COMPARISON OF ORAL AND INTRAVENOUS IRON THERAPY IN GEOPHAGIC BOTSHABELO WOMEN WITH IRON DEFICIENCY ANAEMIA

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This work is dedicated to the Almighty God, the creator of all things.

Father, thank You for the inspiration, wisdom and guidance through the entire process.

My inspiration extract is (Psalm 139):

“Lord, You have searched me.

You know me when I sit down and when I stand up.

You understand my thoughts from far away.

You observe my travels and my rest; You are aware of all my ways.

Before a word is on my tongue, You know all about it, Lord.

You have encircled me, {This} extraordinary knowledge is beyond me.

It is lofty; I am unable to {reach} it…

...For it was You who created my inwards parts; You knit me together in my mother’s womb.

I will praise You, because I have been remarkably and wonderfully made.

Your works are wonderful….”

The Passion Bible, Christian Standard Bible, Holman Bible Production, 2004.

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DECLARATION OF INDEPENDENT WORK

I, LEBOGANG FRANCIS MOGONGOA, hereby declare that this research project submitted to the Central University of Technology, Free State for the degree DOCTOR OF HEALTH SCIENCES: BIOMEