Geophagic	47	**11.21**	2.02	6.00	15.40			
Hct								
Control	36	**38.94**	3.00	30.90	46.70	**<0.0001**	36 –46	%
Geophagic	47	**33.68**	5.27	19.50	44.70			
PLT								
Control	36	**285.14**	66.18	185.0	438.0	0.9832	150-410	10^{9}/l
Geophagic	47	**285.51**	88.55	90.0	480.0			

N= number of participants, SD= standard deviation, Min= minimum, Max= maximum, Ref range=Normal reference range, RBC= red blood cell, Hb= haemoglobin, Hct= haematocrit, PLT= platelet, g/ml= grams per millilitre

The mean Hb and Hct of the geophagic group were below the normal reference range, while the control groups' results were within range. The difference between the two groups for both parameters were statistically significant ($p<0.0001$). Mean Hb (11.21) and mean Hct (33.68) for the geophagic group indicated that more that 50% of the geophagic group had decreased Hb and Hct, therefore rendered anaemic (note: the true percentage from raw data showed Hb: 30/47 {64%}, Hct: 29/47 {62%}). The control group's Hb mean minus 1SD (12.07) and Hct mean minus 1SD (35.94) showed that less than 16% of the participants were anaemic, as depicted in Figure 4.5 (note: that from the raw data 5/36 {14%} for Hb and 6/36 {17%} for Hct). The likelihood of developing anaemia by participants practising geophagia was approximately two times (2.3) more likely in comparison with those not practising geophagia. This association was statistically significant as the general populations' risk factor is between 1.6 and 3.4 (p value<0.0001).

Both mean geophagic and control groups' platelet counts were within the normal reference range (150-410x10^9/l), there was no statistical difference (p=0.9832) between the groups.

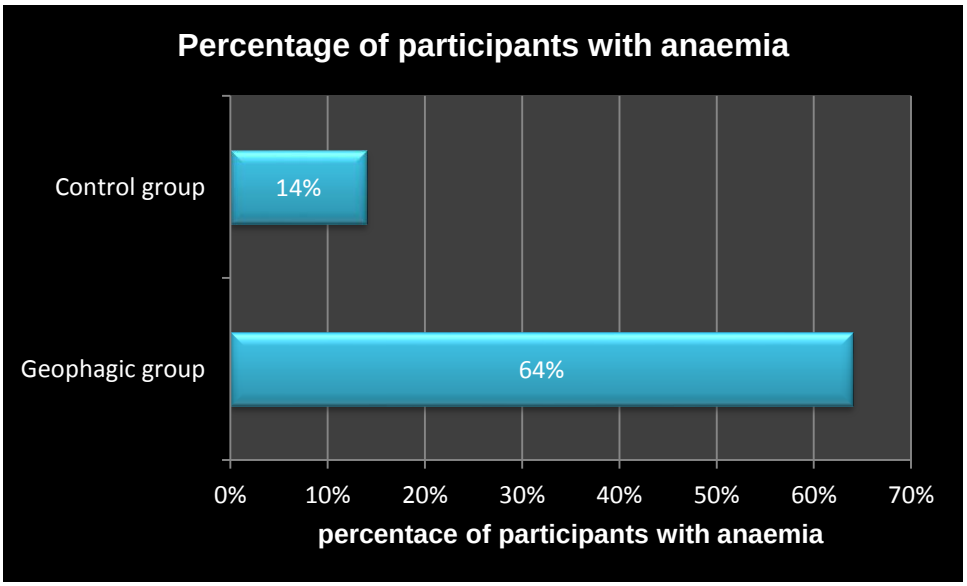

Figure 4.5: Percentage of participants with anaemia

4.3.2 Red cell indices and red cell distribution width

The median red cell indices of the geophagic group were on the lower limit of the normal reference range, which made the geophagic group slightly hypochromic microcytic. The median control group's indices were all within the normal reference range. The indices showed a significant difference between the two groups (p<0.0001), with the geophagic group being lower than the control group. In addition, the geophagic group had approximately 50% participants (validated by the median) with decreased MCV, MCH and MCHC values {note: the true percentage for the geophagic group MCV 26/47 (55%); MCH 21/47(45%) and MCHC 16/47 (34%)} signifying that the red cells of the geophagic group were characteristic of iron deficiency anaemia, by being hypochromic and microcytic (Figure 4.6).

The control group on the other hand had all three parameters' median and lower quartile within the normal reference range, thus reflecting that less than 25% of the control's red cells were hypochromic or microcytic {note: the true percentage MCV 5/36(14%); MCH 2/36(6%) and MCHC 4/36(11%)}. The likelihood of developing hypochromic microcytic red blood cells by participants practising geophagia was roughly two times (1.7) more likely in comparison with those not practising geophagia. This association was statistically significant as the general populations' risk ratio is between 1.3 and 2.2 (p-value=0.0001).

Table 4.6: Red cell indices and RDW for geophagic and control groups

Variable	N	LQ	Median	UQ	Min	Max	p-value	Ref range	Units
MCV									
Control	36	86.50	**90.50**	92.50	67.00	120.00	**<0.0001**	83-101	Fl
Geophagic	47	74.00	**81.00**	90.00	54.00	95.00			
MCH									
Control	36	29.25	**30.70**	31.80	21.30	41.20	**<0.0001**	27-32	Pg
Geophagic	47	24.70	**27.30**	29.80	16.50	32.90			
MCHC									
Control	36	33.80	**34.10**	34.45	31.80	35.40	**<0.0001**	33.0-34.5	g/l
Geophagic	47	32.60	**33.30**	33.80	23.30	34.80			
RDW									
Control	36	12.90	**13.55**	13.95	11.90	18.50	**<0.0001**	11.6-14.0	%
Geographic	47	13.90	**16.00**	18.40	11.60	22.40			

N= number of participants, LQ= Lower quartile, UQ= Upper quartile, Min= Minimum, Max= Maximum, Ref range=Normal reference range, fl= Femtolitre, pg= Pico gram, g/l= grams per litre

The red cell distribution width showed that less than 25% of the control group was above the normal reference range, as the UQ was within the normal reference range {note: the true percentage 9/36 (25%)}. However, the geophagic group showed that less than 75% was increased, as reflected by the lower quartile (LQ) with an increased RDW {note: the true percentage 32/47(68%)}, which is an indication of the

variation in size of the red cells. The difference between the two groups was statistically significant as p-value was <0.0001.

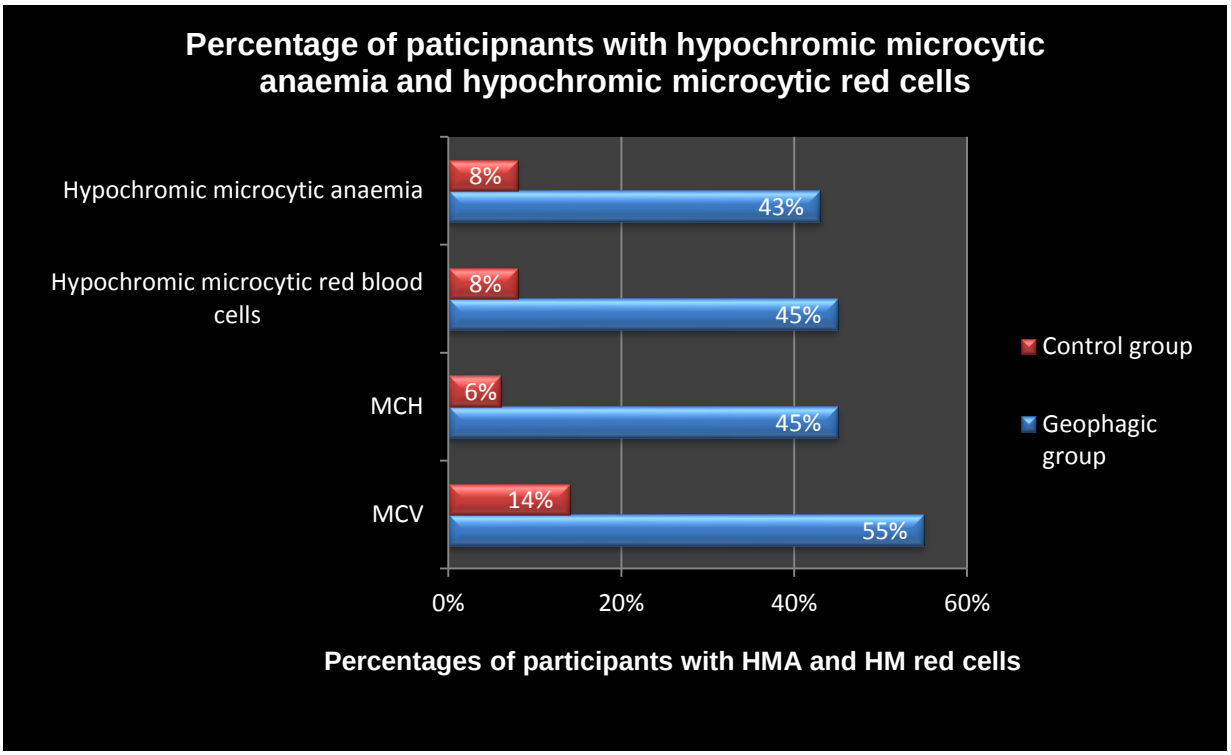

Figure 4.6: Percentage of participants with hypochromic microcytic anaemia and hypochromic microcytic red cells

4.3.3 Summary of red cell parameters and indices

Iron deficiency anaemia (IDA) is characterised by HMA, thus the red cell parameters and indices were summarised by looking at how many participants in both groups had HMA. These were participants with decreased Hb, MCV and MCH. The values were 43% (20/47) for geophagic group contrary to 8% (3/36) for the control group (Figure 4.6). In addition, the likelihood of participants who practise geophagia developing hypochromic microcytic anaemia, with reference to the red cell parameters and red cell indices, was roughly 2 times (1.9) more likely than the non-geophagic participants. This association was statistically significant with range of 1.4 to 2.5 (95% Confidence Interval).

4.4 CHEMICAL ANALYSIS

Chemical analyses were performed to confirm that the HMA was indeed due to iron deficiency. This was performed by Ms van Wyk at the National Health Laboratory Service; serum iron (measuring the iron in circulation), ferritin (measuring stored iron), transferrin (transport protein of iron) and transferrin saturation (the amount iron bound to transferrin) were analysed. In iron deficiency anaemia serum iron, ferritin and transferrin saturation were expected to be decreased, while transferrin concentration was to be increased.

Iron studies results for both groups showed a significant difference between the two groups, with the geophagic group having lower results than the control group, a fact that was authenticated by p-values of <0.0001. In geophagic group less than 75% of participants with decreased serum ferritin {UQ was 17.9 and normal reference range is 15-200µg/l} and more than 75% with decreased serum iron {UQ was 9.00 and range 10-30µmol/l}, which was lower than the normal reference range (note: the true percentages from raw data showed 32/47 {68%} for serum ferritin and 37/47 {79%} for serum iron – Figure 4.7). In comparison, the control groups' results showed more than 25% of the population with decreased serum ferritin and serum iron, as reflected by the LQ {was 11.5 for ferritin and 9.05 for the serum ion}. It must be noted that the true percentages, as reflected by the raw data, was 13/36 {36%} for ferritin and 10/36 {28%} for serum iron (Figure 4.7). Moreover, the likelihood of participants practising geophagia having decreased ferritin levels, in comparison with the one not practising geophagia, was about 2 times more likely. This association was statistically significant as the general populations' risk ratio is between 1.2 and 3.2 (p-value 0.0001).

Table 4.7: Results of ferritin, iron and transferrin saturation for geophagic and control groups

Variable	N	LQ	Median	UQ	Min	Max	p-value	Ref range	Units
Ferritin									
Control	36	11.50	**30.25**	61.60	3.60	175.50	**<0.0001**	15-200	µg/l
Geophagic	47	5.20	**8.90**	17.90	1.20	57.40			
Serum iron									
Control	36	9.05	**12.85**	17.40	3.40	37.50	**<0.0001**	10-30	µmol/l
Geophagic	47	3.80	**5.60**	9.00	1.70	27.80			
Transferrin saturation									
Control	36	10.85	**17.46**	23.95	4.08	64.34	**<0.0001**	16-50	%
Geophagic	47	4.17	**5.54**	10.84	0.98	29.57			

N= number of participants, LQ= Lower quartile, UQ= Upper quartile, Min= Minimum, Max= Maximum, Ref range=Normal reference range, µg/l= micro gram per litre, µmol/l= micro mole per litre

Transferrin saturation results for the control group indicated that 25% {note: true percentage 8/36(22%)} of the participants had less than 10% saturation, as indicated by the LQ {10.85}, whilst the geophagic group had almost 75% {note the true percentage is 35/47(74%)} of the participants with less than 10% transferrin saturation confirmed by UQ {10.80}, as shown in Table 4.7. Transferrin saturation of less than 10% is indicative of iron deficiency. Furthermore, the probability of geophagic participants being iron depleted, meaning having both decreased ferritin and transferrin saturation, in comparison with the non-geophagic participants was roughly 3 times (3.1) more likely. This association was statistically significant, as the general populations' risk ratio is between 1.8 and 5.4 (p-value <0.0001).

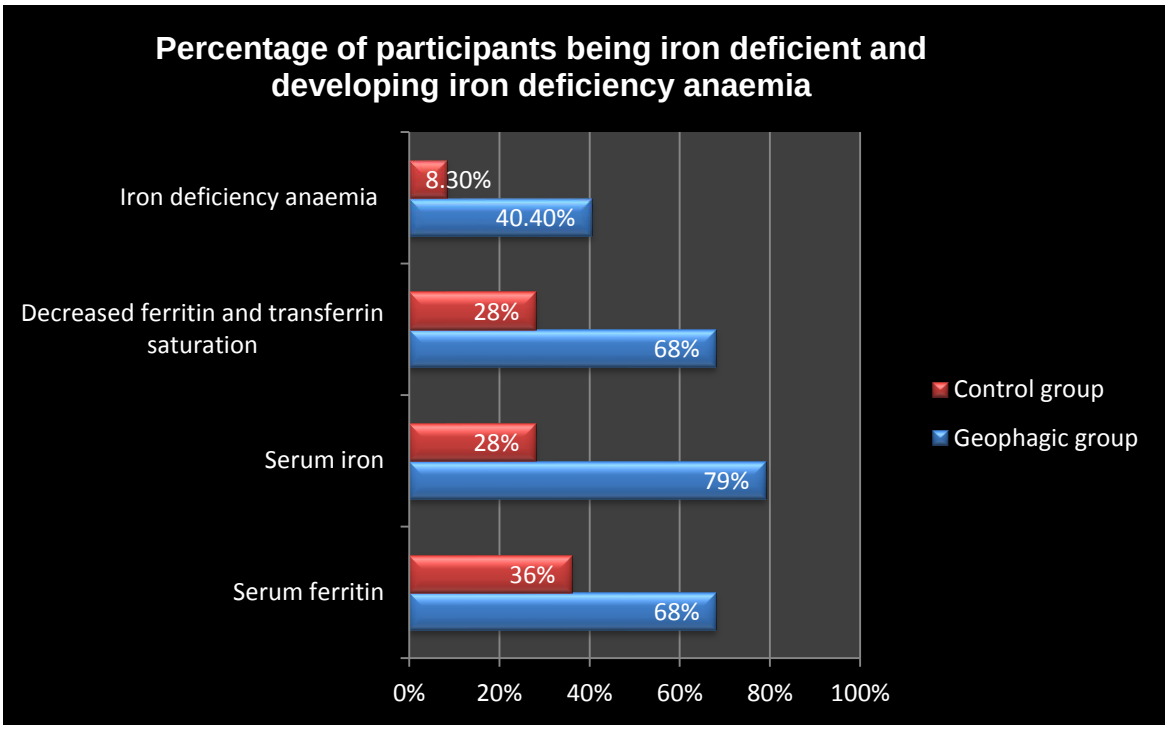

Figure 4.7: Percentage of participants being iron deficient and developing iron deficiency anaemia

Comparison of transferrin results for the control and geophagic groups showed a statistically significant difference ($p < 0.0001$); with the geophagic groups' mean being higher than the mean of the control group. In addition, clinically both the control and geophagic group mean transferrin concentration were within the normal reference range. The geophagic groups' mean plus 1SD (3.62) indicated that almost 84% of the geophagic population had normal transferrin, meaning that less than 16% of the participants had an increased transferrin. However, the control groups' mean plus 2SD (3.78) indicated that approximately 95% of the population showed normal transferrin (note: raw data showed 1/36 {2.8%} of the control and 7/47 {15%} for the geophagic group, with increased transferrin) as indicated in Table 4.8 below.

Table 4.8: Results of transferrin concentration for geophagic and control groups

Variable	N	Mean	SD	Min	Max	p-value	Ref range	Units
Transferrin								
Control	36	**2.74**	0.52	1.90	4.29	<0.0001	1.8 - 3.6g/L	mg/l
Geophagic	47	**3.19**	0.43	2.36	4.45			

SD= standard deviation, Min= minimum, Max= maximum, Ref range=normal reference range

4.4.1 Summary of chemical analysis

IDA is diagnosed when a person has HMA and an iron deficiency state, meaning the following parameters are decreased simultaneously: Hb, MCV, MCH, ferritin and transferrin saturation. The geophagic group presented with 19/47 (40.4%) of the participants who could be diagnosed with IDA (Figure 4.7), in comparison with the control group with 3/36 (8.3%). In addition, the odds ratio of participants practising geophagia developing iron deficiency anaemia, according to the haematological parameters and the iron study results, were about 2 times (1.9) more likely than the non-geophagic participants. This association was statistically significant as the odds ratio of the general populations' risk ratio was between 1.4 and 2.5 (p-value<0.0001).

4.5 INFLAMMATORY AND PARASITIC INDICATORS

The ESR median values for both geophagic and control groups were above the normal reference range. In comparison, the geophagic groups' median was higher than the control groups' median, moreover, this difference was significant ($p = 0.0173$). The median WBC and eosinophil count results of both geophagic and control groups were within the normal reference range and there was no significant difference between the two groups ($p = 0.7476$, $p = 0.1948$ and $p = 0.7300$ respectively).

Table 4.9: ESR, WBC, CRP and eosinophil count of geophagic and control groups

Variable	N	LQ	Med	UQ	Min	Max	p-value	Ref range	Unit
ESR									
Control	36	9.5	**23.5**	48.5	2.0	127.0	**0.0173**	0-12	mm/hr
Geophagic	47	22.0	**40.0**	95.0	3.0	130.0			
WBC									
Control	36	4.35	**5.80**	7.25	3.40	10.70	0.7476	4.0–11.0	$x10^9/L$
Geophagic	47	4.40	**5.30**	6.70	2.30	12.80			
CRP									
Control	36	1.00	**1.85**	5.05	1.00	28.60	0.1948	<5	mg/l
Geophagic	47	1.00	**1.20**	2.90	1.00	21.00			
Eos Ab									
Control	36	0.07	**0.12**	0.17	0.05	0.85	0.7300	<0.45	$X10^9/L$
Geophagic	47	0.07	**0.12**	0.18	0.00	0.94			

N = number of participants LQ =Lower Quartile Med = Median UQ = Upper Quartile Min = Minimum Max = Maximum, Ref range = normal reference range ESR = Erythrocyte sedimentation rate, WBC = White blood cell count, CRP= C-reactive protein Eos Ab= Absolute Eosinophil count, mm/hr: millimetre per hour

CHAPTER 5

DISCUSSION

5.1 INTRODUCTION

This study investigated the unsaid relationship between iron deficiency anaemia and geophagia. In this chapter there is a discussion and comparison of geophagic practices; haematological analysis; chemical analysis; inflammatory and parasitic indicators.

5.2 GEOPHAGIC PRACTICES

This section covered geophagic practices of the geophagic group. This includes: the age of the participants, the clay colour preference of the geophagic group, how much of the geophagic material was consumed, frequency of consumption and how long the participants have been consuming the clay. In addition, the nutritional statuses of participants are included.

5.2.1 Age of participants

As expected, the median age of participants was similar for both the control and geophagic groups (Table 4.1). Thus, it can be deduced that changes observed in other parameters would not have been influenced by the age differences between the two groups. The selection criterion for the participants was females between the ages of 18-45 years who live in Qwa-Qwa. This age range was significant, because studies indicate that women in their child bearing years are prone to practising geophagia due to culture, increased bizarre cravings for certain food and non-food substances (Geissler *et al*., 1998; Luoba *et al*., 2004; Louw *et al*., 2007; Ngozi, 2008; Nyaruhucha, 2009; Momoh *et al*., 2012). Furthermore, Luoba (2004) found that the prevalence for geophagia was higher in women younger than 30 years when compared to those older than 30 years. Their finding was similar to the current

study, as almost 75% of the geophagic population was 30 years or older. In addition, it was important to determine the type of soil consumed, as well as the amount consumed and period of consumption by the participants.

5.2.2 Type of clay consumed according to the participants

This study found that most geophagic participants preferred white clay to yellow, red and other colours of clay. In addition, Theron & Smith (2011) used the Munsell colour classification to categorize the geophagic materials in Qwa-Qwa and found that the most dominating clay colours, from the mining sites and clays sold by vendors in the market place, were grey, white and brown. This finding is supported by Nchito *et al.* (2003), who did a study in Zambia on geophagic materials and also found that white is the clay colour that is consumed the most. In addition, the white geophagic materials from Qwa-Qwa contain quartz and kaoline as the dominating substances, with traces of iron (Ekosse *et al.*, 2010).

5.2.3 Frequency and quantity of clay consumption

The geophagic group has been consuming clay for many years, with some participants consuming it for up to 10 or even 24 years (Table 4.2). From the age of the participants and the years of consumption, it could be deduced that participants consumed clay from a young age. The number of years the participants consumed clay might also contribute to their relation to iron deficiency anaemia status, as the clay could reduce absorption of already bioavailable micronutrients in the system, especially iron. However, the geophagic material does contain micronutrients, such as iron, which may contribute to the body's mineral intake as postulated by Ngole *et al*. (2010) and based on the *in vitro* soil studies. The different reasons people give for consuming clay, such as culture (Ghorbani, 2008); poverty and health (Hawass *et al.*, 1987) could be what causes people to start at a young age.

The frequency of clay consumption, showed a high percentage of participants consuming clay daily (83%), similar to the findings of Ngozi (2008). Bick *et al.* (1993)

stated that in South America clay cakes with impressions of Christ are sold to mothers for children to suck on, which might be the reason for the increased clay consumption. However, in the Republic of South Africa, specifically in Qwa-Qwa, clay is easily accessible because it is sold by vendors at the market place and residential areas that are near the mining sites. This might be the reason for the high amount of clay being consumed. The frequency and quantity of consumption of geophagic materials by women in Qwa-Qwa raises curiosity on the impact clay consumption has on the body. Geophagic materials consumed contain micronutrients such as iron, which could contribute to the iron intake of the women practising geophagia. However, the materials also contain kaoline which has the ability to absorb ferrous and ferric iron in the duodenum, consequently causing or contributing to iron deficiency. This makes the association between iron deficiency anaemia and geophagia probable (von Garnier *et al*., 2008). Thus, a review of nutritional status could assist in determining the reason for the iron status.

5.2.4 Nutritional status

To rule diet out as being the reason for iron deficiency, the nutritional status of all the participants were explored. According to van Onselen & Walsh (2011) the kilojoule intake of both groups was similar and within the recommended range. In addition, the BMI of both groups were similar, indicating that participants of both groups were not malnourished. This was supported by Young *et al.* (2010) who found that economic and/or food intake between geophagic and non-geophagic people was not different and concluded that hunger had no bearing on geophagia in their study. However, mineral intake showed that both groups exhibited increased sodium and decreased calcium intake. Although magnesium was decreased for the geophagic group only, whilst iron and phosphate were within the recommended intake. Therefore, diet did not play a significant role on the effect geophagia has on haematological parameters as dietary iron intake was similar, hence diet was ruled out as the cause of iron deficiency. It should be noted that though that the mineral intake was decreased, as assessed by the food frequency questionnaire. The level

of these minerals (calcium, magnesium) in the participants blood was within the reference range and did not differ significantly for both groups, except in the case of iron (van Wyk *et al.*, 2013).

5.2.5 Summary of the geophagic practices

Participants of the study were all in the specified age range of 18-45.There was no difference between the ages of the two groups, thus age had no impact on the haematological and chemical analyses. Most of the geophagic group consumed 40 grams daily, with 50% (median) of the geophagic group consuming clay for 5 years. In addition, the nutritional status of the geophagic group revealed that their iron intake for both groups was within the recommended daily intake. This raises concern on the impact that geophagia has on the iron status, seeing that the Qwa-Qwa clay contains iron and kaoline. To help solve this problem, haematological parameters and indices were analysed.

5.3 HAEMATOLOGICAL ANALYSIS

This section covered red blood cell parameters (RBC count, Hb and Hct), platelets and red blood cell indices (MCV, MCH, MCHC and RDW).

5.3.1 Red blood cell parameters and platelet counts

There was a statistically significant difference between the red blood cell counts of the geophagic and control groups, but both groups' mean were within the normal reference range (Table 4.5). Red cell count in iron deficiency anaemia cases can be normal or decreased due to the body's compensatory mechanism. As compensation to iron deficiency, the body produces more cells due to increased cell division, thus cells become smaller (Turgeon, 1993:99).

Red cells play an important role in anaemia, as they contain haemoglobin which facilitates oxygen transportation, and a decrease in haemoglobin leads as anaemia.

However, with haemoglobin and haematocrit there was a statistical difference between the two groups. The control group was within the normal reference range with only a small portion of the population (14%) being anaemic, while the geophagic group consisted of the majority of the participants (64%) with decreased Hb and Hct values, thus rendering them anaemic (Figure 4.5). This was confirmed by the odds ratio results, which showed that the odds of someone practising geophagia developing anaemia were about 2 times more likely, with the general population ranging between 1.6 and 3.4.

Literature states that in the case of iron deficiency, haemoglobin (Hb) levels decrease because haem forms an important part of the haemoglobin structure (McDowall, 2005; Hoffbrand *et al*., 2011: 26). Iron is part of the haem molecule (Hoffbrand *et al.,* 2011: 26), thus a decrease in iron would cause a decrease in haem synthesis which in turn causes a decrease in the haemoglobin concentration (Hoffbrand *et al*., 2007: 28). Defective haemoglobin synthesis affects the morphology of red cell size, giving rise to hypochromic microcytic red cells. This appearance in turn impacts on haematocrit (packed cell volume); even though the red cell count is within range, the Hct is decreased because the red cells are microcytic. Similar findings were found in other studies where people practising geophagia had decreased levels of Hb and Hct (Geissler *et al*., 1998, Nchito *et al.,* 2004, von Garnier *et al*., 2008). On the other hand, the control group's mean Hb and Hct were within the normal reference range.

The decrease in Hb and Hct could be due to bleeding, the granular nature of the soil could damage the lining of the mucosa, and thus causing bleeding (Nchito *et al.,* 2004). Therefore, platelet count was performed to partially rule out bleeding as the cause of the anaemia, because an increased platelet count could be associated with bleeding. The mean platelet counts of both the geophagic and control groups were within the normal reference range, with no statistical difference between the two

groups. A normal platelet count for both groups confirms that the loss of iron was not due to bleeding. This finding was supported by von Garnier *et al*. (2008).

Therefore, the geophagic group was anaemic with a low Hb and possibly microcytic cells. The question then would be to classify the anaemia and check if it had the characteristics of iron deficiency anaemia, which are hypochromic microcytic red blood cells.

5.3.2 Red cell indices and red cell distribution width

The median red cell indices, mean cell volume, mean cell haemoglobin and mean cell haemoglobin concentration were within the normal reference range for the control group with 14%, 6% and 11% of the participants with decreased levels (Figure 4.6), respectively. In contrast, the geophagic group had 55%, 45% and 34% of the geophagists with decreased MCV, MCH and MCHC, respectively (Table 4.6). The two groups' results for all indices were significantly different statistically. A decrease in MCV means cells are microcytic with a slight hypochromia as the haemoglobin per cell (MCH) and MCHC gives relative concentration of intracellular haemoglobin, which are decreased in iron deficiency anaemia (IDA) (Hoffbrand *et. al*., 2007: 33).

A case report of a woman diagnosed with iron deficiency anaemia, after the MCV and MCH values were low. Upon investigation of her diet, it was discovered that she practised geophagia. Only when the pica (geophagia) was stopped, and the patient started intravenous iron replacement therapy, was the anaemia, MCV and MCH corrected within a month (von Garnier *et al.,* 2008). In addition, the odds ratio results showed that the likelihood of developing hypochromic microcytic red cells was between 1.3 and 2.2 (95% Confidence Interval) for people practising geophagia, than those that do not practise geophagia (Figure 4.6). If the cells are smaller with less haemoglobin, yet the red cells counts are normal triggers the question whether there was anisocytosis.

RDW provides a measurement of variation in red cell size. The RDW showed a statistical difference between the two groups, which was evident as the geophagic group had 68% of the geophagists with an increased RDW, while the control group had 0% of the participants with increased RDW (Table 4.6). Dodds & Joseph (2006) stated that RDW is a useful parameter in differentiating iron deficiency anaemia from other causes of HMA, like thalassaemia minor. In thalassaemia minor, the RDW is usually normal, as the population of cells is relatively uniform, whereas iron deficiency has an abnormally wide RDW (Best, 2006: 65). The control group had a normal RDW implying that there was little variation amongst the cells. Moreover, the geophagic group had an increased RDW, suggesting the presence of a mixed population of red blood cells.

5.3.3 Summary of haematological findings

The mean red cell parameters and indices of the control group were within the normal reference range, but the geophagic group's results were below the normal reference range. The red cell parameters results indicated that a greater percentage of the participants in the geophagic group were anaemic, while the red cell indices indicated hypochromic microcytic red cells. The red cell's counts were normal; furthermore, the cells were smaller in size, which was confirmed by MCV and lead to the decreased packed cell volume. Increased RDW in the geophagic group showed that there was variation in size, thus there was a mixed population of red blood cells in size. This was confirmed by the odds ratio results which showed that the probability of a geophagic person developing hypochromic microcytic anaemia were between 1.4 and 2.5 higher than someone who is non-geophagic (Figure 4.6). Platelets results partially ruled out bleeding as the cause of anaemia. The most common cause of hypochromic microcytic anaemia is iron deficiency anaemia, but any disorder which affects haemoglobin synthesis may cause HMA. To try and confirm that the HMA was due to IDA, chemical analysis, specifically iron studies, were performed.

5.4 CHEMICAL ANALYSIS

In confirmation of the haematological results, clinical chemical analyses were performed to verify whether iron deficiency was the cause of the hypochromic microcytic anaemia observed in the geophagic group. The statistical results of the chemical analysis (Table 4.7) formed part of the overall research project and were included here to strengthen the haematological results. This encompassed serum iron, ferritin, transferrin saturation and transferrin concentration. Iron study results of the geophagic group indicated decreased levels for serum ferritin in 68% of the participants, 79% for serum iron and 74% with less than 10% transferrin saturation. Moreover, the control group had 36% of the population with decreased serum ferritin, 33% for serum iron and 22% with less than 10% transferrin saturation. The mean transferrin concentration for both geophagic and the control group was within the normal reference range, with 15% of the participants in the geophagic group and 2.8% in the control group having increased concentrations (Table 4.8).

Serum ferritin evaluates total body iron stores. When iron stores are depleted, serum ferritin drops (Hilman & Ault, 2002). In iron deficiency anaemia, serum ferritin is decreased (Lichtin, 2008). Dodds & Joseph (2006) stated that the most useful test in geophagia for the diagnosis of iron deficiency anaemia is ferritin, even though it is an acute phase protein. A case study of a woman practicing geophagia from Cameroon showed that her ferritin levels were lower than the normal reference range (von Garnier *et al.*, 2008). Thus, the results of this current study correlate with von Garnier *et al.* (2008) in that the ferritin levels were decreased. This indicated that the geophagic groups' total iron stores were low. Giessler *et al*. (1998) also found serum ferritin decreased in the geophagic group compared to the control group. In the pilot study done in Qwa-Qwa, Mogongoa *et al*. (2011) found the serum ferritin levels of the geophagic group decreased. This was also confirmed by the odds ratio of the current study of between 1.2 and 3.2 for a geophagic persons' iron stored being depleted, than those of a non-geophagic person (Figure 4.7).

The decreased amount of serum iron in the geophagic group indicated a low amount of iron bound to transferrin, in contrast to normal levels for the control group. Serum iron is the direct measure of the amount of iron bound to transferrin and gives a measure of the current iron supply to the tissues (Hilman & Ault, 2002: 55). In addition, serum iron is affected by diet. Therefore, serum iron is circulating iron and cannot be used to assess stored iron, which is in the form of ferritin and haemosiderin.

The saturation of iron in transport is decreased for the geophagic group, indicating that there was less iron transported in circulation. If transferrin saturation is <10%, it is specific for iron deficiency anaemia (Hilman & Ault, 2002:54; Hoffbrand *et al.*, 2006: 36) and the geophagic group had 75% of the participants with <10% transferrin saturation, which confirms iron deficiency anaemia. The control group showed 22% of the population with less than 10% of transferrin saturation. This indicates that in the control group less people had iron deficiency. In addition, this study found that the likelihood of a geophagic person developing iron deficiency (decreased ferritin and transferrin saturation) was between 1.7 and 5.4 higher than of someone who was not practising geophagia.

Serum transferrin concentration is increased in iron deficiency anaemia, because there is less iron bound to transferrin (Dodds & Joseph, 2006). This statistical difference was due to the fact that the transferrin concentration was increased, meaning the geophagic group had more transferrin in circulation, and with a decrease in serum iron transferrin had less iron to bind with. On the contrary, the control group had only 2.8% of the participants with increased levels of serum transferrin.

5.4.1 Summary of chemical analysis

In iron deficiency anaemia; serum iron, ferritin and transferrin saturation are decreased (Lichtin, 2008). Thus, when looking at the results of all these variables,

the median control group was normal while the geophagic group had a decreased ferritin that indicated depletion of the iron stores, decrease in serum iron indicating a decrease in the amount of iron that could be bound by transferrin (Scherier, 2013). Transferrin carries excess iron into the mitochondria for storage, thus increased transferrin could be due to the decreased iron as there is no iron to transport, and thus the geophagic group was iron deficient (Scherier, 2013). Ferritin, serum iron and transferrin saturation results for both geophagic and control groups support the haematological results in that the control group was not iron deficient. Furthermore, the odds ratio shows that a person practising geophagia is between 1.4 and 2.5 more likely to develop iron deficiency anaemia than a non-geophagic person. However, ferritin being an acute phase protein could be falsely increased, as the iron study results might be affected by inflammation (Thurnham *et al.,* 2010).Thus, inflammatory indicators had to be analysed to rule out falsely elevated ferritin results. One of the causes of IDA is parasitic infections, thus the parasitic infection indicator had to be analysed in order to partly rule out parasitic infection.

5.5 INFLAMMATORY AND PARASITIC INDICATORS

It is important to check inflammatory and infection indicators to rule out any infection or inflammation that may complicate the laboratory diagnosis of iron deficiency anaemia. Infection or inflammation can cause a rise in the ferritin independent of iron stores by causing the release of ferritin in the plasma, resulting in serum ferritin increase. Laboratory analyses performed to rule out inflammation or infection were erythrocyte sedimentation rate (ESR), white blood cell count (WBC) and C-reactive protein (CRP). Furthermore, one expects that if there is an increase in WBC counts, ESR and concentration, it will be an indication of inflammation or infection (Lewis, *et al.*, 2011: 105). The eosinophil count was performed to rule out parasitic infections, as it has been postulated that geophagia increases the likelihood of transmission of orally transmitted parasitic nematodes (Young *et al.*, 2007).

5.5.1 Inflammatory indicators

Inflammatory indicators of both groups showed no statistically significant difference, except for the erythrocyte sedimentation rate (ESR). ESR indicated clinical significance as both groups had an increased ESR in relation to the normal reference range. Even though both groups had an increased ESR, the geophagic groups' ESR was higher than the control group. The increased geophagic group's ESR could be due to the iron deficiency, as red cells are smaller as a result of decreased iron which is a building block for haem, making the red cells smaller and thus settling faster and increasing the rate of sedimentation (Nabili, 2011). In addition, the control groups' increased ESR could also be due to iron deficiency, because 14% of the participants in the control group were found to have decreased MCV, indicating a decrease in size, and the packing of red cells. On the other hand, the geophagic group had 55% of the participants with deceased MCV, which could be the reason why their ESR was higher than that of the control group.

Clinically the two groups were expected to be different in that the geophagic group would have an increased ESR due to inflammation and infection caused by the ingestion of geophagic material. This was expected, due to the studies that show that geophagic material can cause bacterial and parasitic infections (Nchito *et al*., 2004; Brand *et al*., 2009), while the control group would have a normal ESR as inflammation was not expected. An increase in ESR could also be due to rouleaux formation as it can contribute to a high ESR. Rouleaux formation is caused by an increase in plasma protein, which neutralizes the negative charge promoting cell adherence and thus increasing the rate that red blood cells fall (Hoffbrand *et al*., 2011: 335). Increased ESR in both groups was due to iron deficiency; in addition, the geophagic groups' ESR was higher because there were more participants in the group with iron deficiency than the control group. ESR is not a specific test, as it is affected by many conditions such as anaemia (Hoffbrand *et al*., 2007: 333). Thus WBC and CRP had to be performed to rule out or confirm inflammation.

An increase in WBC is indicative of inflammation or infection (Dacie *et al*., 2011: 550-555). All quadrants of white blood cells counts showed no inflammation or infection in both geophagic and control groups; meaning both groups did not have inflammation or infection. CRP is a specific marker for inflammation (Bishop *et al*., 2005: 508, 636, 667). CRP is more sensitive than ESR and WBC, as it has a quick response to inflammation (Dacie *et al.*, 2011). The CRP for both control (22% had increased CRP) and geophagic (17% had increased CRP) groups indicated that the majority of participants did not show signs of inflammation.

5.5.2 Parasitic indicators

An increase in eosinophil count (eosinophilia) is associated with parasitic infections (Hillman & Ault, 2002: 189; Hoffbrand *et al.*, 2011: 319). The eosinophil count for the control and geophagic groups were similar and the p-value attests to this. Clinically the two groups were within the normal reference range, thus partly ruling out parasitic infections. In addition, as part of this current study, geophagic soil samples were collected and analysed for parasite content by Perridge *et al*. (2011). The samples did not contain parasites that were pathogenic to humans. This is in line with the findings of Young *et al.* (2007) who found that geophagia was not a vector of parasitic infection and also added that geophagists tend to consume earth that is free of parasites.

5.5.3 Summary of the inflammatory and parasite indicators

WBC count is an indicator for inflammation or infection, but it is not specific. ESR and CRP are both markers of inflammation. In general ESR does not change as rapidly as CRP, either at the start of inflammation or as it progresses. CRP is not affected by as many factors as the ESR, making it a better maker of inflammation than ESR (Hilliard & Waites, 2002). ESR is also elevated in anaemia. From the inflammatory and infection analysis there was a significant difference in ESR results, although the mean of the geophagic group's ESR was higher than that of the control group. However, with the WBC and CRP it was evident that the groups were not

significantly different, thus infection or inflammation was not more prevalent in one group or the other. Therefore inflammation and infection had no bearing on the iron study findings. On the other hand, the eosinophil count showed no indication of parasitic infection in both groups.

5.6 SUMMARY OF GEOPHAGIC PRACTICES, HAEMATOLOGICAL AND CHEMICAL ANALYSIS

All the participants in this study were within the age of 18-45, with the majority of the geophagic group being 30 years and younger. The nutritional status of both geophagic and control groups revealed that the iron intake for both groups was within the recommended daily intake. In addition, the geophagic group consumed at least 40 grams of geophagic clay on a daily basis. The high consumption might have contributed to the haematological and chemical analysis, as the minority of the control group had hypochromic microcytic anaemia and were iron deficient, whilst the geophagic group had the majority of the participants with hypochromic microcytic anaemia, therefore iron deficient, thus making the geophagic group inclined to having iron deficiency anaemia and the control group inclined to being normal. Inflammatory and parasitic indicators revealed that infection and inflammation had no bearing on the iron studies, and the eosinophil count showed no indication of parasitic infections.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 INTRODUCTION

Geophagia is most commonly practised by females and it is believed that it is because they are prone to iron deficiency. The dominating reason for geophagia is mineral deficiency, because it is believed that geophagic material consists of micro-minerals needed by the body such as iron, calcium and magnesium. These lead to a suspicion that there is a relationship between iron deficiency anaemia and geophagia. The objectives of the study were fulfilled and the study contributed positively towards the bigger geophagia project, as it investigated the association between geophagia and haematological parameters of iron deficiency anaemia amongst geophagic women in Qwa-Qwa. Moreover, this chapter summarises and gives a conclusion to the findings.

6.2 CONCLUSION

6.2.1 Geophagic practices

Participants were within the required age range (18–45 years), in addition there was no significant difference between the two groups, thus age had no bearing on the results. White clay was the most consumed clay colour according to the geophagic participants. White clay from Qwa-Qwa contains kaoline, which has the ability to absorb iron in the duodenum. The majority of the geophagic group consumed approximately 40 grams of clay on a daily basis and the median years of consumption were 5years. Diet was ruled out as the cause of iron deficiency, as the kilojoules and iron intake of all the participants was within the recommended intake guidelines.

6.2.2 Haematological analysis

Red cell parameters and red cell indices signify that less than half (8%) of the control group had hypochromic microcytic anaemia. However, almost half of the geophagic group (43%) was found to have hypochromic microcytic anaemia, (HMA) as revealed by the haemoglobin, the mean cell volume and mean cell haemoglobin which is characteristic of iron deficiency anaemia. The odds ratio for the haematological analysis also showed that the probability of a geophagic person developing hypochromic microcytic anaemia was about two times greater than that of someone who is non-geophagic. Platelets results partially ruled out bleeding as a cause of anaemia. Red cell distribution width showed that the geophagic group (68%) had anisocytosis, whilst the control group had 22% of the participants with anisocytosis.

6.2.3 Chemical analysis

The chemical analysis affirmed that the geophagic group had 75% of participants who were iron deficient, in contrast to the control group with 22% of participants. This was substantiated by the serum ferritin, serum iron, transferrin saturation results of the geophagic and control group. The odds ratio revealed that a person practising geophagia is 3 times more likely to develop iron deficiency. Moreover a person is 2 times more likely to develop iron deficiency anaemia that a non-geophagic person.

6.2.4 Inflammatory and parasitic indicators

The indicators proved that inflammation and infection was not more common in either group, therefore did not complicate the interpretation of iron study results. Inflammatory indicators (WBC, ESR and CRP) ruled out inflammation, whilst eosinophil count showed no indication of parasitic infection for both geophagic and control groups.

6.3 LIMITATIONS

A study that depends solely on human participants consists of certain limitations, such as finding participants willing to partake in the study, which influences the sample number and also the credibility of the information the participants give. In the case of this study, some of the participants were found to be pregnant. Likewise, Qwa-Qwa is a big environment and the distance between the different areas made it difficult to recruit participants.

6.4 RECOMMENDATIONS

It is advised that further investigation must be carried out to determine which one comes first, geophagia or iron deficiency. In addition, the geophagic materials must be analysed for nutritional value. Furthermore, studies must be done to investigate if geophagia is genetic or a learned behaviour.

6.5 THE OVERALL CONCLUSION

All the participants were within the stipulated age range of 18-45years. The nutritional status of both the geophagic and control group revealed that the iron intake of both groups were within the recommended daily intake, even though the geophagic group consumed at least 40 grams of geophagic clay daily. In the geophagic group not all participants were iron deficient, not only as a result of their diet, but it can also be attributed to the geophagic material that they consume which contains kaolinite, as kaolinite is said to have the ability to absorb iron in the duodenum. Kaolinite seems to be a possible source of iron loss, as nutrition, bleeding and parasitic infections were ruled out. There was an association between geophagia and haematological parameters of iron deficiency anaemia amongst geophagic women in Qwa-Qwa, in that geophagic material could contribute to iron deficiency anaemia. The aim of the study was reached in it that the study found that there is an association between geophagia and haematological parameters of iron deficiency amongst geophagic women in Qwa-Qwa.

CHAPTER 7

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APPENDIX: A

Recruitment questionnaire

NATIONAL RESEARCH FOUNDATION

In partnership with

University of Limpopo
Central University of Technology
University of Botswana
University of Swaziland


SOUTH AFRICAN REGIONAL COOPERATION FUND FOR SCIENTIFIC RESEARCH AND TECHNOLOGICAL DEVELOPMENT

NRF Grant UID 63583
Research Project
Human and Enzootic Geophagia in South Africa, Botswana and Swaziland

QUESTIONAIRE RELATED TO HUMAN GEOPHAGIA: ADULT
2009

QUESTIONNAIRE RELATED TO HUMAN GEOPHAGIA: ADULT

INTRODUCTION

The University of Limpopo in the Limpopo Province and the Central University of Technology, Free State in Bloemfontein, South Africa - in collaboration with the Universities of Swaziland and Botswana - are conducting a study to characterise habits related to human and enzootic geophagia in South Africa, Botswana and Swaziland. It is also designed to characterise, in physico-chemical, microbiological, mineralogical and ecological terms, the soils that are preferred by geophagic individuals and animals in these three countries. This exercise is mainly for academic purposes; however, the information gathered may be used generally to improve methods of harvesting geophagic soils that will guarantee the health of geophagic individuals. Strict confidentiality of the information provided is guaranteed at all times, and respondents are therefore urged to cooperate fully with the interviewers in order to facilitate this study.

For office use only

Number ☐☐☐☐☐☐☐ 1-7

Date of interview: _____(dd/mm/yy) ☐☐☐☐☐☐ 8-13

Name of interviewee (optional): _____ ☐☐☐ 14-16

Country:

RSA	Botswana	Swaziland

☐ 17

Region:

Free State		
Limpopo		
North West		
Gauteng		

☐ 18

District: _____ ☐☐ 19-20

A. DEMOGRAPHIC INFORMATION

1. Geographic Information

1. Location: | Rural | Suburban | Urban | ☐ 21

2. Specify town or area: _____ ☐☐ 22-23

2. Personal and Demographic Information

3. Gender | Male | Female | ☐ 24

4. Age: _____(years) ☐☐ 25-26

5. Ethnic Group: ☐ 27

- ☐ Afrikaans
- ☐ English
- ☐ Sesotho
- ☐ Setswana
- ☐ siSwati
- ☐ isiXhosa
- ☐ isiZulu
- ☐ Other, please specify:_____ ☐☐ 28-29

6. Number of children: _____ ☐☐ 30-31

	6.1 Age of Child	6.2 Gender of child		
1			☐☐ 32-33	☐ 34
2			☐☐ 35-36	☐ 37
3			☐☐ 38-39	☐ 40
4			☐☐ 41-42	☐ 43
5			☐☐ 44-45	☐ 46
6			☐☐ 47-48	☐ 49
7			☐☐ 50-51	☐ 52
8			☐☐ 53-54	☐ 55

1

7. Marital status: | Married | Divorced | Single | Widowed | Engaged | Cohabiting | 56

8. Income source:
- ☐ Wage employment — 57
- ☐ Non-wage employment — 58-59
- ☐ Other, please specify:_____ — 60-61

9. Occupation: _____

10. Monthly income: R/Pula_____ — 62-67

11. Highest educational level attained:
- ☐ No schooling
- ☐ Primary — 68
- ☐ Secondary
- ☐ Tertiary

12. Highest grade/standard completed successfully:
_____(if GRADE is applicable) — 69-70
_____(if STANDARD is applicable) — 71-72

13. Number of years in formal education: _____years — 73-74

B. SOCIO-ECONOMIC AND CULTURAL ASPECTS

1. Geophagic Habits

14. Are you presently in the habit of eating soil? | Yes | No | — 75

14.1 If **YES**, how often do you eat soil? — 76
- ☐ Once a month
- ☐ Once a week
- ☐ Once a day
- ☐ More than once a day

14.2 If **YES**, for how long have you been eating soil? _____(years) — 77-78

15. What is/are your reason(s) for eating soil?
- ☐ Standard practice (cultural, traditional, spiritual) — 1
- ☐ Craving — 2
- ☐ Medicinal value — 3
- ☐ Supplement diet — 4
- ☐ Ritualistic — 5
- ☐ When hungry — 6
- ☐ When pregnant — 7
- ☐ Don't know — 8
- ☐ Other, please specify: _____ — 9 — 10-11

16. Do you ever crave soil? | Yes | No | — 12

16.1 If **YES**, how often? — 13
- ☐ Regularly - Monthly
- ☐ Regularly - Weekly
- ☐ Regularly - Daily
- ☐ Only when pregnant

17. When do you crave soil?

- ☐ Pregnant
- ☐ Lactating
- ☐ Both pregnant and lactating
- ☐ Having trouble sleeping
- ☐ Nauseous, but not pregnant
- ☐ Constipated
- ☐ Feeling weak
- ☐ Other, please specify: _____

☐ 14 ☐ 15
☐ 16 ☐ 17
☐ 18 ☐ 19
☐ 20 ☐ 21 ☐☐ 22-23

18. When **pregnant**, how often do you eat soil?

- ☐ Once a month
- ☐ Once a week
- ☐ Once a day
- ☐ Other, please specify: _____

☐ 24

☐☐ 25-26

19. Do you eat any other non-food substance? Yes | No

☐ 27

19.1 If **YES**, name the substance: _____

☐☐ 28-29

20. How often do you eat this substance?

- ☐ Daily
- ☐ More than once a day
- ☐ Weekly
- ☐ Monthly

☐ 30

21. How much of the soil do you eat?

Daily	1	2	3	4	5
More than once a day	1	2	3	4	5
Weekly	1	2	3	4	5
Monthly	1	2	3	4	5

☐ 31
☐ 32
☐ 33
☐ 34

22. Do other people know that you eat clay? Yes | No | Don't know

☐ 35

22.1 If **YES**, who knows about it?

- ☐ Family members
- ☐ Extended family members
- ☐ Friends
- ☐ Other, please specify: _____

☐ 36
☐ 37
☐ 38
☐ 39 ☐☐ 40-41

23. How do people perceive this habit of eating non-food substances?

- ☐ Positive
- ☐ Negative
- ☐ Indifferent
- ☐ Don't know

☐ 42
☐ 43
☐ 44
☐ 45

24. Is this practice of eating soil more common among certain members of the community? Yes | No | Don't know

☐ 46

24.1 If **YES**, specify: _____

☐☐ 47-48

C. INDIGENOUS KNOWLEDGE

25. Which substances are eaten?

- ☐ Soil
- ☐ Clay
- ☐ Soil from termite mounds
- ☐ Other, please specify: _____

☐ 49
☐ 50
☐ 51
☐ 52 ☐☐ 53-54

26. How are the substances eaten?

- ☐ Wet
- ☐ Dry
- ☐ With other food
- ☐ Other, please specify: _____

☐ 55
☐ 56
☐ 57
☐ 58 ☐☐ 59-60

27. What are the traditional names of the substances consumed?

_____ 61-62
_____ 63-64

28. Where do you obtain your preferred substance?

- ☐ From nature — 65
- ☐ Buy it — 66
- ☐ Am given it — 67
- ☐ Other, please specify: _____ — 68 ☐☐ 69-70

28.1 If you **BUY** it, give the brand name: _____ ☐☐ 71-72

28.2 If you **BUY** it, indicate the price per handful: R/Pula _____ ☐☐☐☐ 73-76

29. What is the colour of your preferred substance?

- ☐ Reddish — 1
- ☐ Whitish — 3
- ☐ Blackish — 5
- ☐ Yellowish — ☐ 2
- ☐ Khaki — ☐ 4
- ☐ Other, please specify: _____ — ☐ 6 ☐☐ 7-8

30. Why do you prefer to eat a substance of that specific colour?

- ☐ Taste — 9
- ☐ Tradition / belief — 10
- ☐ Easily accessible — 11
- ☐ Other, please specify: _____ — 12 ☐☐ 13-14

31. Where do you store the substance?

_____ ☐☐ 15-16

32. For how long do you usually store the substance?_____(days) ☐☐☐ 17-19

D. PHYSICO-CHEMICAL, MINERALOGICAL, GEOLOGICAL AND CHEMICAL ASPECTS

33. Where can your preferred substance be found?

- ☐ Hill / mountain — 20
- ☐ Riverbed — 21
- ☐ Termitaria / termite mound — 22
- ☐ Valley — 23
- ☐ Pit / excavation — 24
- ☐ Other, please specify: _____ — 25 ☐☐ 26-27

33.1 If a **termitaria/ termite mound**, from where specifically is the substance collected?

- ☐ From the outer surface of the mound — 28
- ☐ Inside the mound above the surface of the soil — 29
- ☐ Inside the mound below the surface of the soil — 30
- ☐ Does not matter — 31
- ☐ Not sure — 32

34. Is your preferred substance found close to rocks? | Yes | No | Not sure | — 33

34.1 If **YES**, what type of rock?

- ☐ Very hard — 34
- ☐ Hard — 35
- ☐ Soft — 36
- ☐ Very soft — 37

35. Substance-collection method

- ☐ Digging
- ☐ Scooping handfuls
- ☐ Scraping
- ☐ Selective hand-picking
- ☐ Other, please specify: _____

35.1 If **digging**, how deep?_____cm

36. How does the substance feel?

- ☐ Gritty
- ☐ Silky
- ☐ Powdery
- ☐ Does not matter
- ☐ Don't know

37. In what condition is the substance collected? ☐ Wet ☐ Dry ☐ Both

37.1 If **collected wet**, how does the substance feel?

- ☐ Very sticky
- ☐ Sticky
- ☐ Very soapy
- ☐ Soapy
- ☐ None of the above

38. Is the substance processed before being eaten? ☐ Yes ☐ No ☐ Sometimes

38.1 If **YES**, how is it processed?

- ☐ Grinding
- ☐ Pounding
- ☐ Sieving
- ☐ Slurrying
- ☐ Other, please specify: _____

39. Is there any heat treatment of the substance before it is eaten? ☐ Yes ☐ No ☐ Sometimes

39.1 If **YES**, specify the type of heat treatment:

- ☐ Baking
- ☐ Boiling
- ☐ Burning
- ☐ Combination, please specify: _____
- ☐ Other, please specify: _____

E. ECOLOGICAL ASPECTS

40. If applicable, please specify the type of termitaria from which you prefer to collect substances?

- ☐ Mound
- ☐ Tree

40.1 If the substance is collected from a **termite mound** (Section C), describe the preferred height of the mound.

- ☐ < 0.5 m
- ☐ 0.5 – 1 m
- ☐ 1 – 2 m
- ☐ > 2 m

40.2 What is the preferred shape of the mound?

- ☐ Conical
- ☐ Flat topped
- ☐ Dome shaped
- ☐ Other, please specify

☐ 38
☐ 39
☐ 40
☐ 41
☐ 42 ☐☐ 43-44
☐☐☐ 45-47
☐ 48
☐ 49
☐ 50
☐ 51
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☐ 53
☐ 54
☐ 55
☐ 56 ☐☐ 57-58
☐ 59
☐ 60
☐ 61
☐ 62
☐ 63 ☐☐ 64-65
☐ 66 ☐☐ 67-68
☐ 69
☐ 70
☐ 71
☐ 72
☐ 73
☐ 74
☐ 75

40.3 Do you prefer to eat the substance when
- ☐ Newly formed
- ☐ Old
- ☐ Does not matter
- ☐ Not sure

☐ 76

40.4 In what type of terrain do you normally find these mounds?
- ☐ Flat
- ☐ Hilly
- ☐ Undulating
- ☐ Valley
- ☐ Other, please specify: _____

☐ 1
☐ 2
☐ 3
☐ 4
☐ 5 ☐☐ 6-7

40.5 Do you collect the substance from
- ☐ Mound
- ☐ Base of the mound
- ☐ Some distance from the mound
- ☐ Other, please specify: _____

☐ 8

41. If substance is collected from a **tree**, do you prefer it to be a particular type of tree?

Yes	No	Not sure	oes not matt

☐ 9

41.1 If **YES**, name the preferred type of tree: _____

☐☐ 10-11

F. HUMAN HEALTH ASSOCIATED WITH GEOPHAGIA

42. What is your height?_____(cm)

☐☐☐ 12-14

43. What is your weight?_____(kg)

☐☐☐ 15-17

44. Do you think that the substance could be harmful?

Yes	No

☐ 18

44.1 If **YES**, in what way?
- ☐ Constipation
- ☐ Abdominal pains
- ☐ Poisoning the body
- ☐ Causing tooth decay
- ☐ Other, please specify: _____

☐ 19
☐ 20
☐ 21
☐ 22
☐ 23 ☐☐ 24-25

45. Have you ever undergone surgery for a stomach ailment?

Yes	No

☐ 26

45.1 If **YES**,
How many times? _____

☐☐ 27-28

For what reason? _____

_____

☐☐ 29-30

46. Do you think there are harmful elements / parasites present in the substance?

Yes	No

☐ 31

47. Do you know the components of the substance?

Yes	No

☐ 32

47.1 If **YES**, name these components:
- ☐ Vitamins
- ☐ Calcium
- ☐ Iron
- ☐ Salt
- ☐ Other, please specify: _____

☐ 33
☐ 34
☐ 35
☐ 36
☐ 37 ☐☐ 38-39

48. Why do you eat the substance(s) you do?

- ☐ To clean your body
- ☐ For additional nutritional value
- ☐ To protect against infections
- ☐ Don't know
- ☐ Other, please specify: _____

49. Are you often ill (infections like colds, flu, etc.)? ☐ Yes ☐ No

49.1 If **YES**, how often?

- ☐ More than once a month
- ☐ Once a month
- ☐ Once every three months
- ☐ Twice a year
- ☐ Once a year

50. Do you eat these substances when ill? ☐ Yes ☐ No ☐ Sometimes

51. Any medical condition diagnosed/experienced ☐ Yes ☐ No

51.1 If **YES**, which of these?

- ☐ Constant headaches
- ☐ Dizziness
- ☐ Blood in stool
- ☐ Fatigue
- ☐ Chest pains
- ☐ Coughs
- ☐ Muscle pains
- ☐ Tremors
- ☐ Blood in urine
- ☐ Nosebleeds
- ☐ Iron deficiency
- ☐ High Blood pressure
- ☐ Constipation
- ☐ Other, please specify

52. Number of stillborn children (full time)? _____

53. Number of miscarriages? _____

54. Number of children born with abnormalities? _____

55. Name the abnormalities. _____ _____ _____

☐ 40
☐ 41
☐ 42
☐ 43
☐ 44 ☐☐ 45-46
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☐☐ 70-71
☐☐ 72-73

APPENDIX: B

Information Document

Information document

Study title:

Human and Enzootic Geophagia in South Africa, Botswana and Swaziland

Dear participant,
We, the geophagia team (researchers from the University of Limpopo and Central University of Technology, Free State, University of Swaziland and Botswana) are doing research on geophagia (the purposeful ingestion of soils and clays by humans and animals) in Southern Africa. We are asking you to participate in this research project

The study aims to identify and characterize selected soils and clays in Botswana, South Africa and Swaziland that are being ingested by local communities as well as calves, lambs and kids. The study will be directed at identifying soils and clays in target areas, carry out appropriate mineralogical, chemical, microbiological, ecological and environmental health analyses geared towards the documentation of geophagic practices that have been going on for several centuries in these countries. We want to conduct structured questionnaires-response studies which will address aspects related to environmental health and indigenous knowledge (IK) associated with geophagia in these countries. In South Africa the team will conduct structured interviews with 330 adults, 132 children, 110 students and 66 farmers to determine the attitudes and beliefs, standard practice of geophagea, as well as general health status of the respondents. The study will also be conducted in Swaziland and Botswana. In each of Botswana and Swaziland 110 adults, 66 children, 44 students and twenty farmers will be interviewed.

There are no risk being involved in the study, the field worker will ask you a number of questions relating to the practice of geophagia to enable the research team to gain more insight into this practice.

There will be no direct benefits of being involved in the study. However, the study ultimately aims to advance more suitable harvesting techniques of geophagic soils and clays which will aid in reducing down side effects.

You, as participant in the study will be given information on the results of the study once it is completed.

Please note that participation to the study is voluntary and that refusal to participate will involve no penalty. You may discontinue your participation at any time during the study without any penalty.

Efforts will be made to keep personal information confidential. Personal information may be disclosed if required by law. If results are published, this may lead to cohort identification.

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APPENDIX: C

Consent to participate in research

CONSENT TO PARTICIPATE IN RESEARCH

You have been asked to participate in a research study.

You have been informed about the study
by……

You may contact Prof L de Jager at the Central University of Technology, Dr G Ekosse at the University of Limpopo, Dr M Ditlhogo, University of Limpopo or Dr NO Simelane, University of Swaziland any time if you have questions about the research or if you are injured as a result of the research.

You may contact the Secretariat of the Ethics Committee of the Faculty of Health Science, UFS, Bloemfontein, South Africa at telephone number (051) 405 2812 if you have questions about your right as a research subject.

Your participation in this research is voluntary, and you will not be penalized or lose benefits if you refuse to participate or decide to terminate participation.

If you agree to participate, you will be given a signed copy of this document as well as the participant information sheet, which is a written summary of the research.

The research study, including the above information has been verbally described to me.

I understand what my involvement in the study means and I voluntarily agree to participate.

Signature of Participant	Date
Signature of Witness (if available)	Date
Signature of Interviewer	Date

Pampitshana ya Molaetsa

Sehloho sa Thuto:
Batho le Enzootic Geophagia Afrika e Borwa, Botswana le Swaziland

Dumela Monka-karolo
Re le sehlopha sa diphuputso(Baphupotsi hotswa Yunivesithi ya Limpopo, Yunivesithi e bohareng ya thekenoloji, Foreistata, Yuniovesithi ya Swaziland le Botswana) re etsa dipatlisiso ka ho JA mobu le letsopa ke batho le diphoofolo ho la Afrika e Borwa le di naha tse mabapi. Re kopa hore o nke karolo phupotsong/patlisisong ena.

Thuto e ikemiseditse ho temoho le ho hlopolla mefuta ya mebu le letsopa ho la Botswana, Afrika Borwa le Swaziland e Jewang ke Batho metseng le diphoofolo mmoho le bana. Thuto e tla lebiswa ho temoho ya mobu le letsopa di bakeng tse kgethilweng ho ntshetsa hantle mineralogical, dikhemikale, microbiology, ecological le patlisisa ka tsa bophelo ho I setsa ho ngolweng ha ntle ha tlhaello ena ya ho ja mobu e e sa le e etswa ka dilemo lemo naheng tsena. Re rata ho ntshetsa pele dipotso ka tsela ya thuto e tla buwa ka mabaka a amanang le tsa bophelo di bakeng tsa rona le tshebo ya lehae e amanang le ho ja mobu dinaheng tsena. Afrka borwa sehlopha se tlo etsa dipotso le Batho ba baholo ba kabang 330, Bana ba 132, Bithuti ba 110 le bo ramapolasi ba 66 ho fumana hatle tlhaello le mokgwa, le tumelo mmoho le bophello ka kakaretso ho ba nkakarolo. Thuto ena e boetse e tla etsa dipotso tsena Swaziland le Botswana. Ho se seng le se seng sa sebaka 110 Batho ba baholo, 110 Bana, baithuti 44 bo rapolasi ba 22.

Ha ona kotsi ho nkeng karolo ha hao thutong ena, mosebetsi o tla o botsa dipotso tse mmalwa mabapi le tlwaello ya ho ja mobu ho etsa hore patlisiso e fumane le sedi le itseng ka ho ja mobu.

Ha ho naba le moputso/ kgolo (benefits) e itse ho tla ho wena ka nepo thutong ena. Empa thuto e ekemiseditse ho ntlafatsa mekga e nepahetseng ya ho kga mobu le letsopa e tla fokotsa di tla moraho tse itseng.

Wena tjhe ka monka-karolo otla fumana lesedi hang ha thuto e fedile.

Re kopa o ele hloko ho re ho nka-karolo ha hao ho thuto ea ke ka bo ithaopo hape le ho hana ha hao ho se nke karolo ha ho letlo leo re ka o etsang lona (No penalty). Oka e misa kapa wa tlohela nako engwe le engwe ka thuto ntle le kotlo e itseng.

Maikitlaetso a tla etswa ho o sireletsa ditaba tsa hao ele le kunotu ka hohle hohle. Dibata tsa hao ditla fumaneha fela ka tsela ya molao. E bang tse fumangweng thutong di phatlalatswa sena se ka isa ho phatlallatso e itseng empa esa totoballa. (cohort identification)

TUMALLANO HO NKA KAROLO HO PATLISISO

O kupuwe ho nka karolo thutong ea ya patlisiso

O se o fumane lesedi ka thuto ena?
Ka.....

Oka ikopanya le Moporofesa L de Jager Yunivesithing e bohareng ya Foreistata, Ngaka G Ekosse Yunivesithing ya Limpopo, Ngaka M Ditlhogo Yunivesithing ya Limpopo, Ngaka Simelane Yunivesithing ya Swaziland ka nako tsohle ha ona le dipotso ka thuto kapa tse fumangeng patlisisong ena.

Oka boela wa ikopanya le Mokgodi wa komiti ya mekgwa ya bophelo bo bottle le science, UFS, Bloemfontein Afrika Borwa ka mohala nomorong ena (051) 405 2812 ha ona le dipotso ka ditokelo tsa hao tjhe ka monka-karolo.

Ho nka-karolo ha hao ke boithaopo, hape ha ona fumantshwa kotlo kapa wa latlehelwa ke omonyetla oitseng ka ho hana ho nka-karolo kapa ha oka nka qeto ya ho tlohela ebang o ne o se o qadile.

Ha o dumela ho nka-karolo , o tla fumana pampiri ya ho saena le pampitshana ya ditaba mabapi le thuto, e ngotswe ditabana tsa patlilsioso.

Patlisiso , ho kenyeletswa le ditaba tse ngotsweng ka hodimo dibuilwe ho nna. Ke othisisa ho nakakarolo ha ka thutong ho bolellang hape ke e thaopile ho nkakarolo.

Saene ya Monka-karolo	Letsatsi
Saene ya Paki (Ha ho tlokahala)	Letsatsi
Saene ya Motsamaisi/mobutsi	Letsatsi

APPENDIX: D Food frequency questionnaire

GEOPHAGIA 2014

INTERVENTION STUDY

Respondent number: | | | | 1-3

Interviewer:_____ | | 4

(1) Baseline / (2) After Intervention | | 5

QUANTITATIVE FOOD FREQUENCY QUESTIONNAIRE

Greeting

Thank you for giving up your time to participate in this survey. We would like to find out what people usually eat and drink. This information is important to know as it will tell us whether you eat the right foods, and if you are healthy.

Please think carefully about the food and drinks you have consumed during the past 6 months. I will now go through a list of foods and drinks with you and I would like you to tell me:

- if you eat these particular foods,
- how the food is prepared,
- how much of the food you eat at a time, and
- how many times a day you eat it and if you do not eat it every day, how many times a week or a month it is eaten?

To help you to describe the amount of a food, I will show you models and examples of different amounts of the food. Please say which model is the closest to the amount eaten, or if it is smaller, between sizes or bigger than the models. Amounts can also be reported as cups (c), tablespoons (T) or teaspoons (t).

- **THERE ARE NO RIGHT OR WRONG ANSWERS.**
- **EVERYTHING YOU TELL ME IS CONFIDENTIAL.**
- **IS THERE ANYTHING YOU WANT TO ASK NOW?**
- **ARE YOU WILLING TO GO ON WITH THE QUESTIONS?**
- **ENCIRCLE APPROPRIATE ANSWER**

- **Do you use salt in your food during food preparation?** ☐ 6

  (1) Yes (2) No (3) Don't know

- **Are other flavoured salt e.g. Aromat used in food?** ☐ 7

  (1) Yes (2) No (3) Don't know

- **If yes, specify _____** ☐☐ 8-9

- **Do you use beef/chicken stock in your food?** ☐ 10

  (1) Yes (2) No (3) Don't know

- **Do you use laxatives on a regular basis (weekly)?** ☐ 11

  (1) Yes (2) No (3) Don't know

- **If yes, how often?** ☐ 12

  (1) Everyday

  (2) ≥ 3 times per week

  (3) Less then 3 times per week

- **Do you use any nutrient supplements regularly?** ☐ 13

  (1) Yes (2) No (3) Don't know

- **If yes, what kind of nutrient supplement?**

  (1)Vitamins : (1) Yes (2) No ☐ 14

If yes: Specify _____ 15-16

How often? 17

(1) Everyday

(2) ≥ 3 times per week

(3) Less than 3 times per week

If yes, what kind of nutrient supplement?

(2) Minerals: (1) Yes (2) No 18

If yes: Specify _____ 19-20

How often? 21

(1) Everyday

(2) ≥ 3 times per week

(3) Less than 3 times per week

If yes, what kind of nutrient supplement?

(3) Proteins: (1) Yes (2) No 22

If yes: Specify _____ 23-24

How often? 25

(1) Everyday

(2) ≥ 3 times per week

(3) Less than 3 times per week

If yes, what kind of nutrient supplement?

(4) Energy: (1) Yes (2) No | 26

If yes: Specify _____ | 27-28

How often? | 29

(1) Everyday

(2) ≥ 3 times per week

(3) Less than 3 times per week

If yes, what kind of nutrient supplement?

(5) Other: (1) Yes (2) No | 30

If yes: Specify _____ | 31-32

How often? | 33

(1) Everyday

(2) ≥ 3 times per week

(3) Less than 3 times per week

Do you drink coffee and tea (except rooibos) with your meals? | 34

(1) Yes

(2) No

If yes, at which meals? | 35

Breakfast (1) Yes (2) No | 36

Lunch (1) Yes (2) No

Supper (1) Yes (2) No | 37

Do you experience any problems with diarrhea on a regular basis (weekly)? ☐ 38

(1) Yes (2) No

If yes, how often? ☐ 39

(1) Everyday

(2) ≥ 3 times per week

(3) Less than 3 times per week

Do you experience any problems with constipation on a regular basis (weekly)? ☐ 40

(1) Yes (2) No

If yes, how often? ☐ 41

(1) Everyday

(2) ≥ 3 times per week

(3) Less than 3 times per week

EATING HABITS: (FREQUENCY AND TYPE OF CLAY CONSUMPTION)

Do you consume clay? (1) Yes (2) No ☐ 42

How often do you consume clay? ☐ 43

(1) Once a day

(2) More than once a day.

How much clay do you consume at a time (see samples)? ☐☐☐ 44-46

_____g

What kind of clay do you consume most often?

(1) Red ☐ 47

(2) White ☐ 48

(3) Gray ☐ 49

(4) Other, specify _____ ☐ 50

Do you ever consume any other non-food items on a regular basis e.g. chalk, washing powder etc. ☐ 51

(1) Yes

(2) No

If yes, specify:

Non-food items	**Grams**	**How often consumed?** **(1) Everyday** **(2) ≥ 3 times per week** **(3) Less than 3 times per week**			
_____	_____	_____	☐☐	☐☐☐	☐ 52-57
_____	_____	_____	☐☐	☐☐☐	☐ 58-63
_____	_____	_____	☐☐	☐☐☐	☐ 64-69
_____	_____	_____	☐☐	☐☐☐	☐ 70-75

SUMMARY OF FOOD FREQUENCY QUESTIONNAIRE

Respondent number: | | | | 1-3

FOOD	CALCULATIONS	CODE	AMOUNT PER DAY (g)							
										(1-8)
										(9-16)
										(17-24)
										(25-32)
										(33-40)
										(41-48)
										(49-56)
										(57-64)
										(65-72)
										(73-80)
										(1-8)
										(9-16)
										(17-24)
										(25-32)
										(33-40)
										(41-48)
										(49-56)
										(57-64)
										(65-72)
										(73-80)
										(1-8)
										(9-16)
										(17-24)
										(25-32)
										(33-40)
										(41-48)
										(49-56)
										(57-64)
										(65-72)
										(73-80)
										(1-8)
										(9-16)
										(17-24)
										(25-32)
										(33-40)
										(41-48)
										(49-56)
										(57-64)
										(65-72)
										(73-80)
										(1-8)
										(9-16)
										(17-24)
										(25-32)
										(33-40)
										(41-48)
										(49-56)
										(57-64)
										(65-72)
										(73-80)

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT/ DAY
			Per day	Per week	Per month	Seldom/ Never		
Maize-meal porridge	Stiff (pap)						**3400**	
Maize-meal porridge	Soft (slappap)						**3399**	
Maize-meal porridge	Crumbly (phutu)						**3401**	
Sour porridge	Specify ratio Mabella/Maize						**3399**	
Mabella porridge	Stiff, coarse, fine						**3437**	
Mabella porridge	Soft, coarse, fine						**3437**	
Oats porridge	Brand name:						**3239**	
Breakfast cereals	Puffed Wheat, plain						**3325**	
	Corn Flakes, plain						**3243**	
	Weet Bix						**3244**	
	Puffed Rice, sweet						**3372**	
	Specify types usually eaten_____ Brand names of cereals available at home now:							
Milk on porridge or cereal: Circle type usually used	None							
	Whole/fresh						**2718**	
	Sour						**2787**	
	2% fat						**2772**	
	Fat free/skimmed						**2775**	
	Milk blend						**2771**	
	Soy milk						**2737**	
	Condensed (whole,sweet)						**2714**	
	Condensed (skim, sweet)						**2744**	
	Evaporated whole						**2715**	
	Evaporated low fat						**2827**	
	Non-dairy creamer						**2751**	
Is sugar added to porridge or cereal? (Tick box)	None White Brown Syrup Honey Sweetener: type_____						 **3989** **4005** **3988** **3984**	
Is fat added to porridge or cereal? (Tick box)	None Animal fat (butter) Hard margarine Soft margarine Oil Peanut Butter						 **3479** **3484** **3496** **3507** **3485**	
Samp/Maize rice **Samp and beans** **Samp and peanuts**	Bought Self ground Specify ratio (1:1) Specify ratio						**3250** **3725** **3402**	
Rice: specify brands names:	White Brown Sorghum rice						**3247** **3315** **3437**	

Stamped wheat							**3249**	
Pastas	Macaroni Spaghetti Spaghetti in tomato sauce Other:						**3262** **3262** **3258**	

HOW MANY TIMES A WEEK DO YOU EAT PORRIDGE OR BREAKFAST CEREAL AT ANY TIME OF THE DAY (NOT ONLY BREAKFAST)?_____

FOOD	**DESCRIPTION**	**AMOUNT USUALLY EATEN**	**TIMES EATEN**				**CODE**	**AMOUNT /DAY**
			Per day	**Per week**	**Per month**	**Seldom/ Never**		
Bread/Bread rolls	White						**3210**	
Bread slices: thin	Brown						**3211**	
Medium, thick	Whole wheat						**3212**	
Other breads	Specify types e.g. Raisin Maize meal Sweetcorn Rye Other						 **3214** **3278** **3379** **3213**	
Pizza (specify toppings) **Hot Dogs(specify sausage)** **Hamburgers (specify meat)**	Cheese, tomato & onion _____ _____ _____						**3353**	
Are any the following spreads used on bread? **Fat spreads (Tick box)**	Butter Butro Animal fat (beef tallow) Lard Hard margarine (brick) Soft margarine (light) Cooking Fat						**3479** **3523** **3494** **3495** **3484** **3496** **3516**	
Peanut butter							**3485**	
Sweet spreads	Jam Syrup Honey						**3985** **3988** **3984**	
Marmite/ **OXO/** **Bovril**							**4030** **4029** **4029**	
Fish paste **Meat paste**							**3109** **2917**	
Cheese	Specify types: Cottage low-fat cheese Cream cheese Gouda Cheddar Other:_____						 **2760** **2725** **2723** **2722**	

Cheese spreads	Low fat Full fat Specify types						**4310** **2730**	
Atchar							**3117**	
Other spreads: (Specify types)	_____ _____							
Dumpling							**3210**	
Vetkoek							**3257**	
Provita **Crackers (refined)** **Crackers (whole wheat)**							**3235** **3331** **3391**	

Rusks *Home-made:*	Bran Buttermilk White Boerebeskuit, white All-bran Raisins Buttermilk, white Buttermilk, whole wheat Other						**3330** **3329** **3364** **3364** **3380** **3380** **3215** **3255**	
Scones							**3237**	
Muffins	Plain Bran						**3408** **3407**	

HOW MANY TIMES A DAY DO YOU EAT BREAD? _____

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT/ DAY
			Per day	Per week	Per month	Seldom/ Never		
Chicken **Do you eat the chicken with the skin?** **Yes □ No □**	Boiled: with skin without skin						**2926** **2963**	
	Fried: in batter/crumbs Fried, but not coated						**3018** **2925**	
	Roasted/grilled with skin without skin						**2925** **2950**	
Chicken bones stew							**A003**	
Chicken heads, raw							**2999**	
Chicken stew, with veg. & skin							**3005**	
Chicken feet, raw							**2997**	
Chicken offal	Giblets						**2998**	
Chicken pie	Commercial Home-made						**2954** **2954**	
Red meat: Beef	Fried/grilled: with fat without fat						**2908** **2959**	
	Stewed/boiled: with fat without fat						**3006** **2909**	
	Mince with tomato and onion						**2987**	

Red meat: Mutton	Fried/grilled: with fat without fat						**2927** **2934**	
	Stewed/boiled: with fat without fat						**3040** **2916**	
Red meat: Pork	Fried/grilled: with fat without fat						**2930** **2977**	
	Stewed/boiled: with fat without fat						**3046** **3045**	
Red meat: Goat	Fried/grilled: with fat without fat						**4281**	
	Stewed/boiled: plain with veg						**4281** **4282**	
Offal: Specify type:	Intestines: boiled, nothing added						**3003**	
	"Vetderm" fried						**3003**	
	Stewed with vegetables							
	Liver						**2955**	
	Kidney						**2956**	
	Tripe "pens" trotters, head						**3003**	
	Pluck (lungs, heart, gullet)						**3019**	
Specify vegetables used in meat stews (only if not mentioned elsewhere)								
Wors / sausage	Fried						**2931**	
Bacon							**2906**	
Cold meats	Polony						**2919**	
	Ham						**2967**	
	Vienna's canned						**2936**	
	Russian						**2948**	
	Frankfutter						**2937**	
	Other (specify)							
Canned meat	Bully beef						**2940**	
	Other (specify)							
Meat pie	Bought						**2939**	

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT/ DAY
			Per day	Per week	Per month	Seldom/ Never		
Legumes: specify dried beans/peas/ Lentils	Stews & curries (specify) Soups Salad						**3157** **3174**	
Baked beans							**3176**	
Soya products e.g. Toppers/ Imana	Brands at home now Don't know_____ **Show examples**						**3196**	
Fried fish (fresh or frozen fried in sun oil)	With batter/crumbs Without batter/crumbs						**3072** **3060**	
Fresh water fish **Specify type**	**Specify cooking method** Medium fat, batter, fried						**3094**	
Canned fish:								
Pilchards	In brine						**3055**	
	In tomato sauce						**3102**	
	Mashed with fried onion						**A005**	
Sardines	In oil In tomato sauce						**3087** **3087**	
Tuna	In oil In brine						**3093** **3054**	
Mackerel							**3113**	
Salmon							**3101**	
Pickled fish/curried							**3076**	
Do you remove fish bones before eating canned fish	YES ☐ NO ☐							
Fish cakes Specify canned or other	Fried: oil/butter/margarine, commercial						**3080**	
Salted dried fish							**3077**	

Eggs	Boiled/poached Scrambled in: oil butter margarine Fried in: oil butter margarine bacon fat Curried						**2876** **2889** **2886** **2887** **2869** **2868** **2877** **2870** **2902**	

HOW MANY TIMES A WEEK DO YOU EAT MEAT _____,
BEANS _____,
CHICKEN _____,
FISH _____ **AND**
EGGS _____**?**

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT/ DAY
			Per day	Per week	Per month	Seldom/ Never		
Cabbage	Boiled, nothing added						**3756**	
	Boiled with potato and onion and fat						**3813**	
	Fried, in margarine (nothing added)						**3810**	
	Fried, in oil (nothing added)						**3912**	
	Boiled, then fried with potato, onion						**A006**	
	Other:							
Spinach/morogo/imfino/other green leafy vegetables: List names	Boiled, nothing added						**3913**	
	Boiled fat added (margarine)						**3898**	
	Boiled with onion/tomato and fat						**A011**	
	-onion &potato (margarine)						**3901**	
	- onion, tomato & potato							
	- with peanuts							
	Other:							
Tomato and onion 'gravy'/relish/chow	Home made -with fat without fat						**3910** **3925**	
	Canned						**4129**	
Pumpkin Specify type:	Cooked in fat & sugar						**A010**	
	Boiled, little sugar and fat						**A010**	
	Boiled						**4164**	
_____	Other:							
Carrots	Boiled, sugar & fat						**3819**	
	Boiled, nothing added						**3757**	
	Boiled, potato, onion, no fat						**3934**	
	Boiled, potato, onion, margarine						**3822**	
	Boiled, with sugar						**3818**	
	With potato/onion						**3934**	
	Raw, salad (orange juice)						**3711**	
	Chakalaka							
	Other:							
Mealies/Sweet corn	On cob						**3725**	
	Off cob -creamed sweet corn Off cob whole kernel						**3726** **3942**	
Beetroot	Cooked Salad (bought or home-made)						**3698** **3699**	

Potatoes	Boiled with skin						**4155**	
	-without skin						**3737**	
	Baked in skin(flesh and skin) - in skin (flesh only)						**3736** **3970**	
	Mashed - skim milk, margarine						**3875**	
	Mashed - whole milk, margarine						**3876**	
	Roasted in beef fat						**3878**	
	French fries/potato chips (oil)						**3740**	
	Salad (mayonnaise and egg)						**3928**	
	Other:							
Sweet potatoes	Boiled with skin						**3748**	
	without skin						**3903**	
	Baked with skin						**3748**	
	- without skin						**3903**	
	Mashed						**3903**	
	Other:							
Peas	Green, frozen Green, frozen with sugar With sugar and butter Tinned peas						**4146** **3720** **3859** **4149**	
Green peppers	Raw Cooked (stew with oil)						**3733** **3865**	
Brinjal/egg plant	Cooked Fried in oil Stew (oil, onions, tomato)						**3700** **3802** **3798**	
Mushrooms	Raw Sautéed in brick margarine Sautéed in oil						**3842** **3839** **3841**	
Onions	Sauteed in sun oil Sauteed in margarine						**3730** **3844**	
Salad vegetables	Raw tomato						**3750**	
	Lettuce						**3723**	
	Cucumber						**3718**	
	Avocado's						**3656**	
Green Beans	Boiled, nothing added						**3696**	
	Cooked, potato, onion, margarine						**3792**	
	Cooked, potato, onion, no fat						**3933**	
Cauliflower	Boiled							
Other vegetables; specify	_____ _____							

If you fry veg or add fat specify type of fat usually used	Butter Butro Animal fat (beef tallow) Lard Hard margarine (brick) Soft margarine (tub) Soft margarine (light) Sunflower oil						**3479** **3523** **3494** **3495** **3484** **3496** **3524** **3507**	

HOW MANY TIMES A WEEK DO YOU EAT VEGETABLES? _____

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT/ DAY
			Per day	Per week	Per month	Seldom/ Never		
Mayonnaise/ salad dressing	Mayonnaise: bought home-made Cooked salad dressing Salad dressing low-oil Salad dressing French Oil: Olive Sunflower Canola						**3488** **3506** **3503** **3505** **3487** **3509** **3507** **4280**	
Apples	Fresh Canned, unsweetened						**3532** **4216**	
Pears	Fresh Canned, in syrup						**3582** **3583**	
Bananas							**3540**	
Oranges **Naartjie**							**3560** **3558**	
Grapes							**3550**	
Peaches	Fresh Canned, in syrup						**3565** **3567**	
Apricots	Fresh Canned, in syrup						**3534** **3535**	
Mangoes	Fresh						**3556**	
Pawpaw	Raw						**3563**	
Pineapple	Raw Canned (syrup)						**3581** **3648**	
Guavas	Fresh Canned (syrup)						**3551** **3553**	
Watermelon							**3576**	
Spanspek	Orange flesh Green flesh						**3541** **3575**	
Wild fruit/berries (Specify types)	_____ _____ _____ _____ _____							

Dried fruit (also as snacks)	Raisins Prunes (raw) Prunes (cooked with sugar) Peaches (raw) Peach (cooked with sugar) Apples (raw) Dried fruit sweets Other _____ _____ _____						**3552** **3596** **3564** **3568** **3569** **3600** **3995**	
Other fruit	_____ _____ _____ _____	_____ _____ _____ _____	____ ____ ____ ____	____ ____ ____ ____	_____ _____ _____ _____	_____ _____ _____ _____		_____ _____ _____ _____

HOW MANY TIMES A WEEK DO YOU EAT FRUITS? _____

WE NOW WILL ASK YOU QUESTIONS ABOUT WHAT YOU USUALLY DRINK

BEVERAGES	DESCRIPTION	AMOUNT USUALLY TAKEN	TIMES TAKEN				CODE	AMOUNT/ DAY
			Per day	Per week	Per month	Seldom/ Never		
Water							**4042**	
Tea	Ceylon Rooibos						**4038** **4054**	
Coffee							**4037**	
Sugar per cup of tea or coffee	White Brown						**3989** **4005**	
Milk per cup of tea or coffee What type of milk do you put in tea and/or coffee?	Fresh/long life whole						**2718**	
	Fresh/long life 2% Goat						**2772** **2738**	
	Fresh/long life/fat free (skimmed)						**2775**	
	Whole milk powder, reconstituted Specify brand: _____						**2831**	
	Skimmed milk powder, reconstituted Specify brand: _____						**2719**	
	Milk blend, reconstituted Specify brand: _____						**2771**	
	Whitener/non-dairy creamer Specify brand: _____						**2751**	
	Condensed milk (whole)						**2714**	
	Condensed milk (skim)						**2744**	
	Evaporated milk (whole)						**2715**	
	Evaporated milk (low-fat)						**2827**	
	None							
Milk as such: What type of milk do you drink as such?	Fresh/long life/whole Fresh/long life/2% Fresh/longlife/fat free (skimmed) Goat						**2718** **2772** **2775** **2738**	
	Sour / Maas						**2787**	
	Buttermilk						**2713**	

BEVERAGES	DESCRIPTION	AMOUNT USUALLY TAKEN	TIMES TAKEN				CODE	AMOUNT/ DAY
			Per day	Per week	Per month	Seldom/ Never		
Milk drinks Specify brands, Including milk supplements and type of milk used	Nestle Nesquik Milo Flavoured milk Other						**4287** **2735** **2774**	
Yoghurt	Drinking yoghurt Thick yoghurt, plain, fruit						**2756** **2732**	
Squash	SixO Oros Lecol with sugar -artificial sweetener Kool Aid Other_____ _____						**3990** **3982** **3982** **3990** **3982**	
Fruit juice	Fresh/Liquifruit/Ceres/						**2866**	
	"Tropica"/ mixtures with milk						**2791**	
Fruit syrups	Average Guava syrup						**2865** **2864**	
Fizzy drinks Coke, Fanta	Sweetened Diet						**3981** **3990**	
Mageu/Motogo							**4056**	
Alcoholic beverages such as Sorghum beer	Sorghum beer Specify:						**4039**	
Other , specify:	Beer average Wine Cider _____ _____						**4031** **4033** **4057**	

PLEASE INDICATE WHAT TYPES AND AMOUNTS OF SNACKS, PUDDINGS AND SWEETS YOU EAT:

FOODS	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT/ DAY
			Per day	Per week	Per month	Seldom/ Never		
Potato crisps/chips							**3417**	
Peanuts	Roasted, unsalted Roasted, salted						**3452** **3458**	
Cheese curls: Niknaks etc.	Average Savoury						**3267** **3418**	
Popcorn	Plain (no salt and butter) Plain (salt and butter added) Sugar coated						**3332** **3359**	
Raisins (seeds)							**4231**	
Chocolates	Milk						**3987**	

	Kit Kat Peppermint crisp Specify types and names _____						**4024** **3997**	
Candies	Sugus, gums, hard sweets (specify) Peppermint						**3986** **4004**	

Sweets	Toffees Hard boiled Fudge, caramels (specify)						**3991** **3986** **3991**	
Biscuits/cookies	Specify type Home made plain Shortbread, butter Commercial, plain Commercial with filling						**3233** **3296** **3216** **3217**	
Cakes & tarts	Chocolate, plain						**3419**	
Pancakes/ crumpets							**3344**	
Koeksisters							**3231**	
Savouries	Sausage rolls Samoosas - vegetable Samoosa - mutton Biscuits e.g. bacon kips Other: _____						**2939** **3414** **3355** **3331**	
Pudding: jelly							**3983**	
Baked pudding	Plain batter						**3429**	
Instant pudding	Skim milk Whole milk						**3314** **3266**	
Ice cream	Commercial regular Commercial rich Soft serve Sorbet Ice lollies Chocolate coated individual ice creams (e.g. Magnum)						**3483** **3519** **3518** **3491** **3982**	
Custard	Home made, whole milk Ultramel						**2716** **2716**	
Cream	Fresh						**3520/ 3480**	
Other puddings (Specify):	_____							

HOW MANY TIMES A WEEK DO YOU EAT SNACK FOODS? _____

SAUCES / GRAVIES / CONDIMENTS

FOODS	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT/ DAY
			Per day	Per week	Per month	Seldom/ Never		
Tomato Sauce Worcester sauce							**3139 4309**	
Chutney	Fruit						**3168**	
	Tomato						**3114**	
Pickles							**3866**	
Packet soups							**3158**	
Beef/chicken stock							**4029**	
Others:								

WILD BIRDS, ANIMALS, INSECTS OR FRUITS AND BERRIES (hunted or collected in rural areas or on farms: (specify)								

- **PLEASE MENTION ANY OTHER FOODS YOU EAT MORE THAN ONCE EVERY TWO WEEKS WHICH WE HAVE NOT TALKED ABOUT AND OR FOODS EATEN IN OTHER HOMES OR PLACES DURING THE PAST WEEK**

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/ Never		

- **ARE THERE ANY FOODS THAT YOU EAT WHICH WE HAVEN'T TALKED ABOUT? PLEASE LIST THEM.**

FOODS	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT/ DAY
			Per day	Per week	Per month	Seldom/ Never		

THANK YOU FOR YOUR CO-OPERATION AND PATIENCE.
GOOD BYE!

ADAPTED FROM THE QUESTIONNAIRES OF THE THUSA STUDY (WITH ACKNOWLEDGEMENT TO THE RESEARCH GROUP OF PUCHO) AND THE NATIONAL FOOD CONSUMPTION SURVEY

APPENDIX: E Raw data

Num	Group	NR	Number	Age	ESR	Wbc	Rbc	Hb
1	Patient	ADCI 7001	SAQA 65	20	31 H	5.5 N	4.35 N	10 L
2	Patient	ADCI 7004	SAQA 68	22	63 H	4.8 N	3.56 H	9.2 L
3	Patient	ADCI 7011	SAQA 71	28	121 H	7.7 N	4.01 N	8 L
6	Patient	ADCI 7014	SAQA 74	22	119 H	2.9 L	3.7 L	7.2L
7	Patient	ADCI 7022	SAQA 79	18	42 H	4.8 N	4.8 N	13.8 L
8	Patient	ADCI 7023	SAQA 80	19	48	6.4	4.17	11
9	Patient	ADCI 7025	SAQA 87	24	22	5.3	4.55	14.3
10	Patient	ADCI 7026	SAQA 89	30	28	6.6	4.17	11.9
11	Patient	ADCI 7010	SAQA 93	25	126	3.6	3.63	9.1
12	Patient	ADCI 7009	SAQA 94	27	109	4.4	4.56	12.4
13	Patient	ADCI 7008	SAQA 95	30	118	3.7	3.74	11.6
14	Patient	ADCI 7017	SAQA 96	34	57	4.5	3.97	13
15	Patient	ADCI 7018	SAQA 97	20	24	9.1	4.53	13.5
16	Patient	ADCI 7028	SAQA 98	21	35	4.4	4.15	11.8
17	Patient	ADCI 7031	SAQA 99	18	5	6.5	4.66	12.8
18	Patient	ADCI 7029	SAQA 100	18	34	7.7	4.5	10.7
19	Patient	ADCI 7032	SAQA 101	30	30	5.2	4.54	11.3
20	Patient	ADCI 7040	SAQA 102	18	48	6.7	4.05	8.5
21	Patient	ADCI 7042	SAQA 103	37	95	6.7	4.33	9.4
22	Patient	ADCI 7037	SAQA 104	35	99	3.7	4.27	11
23	Patient	ADCI 7039	SAQA 105	35	103	2.3	3.72	9.2
24	Patient	ADCI 7047	SAQA 106	18	25	7.4	3.65	10.8
25	Patient	ADCI 7046	SAQA 107	18	42	4.3	4.14	12.9
26	Patient	ADST 1907	SAQA 108	22	66	4.4	4.12	12.6
27	Patient	ADST 1906	SAQA 109	18	45	4.1	4.19	11
28	Patient	ADST 1900	SAQA 110	40	99	6	4.45	12.2
29	Patient	ADST 1902	SAQA 111	26	6	5.2	4.31	12.8
30	Patient	ADST 1901	SAQA 112	33	18	4.9	3.96	11.9
31	Patient	ADCI 7038	SAQA 113	22	5	5	4.07	11.6
32	Patient	ADCI 7036	No number	26	9	8.3	4.17	12.8
33	Patient	ADST 1909	SAQA 114	26	113	5	4.03	9.9
34	Patient	ADST 1911	SAQA 116	23	10	2.9	4.26	8.9
35	Patient	ADST 1913	SAQA 117	21	11	4.5	4.25	11.7
36	Patient	ADST 1921	SAQA 118	30	38	9.7	3.91	11.2
37	Patient	ADST 1922	SAQA 119	22	44	9	4.31	10.8
38	Patient	ADST 1923	SAQA 120	23	22	12.8	4.26	14
39	Patient	ADST 1924	SAQA 121	22	130	7.3	3.43	7.6
40	Patient	ADST 1925	SAQA 122	30	16	7.6	4.36	12.8
41	Patient	ADST 1926	SAQA 123	24	30	7.4	4.93	12.6
42	Patient	ADST 1928	SAQA 124	24	44	6.4	4	10.5
43	Patient	ADST 1930	SAQA 125	20	11	6.4	4.76	15.4

Num	Group	NR	Number	Age	ESR	Wbc	Rbc	Hb
44	Patient	ADST 1932	SAQA 126	43	70	4.6	3.74	10.5
45	Patient	ADST 1933	SAQA 127	18	34	5.6	3.63	6
46	Patient	ADST 1938	SAQA 128	41	123	2.9	4.14	12.7
47	Patient	ADST 1940	SAQA 130	23	40	4.2	4.46	9.5
48	Patient	ADST 1941	SAQA 131	20	3	4	4.13	13
49	Patient	ADST 1943	SAQA 129	22	19	4.4	4.45	14.4
1	Control	ADCI 7027	SAQAC 00	18	6	4.5	4.43	13.1
3	Control	ADCI 7041	SAQAC 02	18	50	5.9	4.05	13.3
4	Control	ADCI 7043	SAQAC 03	39	51	3.5	4.12	12.7
5	Control	ADCI 7030	SAQAC 04	18	16	5.8	4.2	13
6	Control	ADCI 1905	SAQAC 08	30	127	4.2	4.45	11
7	Control	ADST 1904	SAQAC 09	26	15	8.4	4.26	13.6
8	Control	ADCI 7020	SAQAC 10	19	10	10.2	4.93	15.9
9	Control	ADST 1903	SAQAC 11	22	65	3.6	3.74	11.2
10	Control	ADCI 7003	SAQAC 13	21	3	8.5	4.05	12.7
11	Control	ADCI 7000	SAQAC 64	32	65	6.5	3.35	13.8
12	Control	ADCI 7002	SAQAC 65	29	23	9.1	4.71	13
13	Control	ADCI 7005	SAQAC 68	20	14	3.9	4.48	13.4
15	Control	ADCI 7015	SAQAC 72	21	30	4.4	4.59	13.2
16	Control	ADCI 7016	SAQAC 74	24	46	4.4	4.63	14.1
17	Control	ADCI 7024	SAQAC 79	32	84	4.3	4.28	12.4
18	Control	ADCI 7021	SAQAC 80	18	9	6	4.32	13.4
19	Control	ADCI 7007	SAQAC 93	23	65	6.3	4.64	13.9
22	Control	ADCI 7006	No number	29	24	7.4	4.64	15.3
23	Control	ADCI 7034	No number		30	5	4.36	10.1
24	Control	ADCI 7035	No Number		8	3.4	5.01	13.9
25	Control	ADST 1908	SAQAC 15	33	89	4.6	4.09	12.7
26	Control	ADST 1912	SAQAC 21	44	22	8	4.1	13.3
27	Control	ADST 1914	SAQAC 16	35	36	6.3	4.25	13.1
28	Control	ADST 1915	SAQAC 20	18	2	7.1	4.34	12.9
29	Control	ADST 1916	SAQAC 17	18	24	5.5	5.21	11.1
30	Control	ADST 1917	SAQAC 23	34	36	4	4.25	13.7
31	Control	ADST 1918	SAQAC 22	27	3	4.2	4.44	13.6
32	Control	ADST 1919	SAQAC 19	22	18	8.6	4.75	14.6
33	Control	ADST 1920	SAQAC 18	20	14	5	4.44	14.4
34	Control	ADST 1927	SAQAC 24	31	48	5	3.59	12.4
35	Control	ADST 1929	SAQAC 25	23	20	6.1	4.31	13.4
36	Control	ADST 1934	SAQAC 27	30	49	8.5	4.31	13.5
37	Control	ADST 1935	SAQAC 26	25	3	10.7	4.58	14.6
38	Control	ADST 1937	SAQAC 29	40	8	3.6	4.3	13.7
39	Control	ADST 1939	SAQAC 30	23	27	5.8	4.43	12.2
40	Control	ADST 1942	SAQAC 28	18	5	6.4	4.84	14.6

NR	Hct	MCV	MCH	MCHC	RDW	CRP	Plt	Ferr	Iron
ADCI 7001	31.3 H	72 L	23 L	31.9 L	18.5	12.2	347	12.6 L	5.6 L
ADCI 7004	28.2 H	79 L	26 L	32.8 L	15.9	3.9	419	9.3 L	2.8 L
ADCI 7011	25.4 H	63 L	20.1 L	31.7 L	18.4	4	352	8.9 L	3.5 L
ADCI 7014	24.2 H	66 L	19.5 L	29.8 L	18.1	11.4	90	6.8 L	2.2 L
ADCI 7022	40.3 H	84 N	28.7 N	34.1	15.6	11.6	216	10.8	6.8
ADCI 7023	33.9	81	26.3	32.4	16	3.5	221	5	4.4
ADCI 7025	42.3	93	31.4	33.8	13.2	1.8	346	20.1	7.9
ADCI 7026	35.7	86	28.6	33.4	14.3	<1.0	359	5.7	4
ADCI 7010	27.7	76	25.1	32.9	16.3	1.5	271	11.6	4.6
ADCI 7009	37.3	82	27.2	33.3	12.7	3.9	188	19.4	5.3
ADCI 7008	34.2	91	31.1	34	13.9	2.9	213	24.4	10.5
ADCI 7017	38.7	97	32.7	33.5	16.6	<1.0	253	22.1	5.6
ADCI 7018	40.2	89	29.8	33.5	13.1	6.7	323	51	7.9
ADCI 7028	35.1	84	28.4	33.7	16.3	<1.0	265	6.1	5.6
ADCI 7031	38.2	82	27.6	33.6	12.4	1.4	310	14.2	12
ADCI 7029	32.6	72	23.8	32.9	15.1	2.8	379	3.7	3.7
ADCI 7032	33.9	75	24.9	33.3	18.6	1.3	191	5	3.8
ADCI 7040	27.7	68	21.1	30.8	18.3	1.1	473	4.6	2.7
ADCI 7042	28.9	67	21.7	32.4	17.5	19.8	356	3.7	5.9
ADCI 7037	33.2	78	25.7	33.1	18.2	<1.0	293	7.4	4.5
ADCI 7039	28.4	76	24.7	32.3	19.1	1.8	273	12.2	6.2
ADCI 7047	32.4	89	29.7	33.5	15.9	<1.0	270	7.6	5.5
ADCI 7046	37.6	91	31.2	34.4	13.4	<1.0	202	29.2	9.1
ADST 1907	37.7	91	30.5	33.3	13.6	1.5	413	15.9	10.2
ADST 1906	33.4	80	26.2	33	16.7	<1.0	480	18.7	3.7
ADST 1900	36.2	81	27.3	33.6	15.2	<1.0	227	18.1	13.4
ADST 1902	39	90	29.7	32.8	12.8	1.2	356	31.9	23.2
ADST 1901	35.9	91	30.1	33.2	18.6	1.1	336	9.7	6.5
ADCI 7038	34.3	84	28.4	33.7	15.5	<1.0	312	4.8	4.5
ADCI 7036	37.3	90	30.6	34.2	12.9	<1.0	243	13.3	6.5
ADST 1909	29.6	74	24.6	33.5	19.3	6	219	6.9	3.6
ADST 1911	27.9	65	20.5	31.4	19.7	<1.0	386	2.1	4.1
ADST 1913	34.7	82	27.6	33.8	17.3	2.2	224	8.9	2.6
ADST 1921	32.4	83	28.6	34.5	15.2	1.5	242	12.2	11.1
ADST 1922	32.3	75	25	33.3	16	21	138	5.2	3.8
ADST 1923	40.5	95	32.9	34.6	11.6	1.4	327	26.2	15.6
ADST 1924	24.7	72	22.2	30.8	16.6	<1.0	414	4.1	2.4
ADST 1925	37.1	85	29.4	34.6	14	<1.0	233	16.6	16.9
ADST 1926	38.2	77	25.7	33.1	22.4	<1.0	338	5	9
ADST 1928	31.1	78	26.2	33.8	19.2	<1.0	443	2.5	5.3
ADST 1930	44.7	94	32.3	34.4	13.3	<1.0	187	57.4	27.8
ADST 1932	31.4	34	28	23.3	17.8	<1.0	324	6.1	4.3
ADST 1933	19.5	54	16.5	30.6	20.5	<1.0	342	1.2	1.7
ADST 1938	37.2	90	30.6	34	14.6	14	247	17.9	6.5
ADST 1940	29	65	21.2	32.6	19.1	<1.0	276	7.3	2.4
ADST 1941	37.3	90	31.5	34.8	15.1	<1.0	132	8.3	8.6

NR	Hct	MCV	MCH	MCHC	RDW	CRP	Plt	Ferr	Iron
ADST 1943	42.1	95	32.4	34.1	13.5	<1.0	196	22.2	16.4
ADCI 7027	37.8	85	29.5	34.6	13	<1.0	276	19.2	8.9
ADCI 7041	38.4	95	32.9	34.6	12.3	1.2	377	26.7	12.8
ADCI 7043	36.9	89	30.7	34.3	14.8	1.5	247	11.9	11.4
ADCI 7030	38.2	91	30.9	34	12.9	<1.0	336	11.7	9.2
ADCI 1905	34.6	78	24.8	31.8	16	11.3	195	11.3	12.2
ADST 1904	40.1	94	31.8	33.8	13	<1.0	438	83.9	19.7
ADCI 7020	46.7	95	32.3	34.1	13.6	1.4	389	57.5	21.6
ADST 1903	34	91	29.8	32.8	13.5	23.4	253	108.1	6.5
ADCI 7003	37.3	92	31.3	33.9	13.7	1	294	7.2	7
ADCI 7000	40.1	120	41.2	34.3	12.4	2.1	305	78.9	37.5
ADCI 7002	39.1	83	27.5	33.2	14.3	5.5	312	7.4	5.1
ADCI 7005	39.4	88	29.9	34	13.6	3.7	260	66.7	16.3
ADCI 7015	38.6	84	28.8	34.2	14.6	1.1	250	10.2	5.8
ADCI 7016	41.9	91	30.6	33.8	12.9	<1.0	229	37.7	16
ADCI 7024	36.6	86	29	33.9	13.8	1.3	247	6.7	11.9
ADCI 7021	39.6	92	31	33.8	12.4	1.1	235	37.1	20.5
ADCI 7007	40.4	87	30	34.5	12.7	28.6	253	94.3	3.4
ADCI 7006	44.9	97	33	34.1	12.6	2.4	261	48.3	22.7
ADCI 7034	30.9	71	23.1	32.6	17.3	<1.0	392	6.2	4.6
ADCI 7035	41	82	27.9	34	13.7	3.2	207	175.5	11.8
ADST 1908	36.7	90	31	34.6	13.8	4.6	218	102	12.9
ADST 1912	39.4	96	32.5	33.8	11.9	2.7	293	54.1	15.1
ADST 1914	39.2	92	30.8	33.5	13	6.1	333	27.7	16.6
ADST 1915	38.6	89	29.6	33.3	13.7	<1.0	185	9.3	21.6
ADST 1916	34.9	67	21.3	31.8	18.5	<1.0	321	3.6	5
ADST 1917	39.4	93	32.2	34.7	13.2	2.5	345	33.4	12.3
ADST 1918	39.6	89	30.6	34.3	14.3	5.7	190	85.3	17.4
ADST 1919	42.4	89	30.7	34.4	13.6	6.3	225	17	15
ADST 1920	40.7	92	22.4	35.4	13.6	<1.0	237	58.6	18.8
ADST 1927	35.9	100	34.4	34.4	13.1	24	349	14.8	14
ADST 1929	38.8	90	31	34.5	14.1	<1.0	284	13.1	20.4
ADST 1934	39	91	31.3	34.6	13.5	2.5	244	64.6	11.6
ADST 1935	42.4	93	31.9	34.4	12.6	4.5	221	51.5	15
ADST 1937	39.7	92	31.8	34.5	12.9	1.6	419	25.1	17.4
ADST 1939	36.1	82	27.5	33.7	15.3	5.5	303	6.3	5.6
ADST 1942	42.4	88	30.1	34.4	12.5	<1.0	342	32.8	10.4

NR	Tfn	TfnSat	MPV	PCT	PDW	Lym_Perc	LymAb	Mon_Perc	Mon Ab	Neu_perc
ADCI 7001	3.59	5.45961	8.8	0.306	16.3	40	2.19	9.4	0.51	47.6
ADCI 7004	3.48	2.816092	8.1	0.338	12.8	37.1	1.79	12.1	0.58	46.6
ADCI 7011	3.56	3.441011	8.2	0.288	0	33.5	2.56	9.2	0.7	0
ADCI 7014	4.45	1.730337	8.7	0.078	0	38	1.11	11	0.32	47.7
ADCI 7022	3.13	7.603834	10	0.216	18.3	40.6	1.96	7.1	0.34	49
ADCI 7023	3.48	4.425287	10.6	0.234	20.3	43.5	2.8	8.7	0.56	46.4
ADCI 7025	3.15	8.777778	8.2	0.284	12.3	48.9	2.57	5.1	0.27	43
ADCI 7026	3.47	4.034582	9	0.321	14.3	32.6	2.16	9.9	0.66	55.6
ADCI 7010	3.18	5.062893	9.3	0.253	17.3	37.4	1.33	8.9	0.32	52.4
ADCI 7009	2.58	7.18992	9.6	0.181	16.8	40.5	1.78	9.1	0.4	48.7
ADCI 7008	2.36	15.57203	8.7	0.185	14.8	35.6	1.31	8.5	0.31	53.8
ADCI 7017	3.06	6.405229	8.6	0.217	14.3	46	2.06	8.5	0.38	42.3
ADCI 7018	2.55	10.84314	9.3	0.302	16.5	50.5	4.57	6.7	0.61	40.4
ADCI 7028	3.54	5.536723	9.3	0.248	17	48.2	2.12	7.9	0.35	42.3
ADCI 7031	2.81	14.94662	8.4	0.262	13.5	44.9	2.94	5.5	0.36	46.3
ADCI 7029	3.43	3.77551	8.7	0.33	14.3	43.4	3.32	7.8	0.6	46.5
ADCI 7032	3.19	4.169279	8.4	0.162	12.8	51.3	2.69	9.5	0.5	35.2
ADCI 7040	3.45	2.73913	9.5	0.448	16	26.2	1.74	7.2	0.48	60.5
ADCI 7042	2.61	7.911877	8.4	0.301	13	36.1	2.42	5.6	0.38	55.8
ADCI 7037	3.17	4.968454	9.3	0.274	16	53.9	1.99	10.4	0.38	25
ADCI 7039	3.22	6.73913	8.6	0.234	14.8	58.7	1.38	11.6	0.27	25.9
ADCI 7047	3.94	4.885787	9.5	0.256	15.5	39.2	2.9	5.6	0.41	53.5
ADCI 7046	2.74	11.62409	9	0.181	15	52.8	2.28	7.1	0.31	36.8
ADST 1907	2.67	13.30779	7.7	0.317	10.5	49.5	2.15	8.5	0.37	40.4
ADST 1906	3.05	4.245902	8.1	0.387	11.3	49.6	2.03	6.8	0.28	39.7
ADST 1900	2.79	16.81004	8.3	0.189	13.3	49.9	2.99	6	0.36	41.7
ADST 1902	3.28	24.7561	7.2	0.255	9.5	51.9	2.7	7.7	0.4	39.1
ADST 1901	3.53	6.444759	8.2	0.276	12.8	55.2	2.69	8.3	0.4	30.3
ADCI 7038	3.22	0.978	8.7	0.272	14	49.7	2.5	7.1	0.36	40.3
ADCI 7036	2.86	7.954545	9.5	0.23	16	37.2	3.08	7.7	0.64	50.1
ADST 1909	2.8	4.5	10.2	0.225	22.3	29.8	1.5	5.6	0.28	62.6
ADST 1911	3.16	4.541139	7.8	0.301	0	53.1	1.55	9	0.26	34.8
ADST 1913	3.6	2.527778	10.8	0.241	22	50.3	2.24	9	0.4	34.4
ADST 1921	3.56	10.91292	8.3	0.201	13.5	34.5	3.36	4.3	0.42	57.5
ADST 1922	3.23	4.117647	11	0.152	20.8	33.4	3.02	7.3	0.66	55.7
ADST 1923	3.48	15.68966	7.9	0.259	11	24.3	3.1	4.5	0.57	62.9
ADST 1924	3.24	2.592593	8.8	0.366	15.5	33.3	2.42	6.3	0.46	57.9
ADST 1925	2.43	24.34156	9.9	0.229	17	28.8	2.18	7.5	0.57	59.5
ADST 1926	3.62	8.701657	8.6	0.29	0	36	2.65	5.5	0.4	55.7
ADST 1928	3.66	5.068305	7.6	0.335	11	38.9	2.48	6.8	0.43	52.5
ADST 1930	3.29	29.57447	8.8	0.165	16.5	33.4	2.15	7.3	0.47	55.9
ADST 1932	3.3	4.560606	8.3	0.269	12.8	40.6	1.88	13.3	0.61	41.4
ADST 1933	3.83	1.553525	9.2	0.315	0	53.4	2.99	4.5	0.25	39.3
ADST 1938	2.65	8.584906	9.6	0.238	15.5	29.2	0.85	16.3	0.48	47.6
ADST 1940	3.21	2.616822	9.3	0.258	0	44.2	1.85	6.9	0.29	46.9
ADST 1941	3.06	9.836601	9.6	0.127	20.5	52.1	2.11	7.2	0.29	37.9

NR	Tfn	TfnSat	MPV	PCT	PDW	Lym_Perc	LymAb	Mon_Perc	Mon Ab	Neu_perc
ADST 1943	2.63	21.8257	10.1	0.198	19.5	38.1	1.66	7.4	0.32	49
ADCI 7027	2.84	10.96831	8.3	0.229	12.8	52.5	2.37	8.8	0.4	35.4
ADCI 7041	2.87	15.6076	8.1	0.304	12	57	3.34	7.5	0.44	32.3
ADCI 7043	3.35	11.91045	8.1	0.199	12.3	46.4	1.61	8.5	0.29	41.8
ADCI 7030	3.28	9.817073	9.7	0.327	16.5	44.6	2.6	8.5	0.49	44.8
ADCI 1905	2.84	15.03521	8.7	0.17	15.5	25.9	1.08	11.7	0.49	60.7
ADST 1904	2.2	31.34091	7.6	0.331	9.3	59.4	5	6.3	0.53	24.2
ADCI 7020	2.65	28.5283	8.8	0.343	14	26.8	2.73	3.7	0.38	66.9
ADST 1903	2.12	10.73113	7.6	0.191	10.5	51.3	1.83	9	0.32	37.2
ADCI 7003	3.18	7.70403	7.3	0.214	9.8	26.2	2.22	11.4	0.97	57.3
ADCI 7000	2.04	64.33824	7.4	0.226	11	38.5	2.49	5.9	0.38	51.6
ADCI 7002	3.28	5.4427	9.2	0.288	16.3	28.5	2.61	4.5	0.41	64.7
ADCI 7005	2.77	20.59567	7.8	0.203	11.5	42.3	1.64	10.1	0.39	45.2
ADCI 7015	2.98	6.812081	8.6	0.214	13.3	46.7	2.07	4.7	0.21	42.9
ADCI 7016	2.54	22.047724	8.4	0.193	13.3	53.1	2.35	7.2	0.32	36.3
ADCI 7024	2.97	14.02357	9	0.221	15	38.8	1.66	7.4	0.32	49.7
ADCI 7021	2.41	29.77178	8.4	0.198	12.3	44.3	2.67	7	0.42	46.9
ADCI 7007	1.9	6.263158	7.8	0.198	12.5	29.4	1.84	7.9	0.49	61
ADCI 7006	2.53	31.40316	7.7	0.2	10.8	41.4	3.07	7.4	0.55	48.5
ADCI 7034	3.71	7.954545	8.2	0.321	12.3	52.4	2.62	5.1	0.25	38.1
ADCI 7035	2.42	17.06612	9.1	0.188	16	59.6	2.01	10.5	0.35	26.4
ADST 1908	2.01	22.46269	8.3	0.182	12.3	51.8	2.4	7	0.32	38.6
ADST 1912	2.63	20.09504	8.3	0.244	12.8	34.8	2.77	5.7	0.45	57.6
ADST 1914	2.43	23.90947	7.7	0.256	11	43.3	2.74	5.8	0.37	47.5
ADST 1915	3.15	24	10.1	0.187	17.8	24.8	1.77	6	0.43	65
ADST 1916	4.29	4.079254	10.9	0.349	0	54.7	3.03	7.3	0.4	35.4
ADST 1917	2.41	17.86307	7.2	0.248	9.8	51.7	2.07	8.6	0.34	37.7
ADST 1918	2.21	27.55656	8.8	0.168	15	47.7	2.02	7.2	0.3	42.6
ADST 1919	3.31	15.86103	7.8	0.175	11.8	36	3.09	7.9	0.68	45.1
ADST 1920	2.45	26.85714	7.6	0.179	10.5	44.8	2.24	8.3	0.42	44.4
ADST 1927	2.19	22.37443	8.3	0.29	12.8	35	1.75	9.5	0.48	52.7
ADST 1929	3.1	23.03226	9.1	0.258	16.5	48.6	2.97	5.8	0.35	43.1
ADST 1934	2.73	14.87179	9.3	0.228	16.3	32	2.72	6.1	0.52	58.1
ADST 1935	2.24	23.4375	10.9	0.241	22	33.8	3.61	4.9	0.52	58.2
ADST 1937	2.31	26.36364	7.9	0.333	12.3	33.8	1.2	7.4	0.26	53.3
ADST 1939	3.18	6.163522	9.2	0.279	14.3	40.5	2.35	9.1	0.53	48.8
ADST 1942	3.02	12.05298	7.8	0.267	11.5	35.4	2.28	5.5	0.35	56.3

NR	Neu Ab	Eos%	Eos Ab	Bas%	Bas Ab	Aly%	AlyAb	LIC%	LIC Ab	TYPE_OF_SOIL
ADCI 7001	2.61	2.2	0.12	0.8	0.04	0.6	0.03	1.6	0.08	clay whitish
ADCI 7004	2.25	3.7	0.18	0.5	0.02	1	0.05	0.4	0.02	soil whitish
ADCI 7011	0	0	0	0.5	0.04	1.3	0.1	2	0.15	soil whitish
ADCI 7014	1.39	2.5	0.07	0.8	0.02	1	0.03	1.3	0.04	clay yellowish
ADCI 7022	2.37	2.7	0.13	0.6	0.03	1.2	0.06	0.6	0.03	clay whitish
ADCI 7023	2.99	0.8	0.05	0.6	0.04	1.4	0.09	1.3	0.08	clay yellowish
ADCI 7025	2.26	2.1	0.11	0.9	0.05	1.1	0.06	0.7	0.04	clay whitish
ADCI 7026	3.69	1.4	0.09	0.5	0.03	0.6	0.04	0.8	0.06	clay whitish
ADCI 7010	1.87	0.7	0.02	0.6	0.02	1.3	0.05	1.1	0.04	clay whitish yellowish
ADCI 7009	2.14	0.8	0.04	0.9	0.04	1.8	0.08	1.8	0.08	clay and soil whitish
ADCI 7008	1.98	1.5	0.06	0.6	0.02	0.9	0.03	4	0.14	clay and soil whitish yellowish
ADCI 7017	1.9	2.6	0.12	0.6	0.03	1	0.04	0.4	0.02	soil whitish
ADCI 7018	3.66	1.5	0.14	0.9	0.08	0.9	0.08	0.7	0.06	soil whitish
ADCI 7028	1.86	1	0.04	0.6	0.03	1	0.04	0.05	0.02	soil whitish
ADCI 7031	3.03	2.6	0.17	0.7	0.05	0.7	0.05	1	0.06	clay whitish
ADCI 7029	3.56	1.6	0.12	0.7	0.05	1	0.08	0.8	0.06	soil clay, reddish-whitish khaki
ADCI 7032	1.84	2.5	0.13	1.5	0.08	1.8	0.09	1.3	0.07	soil whitish
ADCI 7040	4.02	5.6	0.37	0.5	0.03	0.8	0.05	0.9	0.06	soil whitish
ADCI 7042	3.74	2.1	0.14	0.4	0.03	0.6	0.04	0.2	0.01	clay whitish
ADCI 7037	0.92	10.2	0.38	0.5	0.02	1.5	0.05	1.1	0.04	soil whitish
ADCI 7039	0.61	3	0.07	0.8	0.02	1.6	0.04	1.2	0.03	soil whitish
ADCI 7047	3.96	1.1	0.08	0.6	0.04	1.1	0.08	0.6	0.04	soil whitish
ADCI 7046	1.59	2.3	0.1	1	0.04	1.3	0.06	1.2	0.05	soil whitish
ADST 1907	1.76	0.9	0.04	0.7	0.03	0.8	0.03	0.3	0.01	soil whitish
ADST 1906	1.62	3.3	0.13	0.6	0.02	1	0.04	0.6	0.03	soil whitish
ADST 1900	2.5	1.7	0.1	0.7	0.04	0.9	0.05	0.6	0.04	clay whitish
ADST 1902	2.03	0.6	0.03	0.7	0.04	1.1	0.06	0.9	0.05	clay whitish
ADST 1901	1.48	5.6	0.27	0.6	0.03	0.8	0.04	1.6	0.08	clay whitish
ADCI 7038	2.02	2.4	0.12	0.5	0.03	0.9	0.05	0.7	0.03	soil whitish
ADCI 7036	4.15	3.6	0.3	1.4	0.12	0.9	0.07	1.3	0.11	clay whitish
ADST 1909	3.14	1.4	0.07	0.6	0.03	0.4	0.02	1.2	0.06	Clay- yellow
ADST 1911	1.02	2.5	0.07	0.6	0.02	0.5	0.02	0.8	0.02	greyish
ADST 1913	1.53	5.5	0.24	0.8	0.04	1.1	0.05	0.9	0.04	Clay- yellow
ADST 1921	5.59	2.9	0.28	0.8	0.08	0.7	0.07	0.4	0.04	clayreddish/yellow
ADST 1922	5.04	3	0.27	0.6	0.05	1	0.09	0.7	0.06	clay yellowish
ADST 1923	8.02	7.4	0.94	0.9	0.11	0.4	0.05	1	0.12	clay whitish khaki
ADST 1924	4.21	1.9	0.14	0.6	0.04	1	0.07	0.5	0.04	clay whitish
ADST 1925	4.5	3.2	0.24	1	0.08	0.8	0.08	0.9	0.07	any
ADST 1926	4.1	1.8	0.13	1	0.07	0.5	0.04	0.7	0.05	reddish
ADST 1928	3.35	1.3	0.08	0.6	0.04	0.7	0.05	0.6	0.04	clay and soil khaki
ADST 1930	3.59	2.1	0.13	1.3	0.08	0.7	0.05	1.1	0.07	reddish
ADST 1932	1.91	3.9	0.18	0.8	0.04	0.5	0.02	0.8	0.04	whitish
ADST 1933	2.2	1.9	0.11	0.9	0.05	0.5	0.03	0.7	0.04	clay whitish
ADST 1938	1.39	6.2	0.19	0.7	0.02	1.9	0.05	0.9	0.03	clay whitish
ADST 1940	1.96	1.6	0.07	0.4	0.02	1.3	0.05	0.4	0.02	soil khaki
ADST 1941	1.53	2	0.08	0.8	0.03	1.2	0.05	0.6	0.02	soil khaki

NR	Neu Ab	Eos%	Eos Ab	Bas%	Bas Ab	Aly%	AlyAb	LIC%	LIC Ab	TYPE_OF_SOIL
ADST 1943	2.14	4.8	0.21	0.7	0.03	1	0.04	0.9	0.04	whitish soil
ADCI 7027	1.6	2.4	0.11	0.9	0.04	1.3	0.06	1	0.04	No info= controls
ADCI 7041	1.89	2.5	0.15	0.7	0.04	1.1	0.07	0.6	0.03	No info= controls
ADCI 7043	1.45	2.5	0.09	0.8	0.03	1	0.03	0.3	0.01	No info= controls
ADCI 7030	2.61	1.3	0.08	0.8	0.05	1.2	0.07	0.8	0.04	No info= controls
ADCI 1905	2.54	1.3	0.05	0.4	0.02	1.3	0.06	0.3	0.01	No info= controls
ADST 1904	2.04	8.9	0.75	1.2	0.1	1	0.08	0.7	0.06	No info= controls
ADCI 7020	6.81	1.6	0.16	1	0.1	0.8	0.09	1.6	0.16	No info= controls
ADST 1903	1.32	2.1	0.07	0.4	0.01	0.6	0.02	0.7	0.02	No info= controls
ADCI 7003	4.86	4.1	0.35	1	0.08	0.5	0.04	2.4	0.2	No info= controls
ADCI 7000	3.33	3.3	0.21	0.7	0.05	0.8	0.05	0.8	0.05	No info= controls
ADCI 7002	5.92	1.6	0.15	0.7	0.06	0.5	0.04	1.4	0.13	No info= controls
ADCI 7005	1.75	1.6	0.06	0.8	0.03	1.5	0.06	0.8	0.03	No info= controls
ADCI 7015	1.9	5.2	0.23	0.5	0.02	0.8	0.04	0.6	0.03	No info= controls
ADCI 7016	1.61	2.6	0.12	0.8	0.04	1.3	0.06	0.3	0.01	No info= controls
ADCI 7024	2.13	3.6	0.15	0.5	0.02	1.1	0.05	0.7	0.03	No info= controls
ADCI 7021	2.83	1.2	0.07	0.6	0.04	0.9	0.06	0.7	0.04	No info= controls
ADCI 7007	3.81	1	0.06	0.7	0.04	0.7	0.05	1.1	0.07	No info= controls
ADCI 7006	3.59	1.6	0.12	1.1	0.08	0.9	0.07	0.7	0.05	No info= controls
ADCI 7034	1.9	3.5	0.17	0.9	0.04	0.8	0.04	0.8	0.04	No info= controls
ADCI 7035	0.89	2.5	0.08	1	0.03	2	0.07	0.7	0.02	No info= controls
ADST 1908	1.79	1	0.05	1.6	0.07	1.2	0.05	1	0.05	No info= controls
ADST 1912	4.58	1	0.08	0.9	0.07	0.5	0.04	1.1	0.08	No info= controls
ADST 1914	3	2.3	0.15	1.1	0.07	0.7	0.04	0.6	0.04	No info= controls
ADST 1915	4.64	3.6	0.26	0.6	0.04	0.5	0.03	0.7	0.05	No info= controls
ADST 1916	1.96	1.8	0.1	0.8	0.04	0.9	0.05	0.6	0.03	No info= controls
ADST 1917	1.51	1.3	0.05	0.7	0.03	1.3	0.05	1.6	0.06	No info= controls
ADST 1918	1.8	1.6	0.07	0.9	0.04	1	0.04	0.6	0.03	No info= controls
ADST 1919	3.87	9.9	0.85	1.1	0.09	0.8	0.07	0.9	0.07	No info= controls
ADST 1920	2.22	1.3	0.07	1.2	0.06	0.8	0.04	0.6	0.03	No info= controls
ADST 1927	2.64	1.8	0.09	1	0.05	1	0.05	0.9	0.5	No info= controls
ADST 1929	2.63	1.7	0.1	0.8	0.05	0.9	0.06	0.9	0.06	No info= controls
ADST 1934	4.95	2.8	0.24	1	0.09	0.7	0.06	0.7	0.06	No info= controls
ADST 1935	6.22	2	0.21	1.1	0.12	0.8	0.08	0.9	0.09	No info= controls
ADST 1937	1.9	4.7	0.17	0.8	0.03	1.1	0.04	0.5	0.02	No info= controls
ADST 1939	2.83	0.9	0.05	0.7	0.04	0.6	0.03	0.8	0.05	No info= controls
ADST 1942	3.62	2.1	0.14	0.7	0.05	0.9	0.06	0.4	0.03	No info= controls

NR	YEARS_CONSUMING	HOW_OFTEN	QUANTITY
ADCI 7001	2	daily	2
ADCI 7004	3	daily	1
ADCI 7011	1	daily	4
ADCI 7014	1	weekly	6
ADCI 7022	3	daily	0.25
ADCI 7023	6	daily	2
ADCI 7025	4	daily	5
ADCI 7026	2	daily	1
ADCI 7010	5	daily	4
ADCI 7009	7	daily	5
ADCI 7008	5	daily	5
ADCI 7017	20	daily	5
ADCI 7018	5	daily	4
ADCI 7028	5	daily	4
ADCI 7031	3	monthly	1
ADCI 7029	4	daily	3
ADCI 7032	7	daily	5
ADCI 7040	3	daily	2
ADCI 7042	1	monthly	1
ADCI 7037	10	daily	5
ADCI 7039	2	daily	5
ADCI 7047	4	daily	3
ADCI 7046	3	daily	2
ADST 1907	7	weekly	1
ADST 1906	1	daily	1
ADST 1900	4	daily	5
ADST 1902	13	daily	4
ADST 1901	9	daily	4
ADCI 7038	5	daily	0.25
ADCI 7036	3	daily	2
ADST 1909	12	monthly	0.5
ADST 1911	7	monthly	3
ADST 1913	2	daily	4
ADST 1921	10	daily	2
ADST 1922	6	daily	2
ADST 1923	1	monthly	4
ADST 1924	1	daily	1
ADST 1925	3	weekly	1
ADST 1926	1	daily	2
ADST 1928	9	daily	3
ADST 1930	6	daily	1
ADST 1932	5	daily	3
ADST 1933	13	daily	1
ADST 1938	24	daily	4
ADST 1940	6	daily	1
ADST 1941	6	daily	2

NR	YEARS_CONSUMING	HOW_OFTEN	QUANTITY
ADST 1943	6	daily	1
ADCI 7027	No info= controls	No info= controls	No info= controls
ADCI 7041	No info= controls	No info= controls	No info= controls
ADCI 7043	No info= controls	No info= controls	No info= controls
ADCI 7030	No info= controls	No info= controls	No info= controls
ADCI 1905	No info= controls	No info= controls	No info= controls
ADST 1904	No info= controls	No info= controls	No info= controls
ADCI 7020	No info= controls	No info= controls	No info= controls
ADST 1903	No info= controls	No info= controls	No info= controls
ADCI 7003	No info= controls	No info= controls	No info= controls
ADCI 7000	No info= controls	No info= controls	No info= controls
ADCI 7002	No info= controls	No info= controls	No info= controls
ADCI 7005	No info= controls	No info= controls	No info= controls
ADCI 7015	No info= controls	No info= controls	No info= controls
ADCI 7016	No info= controls	No info= controls	No info= controls
ADCI 7024	No info= controls	No info= controls	No info= controls
ADCI 7021	No info= controls	No info= controls	No info= controls
ADCI 7007	No info= controls	No info= controls	No info= controls
ADCI 7006	No info= controls	No info= controls	No info= controls
ADCI 7034	No info= controls	No info= controls	No info= controls
ADCI 7035	No info= controls	No info= controls	No info= controls
ADST 1908	No info= controls	No info= controls	No info= controls
ADST 1912	No info= controls	No info= controls	No info= controls
ADST 1914	No info= controls	No info= controls	No info= controls
ADST 1915	No info= controls	No info= controls	No info= controls
ADST 1916	No info= controls	No info= controls	No info= controls
ADST 1917	No info= controls	No info= controls	No info= controls
ADST 1918	No info= controls	No info= controls	No info= controls
ADST 1919	No info= controls	No info= controls	No info= controls
ADST 1920	No info= controls	No info= controls	No info= controls
ADST 1927	No info= controls	No info= controls	No info= controls
ADST 1929	No info= controls	No info= controls	No info= controls
ADST 1934	No info= controls	No info= controls	No info= controls
ADST 1935	No info= controls	No info= controls	No info= controls
ADST 1937	No info= controls	No info= controls	No info= controls
ADST 1939	No info= controls	No info= controls	No info= controls
ADST 1942	No info= controls	No info= controls	No info= controls

APPENDIX: F Published article

IGCP Project 545: Clays and Clay Minerals in Africa
NRF Grant UID 63583: Human and Enzootic Geophagia Southern Africa
NRF Grant No 73657: Clays and Clay Minerals in South Africa

AN INNOVATIVE PERSPECTIVE ON THE ROLE OF CLAYS AND CLAY MINERALS, AND GEOPHAGIA ON ECONOMIC DEVELOPMENT

BOOK OF CONFERENCE PROCEEDINGS

1st INTERNATIONAL CONFERENCE ON CLAYS AND CLAY MINERALS IN AFRICA

and

2nd INTERNATIONAL CONFERENCE ON GEOPHAGIA IN SOUTHERN AFRICA

(1st ICCCM & 2nd ICGSA)

2011

G-IE Ekosse, L de Jager and VM Ngole

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Central University of Technology, Free State, South Africa

1st ICCCM & 2nd ICGSA

DISCLAIMER

This book contains abstracts and completed papers which were presented at 1st International Conference on Clays and Clay Minerals in Africa and the 2nd International Conference on Geophagia in Southern Africa (1st ICCCM & 2nd ICGSA) that took place in Bloemfontein, South Africa.

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Association between Geophagia and Haematological Parameters of Iron Deficiency Anaemia in Geophagic Qwa-Qwa Women

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Abstract

Geophagia is the consumption of soil/clay practiced worldwide in many cultures for different reasons. The compulsion is thought to be associated with disorders of mineral balance. Iron deficiency anaemia can be categorised as hypochromic microcytic anaemia. The possible reason for the association between geophagia and iron deficiency anaemia (IDA) includes among others, that soil or clay could decrease the bioavailability of iron for absorption in the body through kaolinite. This undefined relationship between IDA and geophagia was investigated in QwaQwa women because this relationship has not been investigated in South Africa. In this case control study, blood sample was drawn from thirty-six women who do not consume soil representing the control group, and forty-seven women who consumed soil who served as the geophagic group. All the participants were from the same area or living under the same environmental conditions so that a similar diet was expected, were informed about the study and had to sign a consent form before the collection of blood sample. To determine the prevalence of IDA full blood count and iron studies (serum iron, ferritin and transferrin saturation) were performed. The mean haemoglobin (Hb) results were 13.2g/dl and 11.2g/dl for control and geophagic groups respectively; mean cell volume (MCV) results were 91fl and 81fl for control and geophagic group respectively while the mean cell haemoglobin (MCH) were 30.7pg and 27.3pg for control and geophagic group respectively. The mean haemoglobin (Hb), MCV and MCH for geophagic group were decreased below the reference range; whilst for the control group, all three parameters were within the reference range. This difference in mean Hb, MCV and MCH was statistically significant ($P<0.0001$) A statistically significant difference ($P<0.0001$) was observed when the median ferritin concentration values (30.3µg/l and 8.9µg/l) and mean transferrin saturation (17.5% and 5.5%) for the control and geophagic groups respectively were compared. Haemoglobin, MCV, MCH, ferritin and transferrin saturation results for both geophagic and control group indicate that the control group is normal while the geophagic group had IDA with hypochromic microcytic cells. In conclusion, IDA is associated with geophagia, and geophagia predisposes or contributes to the development of iron deficiency.

Keywords: *Geophagia; iron deficiency anaemia; QwaQwa, hypochromic microcytic anaemia; ferritin.*

1. Introduction

The puzzling phenomenon of pica has been recognized and described for centuries (Woywodt & Kiss, 2002). Pica is not always referred to as craving or compulsive eating of non-food substances but also involves the consumption of dozens of other substances, including clay, dirt, sand, and stones (Halsted, 1968; Louw *et al.*, 2007). Pica has been observed in ethnic groups worldwide, in both primitive and modernized cultures, and in all age groups (Louw *et al.*, 2007). Pica in humans has many different subgroups, defined by the substance that is ingested. Some of the most commonly described types of pica are eating earth, soil or clay (geophagia) and ice (pagophagia) (Ellis, 2006). Uncontrollable consumption of large quantities of earth even to a point of death in referred to as geomania (Bick *et al.*, 1993). Geophagia originates from Greek words geo-(earth) and phag-(eat) (Thompson, 1995).

Geophagia is a self-willing behaviour by humans and animals, of consuming soil/clay like substances such as clay and chalk (Woywodt & Kiss, 2002). Geophagia has been associated with pregnancy, e.g. in Malawi; geophagia is taken as a sign of pregnancy (Ghorbani, 2008). Social and economic: mostly in developing countries earth consumption is due to poverty (Ghorbani, 2008), while this theory is not supported by Young *et al.* (2007). Amongst others, nutritional, psychological, physiological, pharmacological and cultural reasons; are other reasons people give for the practice of geophagia, with health reasons being most prominent (Feldman, 1968; Young *et al.*, 2007). Most people claim that earth/clay provides the minerals that they need; these minerals include calcium, iron and magnesium (Hooda *et al.*, 2002). Geophagia in some literature is said to be the practice of eating earthy substances (as clay) that in humans is performed especially to augment a scanty or mineral-deficiency diet or as part of a cultural tradition (Abrahams *et al.*, 2005).

In 1968 Halsted already stated that geophagia is seen as a medical problem, on the other hand proven to be a medicament thought to be beneficial for 2000 years (Bick *et al*., 1993). Ellis (2006) links severe cases of pica to nutritional cases that include iron deficiency. In some countries or even ethnic groups geophagia in women is accepted and passed down generations. It is believed that geophagia is beneficial in women especially those in child bearing ages as a source of iron. Geophagia is missed by doctors and not commonly reported but when reported frequently in about 50% of patients it is associated with severe iron deficiency anaemia (Bick *et al*., 1993).

Iron deficiency anaemia is the most common cause of anaemia resulting when the rate of iron loss exceeds the rate of uptake and assimilation (Webster, 2009). Anaemia is dependent on the haemoglobin concentration; taking in consideration the age, sex and altitude of residence (Webster, 2009).Anaemia whether due to iron deficiency or not, is primarily quantitative (Bick *et al*., 1993). Shortage of iron leads to a limited haemoglobin production which with time affects the production of red blood cells (Lombard, 2009). Even in the absence of anaemia, iron deficiency can still be present. Extreme stages of iron deficiency can lead to iron deficiency anaemia. When the iron stores in the body are depleted and red blood cells are microcytic and hypochromic this is called iron deficiency anaemia (Webster, 2009). Due to the menstrual cycle that occurs monthly, women in their child bearing age are more prone to iron deficiency.

von Garnier *et al*. (2008) and Barton *et al*. (2010) established the undefined relation between pica and iron deficiency anaemia but the link specifically of geophagia, a form of pica, and iron deficiency anaemia has not been conclusively established. This link has been alluded to by mostly individual case studies (von Garnier *et al*., 2008 and Barton *et al*., 2010) while a population based study mostly link geophagia with anaemia[10] and not with iron deficiency anaemia. According to a case report by von Garnier *et al.,* (2008) pica was reported to be associated with severe IDA in up to half of the patients; however, it was unclear whether pica causes or is the consequence of IDA. Nchito *et al.* (2004) in a case study done on Zambian school children established that there is a link between iron deficiency anaemia and geophagia The aim of the study was to investigate the correlation between haematological parameters and iron deficiency anaemia in geophagic Qwa-Qwa women.

2. Methodology

In this case control study, the participants were randomly recruited from sub-urban areas of Qwa-Qwa. The participants where preferably from the same house hold or living in the same environmental conditions. The recruitment was done by a field worker by means of a questionnaire months in advance, moreover, some participants were recruited during blood samples collection. Blood samples for laboratory analysis were collected on two different visits to Qwa-Qwa.

A total of 83 participants complied with the selection criteria, and the control and geophagic group were composed of 36 and 47 participants, respectively. The selection used was as follows: (1) participants were permanent female residents of Qwa-Qwa. (2) Pregnant and lactating females were excluded because of their increased requirements for iron due to the baby. (3) Only women within the age range of 18 - 45 years were included in the study.

This study was approved by the ethical committee of the University of the Free State (reference Etovs 104/08). The scientific importance and expectations of the research were explained to the participants, in their language of preference. The informed consent document, recruitment and food frequency questionnaires were completed by participants. These questionnaires gathered information about the type of soil consumed, the period of consumption together with the quantity consumed as well as the colour of soil. The laboratory tests were performed on site within four hours of blood collection. All participants with abnormal full blood counts and iron status were advised to visit their local clinic.

From each participant, five millilitres of blood was collected into ethylenediaminetetra-acetic acid (EDTA) tubes for full blood counts (FBC) and ESR determination and ten millilitres of clotted blood samples for iron studies (ferritin, serum iron, transferrin saturation, transferrin and C-reactive studies). The FBC were performed using the ABX PENTRA 60 which uses current impedance changes; spectrophotometry; double hydrodynamic sequential system coupled with cytochemistry and measuring of transmitted light; to measure the different parameters of the FBC. ESR was measured using the SEDIPLAST® which is a closed system based on the Westergren method. The Westergren

method is considered the gold standard and it is recommended by the International Council for Standardization in Haematology (ICSH). The iron studies were performed at Universitas Tertiary service laboratory, National Health Laboratory Services, Bloemfontein. Serum iron and transferrin were done on the Beckman Coulter CX9 and the transferrin saturation automatically calculated. The ferritin studies were performed on the Siemens Advia Centaur using a method as described by the UCSF Clinical Labs- Chemistry.

Data was captured electronically in Microsoft Excel. Any further analysis was done by a biostatistician using SAS Version 9.1.3. Descriptive statistics namely frequencies and percentages were calculated for categorical data. Means and standard deviations or medians and percentiles were calculated for numerical data. Medians were used when data did not form a Gaussian curve. Means of the control and geophagic group were compared using the t-test. Medians of the control and geophagic group were compared using the Kruskal-Wallis geophagic. A significance level of 0.05 was used throughout the study.

3. Results

The data is summarised as geophagic practices which describes age and consumption properties, followed by inflammatory and haematological findings. Finally, the clinical chemistry results are presented.

3.1. Geophagic practices

The geophagic group consisted of 47 women with a median age of 23 and an age range of 18- 43 years, whilst the control group had 36 women with a median age of 24 and a range of 18- 44years. There was no statistical significant change observed between the two groups ($p = 0.7914$). The geophagic and control groups consumed similar as determined by the food frequency questionnaires (data not presented). Analysis of the questionnaire revealed that 43% of the participants in the geophagic group preferred white clay with 29% consuming white soil and 17% yellow clay. Eighty- three percent of the geophagic group consumed soil/clay daily with 6% consuming weekly and 11% consuming soil/clay monthly. The median number of bags consumed was 2 with a maximum of 6bags and a minimum of 0.25bags. However the numbers of year participants have been consuming was high, median number of years was 5years with 24years as the maximum and 1year as the minimum.

3.2. Haematological and inflammatory indicators

The haematological analysis entails red cell parameters {red blood cell count (RBC), haemoglobin (Hb) & haematocrit (HCT)}, red cell indices {mean cell volume (MCV) & mean cell haemoglobin (MCH)}, other FBC parameters {platelets (PLT), white blood cell count (WBC) & eosinophil count (Eos AB)}, and inflammatory markers {erythrocyte sedimentation rate (ESR) & C-reactive protein (CRP)}., The median haematological results of both control and geophagic groups showed an increase in ESR. A significant difference ($P<0.05$) in the median increase in ESR was observed when both groups were compared (Table1).

Table 1: Inflammatory indicators and red cell indices for control and geophagic groups

Laboratory tests	Reference range	Control group median & (min – max)	Geophagic group median & (min – max)	p-value
	0 – 12mm/h	24↑ (2.0 – 127.0)	40 ↑(3 – 130)	0.0173*
	4 – 11x10^9/l	5.8 (3.4 – 10.7)	5.3 (2.3 – 12.8)	0.7476
	<5mg/l	1.9 (1.0 – 28.6)	1.2 (1.0 – 21.0)	0.1948
	<45x10^9/l	0.12 (0.05- 0.85)	0.12 (0.03 – 0.94)	0.7300
	80 – 100fl	91 (67- 120)	81 (34 – 95)	<0.0001*
	27 – 32pg	30.7 (21.3 – 41.2)	27.3 (16.5 – 32.9)	<0.0001*

*p<0.05 meaning there is a significant difference between the median values of the control group and the patient group. Min = minimum, max = maximum Erythrocyte Sedimentation Rate= ESR, White Blood Cell= WBC, C-reactive protein= CRP, Eos Ab= Eosinophil Absolute count, Mean Cell Volume= MCV, Mean Cell Haemoglobin= MCH

On the other hand; WBC, CRP and Eos AB for both groups were within reference ranges and no significant difference between the groups was observed as indicated by p-values >0.05 (Table 1). On the contrary, the MCV and MCH for both the control and geophagic groups were within the reference range, although the geophagic group had low normal values. Both the red cell indices showed a significant difference between the two groups as the p-value was <0.05 (Table 1).

The mean RBC, HCT and Hb for the control group were within the reference range while the HCT and Hb for the geophagic group were below the reference range. There was a significant difference for all the red cell parameters with the geophagic group having lower mean red cell indices when compared with the control group, (Table 2). In addition, the mean PLT counts for both groups were within the reference range and there was no significant difference between the groups.

Table 2: Mean results of red cell parameters and platelet count for control and geophagic group

Laboratory tests	Reference range	Control group mean & (min-max)	Geophagic group mean & (min- max)	p- value
RBC	$3.8 - 4.8x10^{12}/l$	4.37 (3.35 – 5.21)	4.17 (3.43 – 4.98)	0.0114*
Hb	12 – 15g/dl	13.2 (10.1 – 15.9)	11.2↓ (6.0 – 15.4)	<0.0001*
HCT	36 – 46%	38.5 (30.9 – 46.7)	33.7↓ (19.5 – 44.7)	<0.0001*
PLT	$150 - 400x10^{9}/l$	285 (185- 438)	285 (90 – 480)	0.98323

*p<0.05 meaning there is a significant difference between the median values of the control group and the patient group, Red Blood Cell= RBC, Haemoglobin= Hb, Haematocrit= Hct, Platelet= PLT, Minimum= Min, Maximum= Max

3.3. Clinical chemistry findings

The chemical analysis encompassed serum iron, transferrin saturation and ferritin. The median results of ferritin, serum iron and transferrin saturation for the control group were within reference range, while the geophagic groups' median ferritin, serum iron and transferrin saturation results were below the reference range, as depicted in Figure 1, 2 & 3. There was a significant difference for all iron study parameters as p-values were <0.0001. One participant in the control group had an increased serum iron value (Figure 1). It can also be noted that one participant in the geophagic group had an increased transferring saturation percentage (Figure 2).

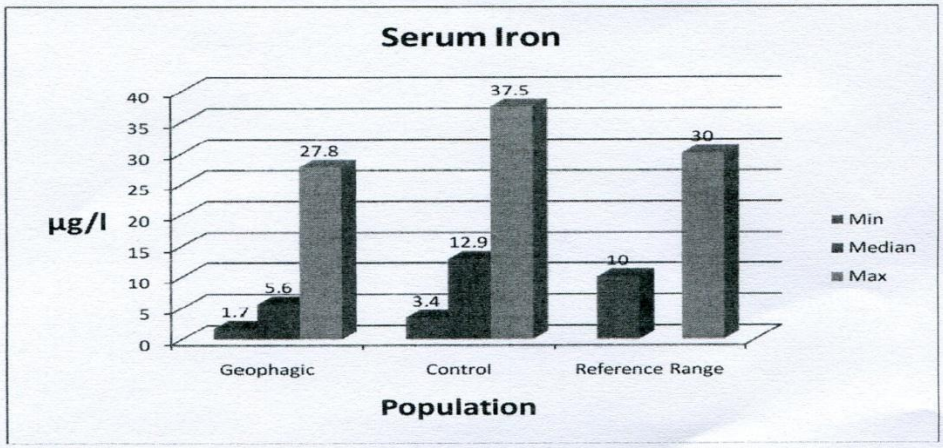

Figure 1: Serum iron results for both geophagic and control group

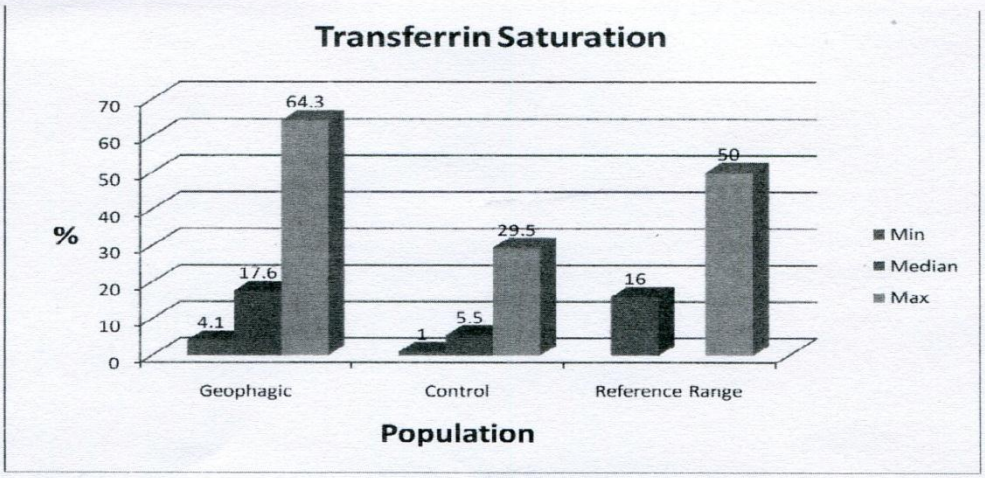

Figure 2: Transferrin Saturation results for both geophagic and control groups

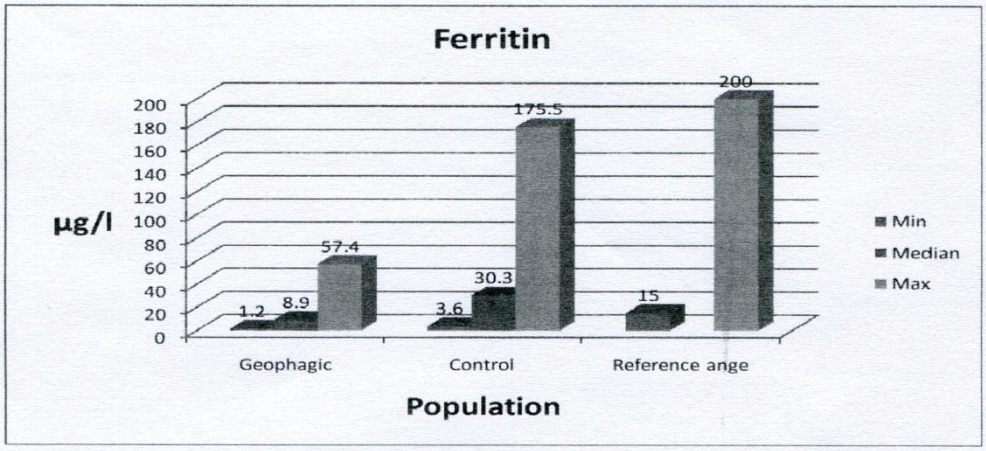

Figure 3: Ferritin results for both geophagic and control groups

4. Discussion

Geophagic practices illustrated that both control and geophagic groups had participants in the same age range supported by p-value> 0.05. This age range is significant because most studies indicate that women in their child bearing years are prone to practising geophagia due to the bizarre cravings (Louba & Geisser, 2004; Ngozi, 2008; Nyaruhucha, 2009). Luoba & Geisssler (2004) found that the prevalence of geophagia was higher in women below 30 years when compared with those above 30 years; this was also supported by this study. In a study conducted in Zambia by Nchito *et al.* (2004) the most commonly consumed earth type was brown and white earth; similarly the most commonly consumed amongst Qwa-Qwa women was white. Most geophagic participants (83%) consumed soil/clay on a daily basis, because it is sold by vendors in the market place and residential areas are near mining sites, thus it is

easily accessible. The high percentage of soil consumption in Qwa-Qwa women raises curiosity on the impact soil/clay consumption has in the body

Young *et al.* (2007) postulated that geophagia increases the likelihood of orally transmitted parasitic nematodes. This means that inflammation could be the reason for decreased serum iron and transferrin saturation but increased ferritin levels [25], thus affecting the iron study results. To rule out the effect of inflammation on iron status indicators ESR, CRP and WBC were assessed. As indicated in Table 1 the two groups had an increased ESR with the geophagic group having an even higher median ESR reading. This may indicate inflammation; however, ESR is affected by many conditions including anaemia. ESR as a measure of inflammation is not conclusive, thus CRP and WBC were performed. Analyses of the results revealed no sign of inflammation as CRP and WBC for both groups were normal with no significant difference between the two groups.

According to Young *et al.* (2007) soil consumption can lead to parasitic infection which can cause bleeding; in addition, bleeding is the most common cause of IDA. Thus the eosinophil and platelet count were investigated. For both groups there was no significant difference, moreover the two groups had normal eosinophil counts; indirectly ruling out parasitic infections. An increase in the platelet count can be associated with bleeding. Both geophagic and control groups' platelet results were within the reference range demonstrating that there were no bleeding tendencies (although not measured directly). This was also observed by von Garnier *et al.* (2008) who observed that anaemia caused by geophagia was not accompanied by bleeding.

Iron deficiency anaemia is characterised by a decrease in the haematological values which makes it hypochromic microcytic anaemia (HMA) coupled with a decrease in iron study results. Microcytic means a decrease in cell size, measured by MCV; whilst the amount of haemoglobin in a cell is hypochromia which is measured by MCH. A decrease in Hb is defined as anaemia (Hoffbrand *et al.*, 2006). The red cell indices MCV and MCH in Table 1 indicate that there was a significant difference between the two groups as supported by p-value< 0.05 for both indices. The control and geophagic group had normal MCV and MCH readings and although normal for the geophagic group they were on the lower limit of the reference range, therefore the geophagic group, even though appearing normal, indicate a decrease in cell size and haemoglobin content which makes the geophagic group slightly hypochromic microcytic red cells. The Hb readings in Table 2 indicated a significant difference between the two groups with the geophagic group having the lower reading. The Hb results for the control group were within reference range while the geophagic groupwas decreased. Therefore pointing out that the geophagic group was anaemic.

The mean results of the RBC for both geophagic and control groups were normal, and this was expected as hypochromic microcytic anaemia leads to increased red cell formation (Hoffbrand *et al.*, 2006). There was a significant difference in the RBC counts for the two groups. The HCT results in Table 2 indicated a significant difference between the groups. HCT and MCV results being low for the geophagic group could be the reason for the increased ESR; the geophagic group had smaller cells thus increasing the rate of cell sedimentation.

Geophagia has been associated with iron deficiency and anaemia but no causal relationship has been established[19]. In the assessment of IDA the iron indices analysis are important. A decrease in the serum iron, saturated transferrin and ferritin help in stating if the anaemia was due to the lack of iron in the circulation or not. A decrease in the serum iron, saturated transferrin and ferritin help in stating if the anaemia was due to the lack of iron in the circulation or not. Results of the median iron indices in figure 1, 2 and 3 exhibited the control group with normal, serum iron, transferrin saturation and ferritin; hence the control group was not iron deficient. On the contrary the geophagic group results of the serum iron, transferrin saturation and ferritin was decreased. The decrease indicates that the geophagic group is iron deficient consequently affirming that the geophagic group had iron deficiency anaemia. These results were in agreement with the reported case studies (von Garnier *et al.*, 2008 and Barton *et al.*, 2010). One participant in the control group had an increased serum iron value (Figure 1). Serum iron concentration is affected by diet, thus an increased value could be reflection of the current situation. In addition it could also be due to ineffective erythropoeisis, iron overload and liver disease (Kaplan *et al.*, 1995). These above-mentioned reasons could also be the cuase of the increased saturation percentage in one geophagic group participant (Figure 2).

The control group was neither anaemic nor iron deficient as expected since they are not ingesting soil/clay. However, the geophagic group had hypochromic microcytic anaemia accompanied by the iron deficiency, thus making

the geophagic group to have IDA. These findings are supported by a number of studies (Kalantar-Zadeh *et al.*, 2004; von Garnier *et al*., 2008). The theory that kaolinite absorbs iron from the duodenum; can imply that geophagia enhances IDA than contributing towards the addition of iron in the body as the people consuming iron believe.
In conclusion, the control group was found to be normal whilst the geophagic group was found to have iron deficiency anaemia. This clearly indicates that geophagia amongst Qwa-Qwa women is associated with abnormal haematologic findings of IDA. These findings still bring up the question of which one occurs first: IDA or geophagia, further studies are needed in this area.

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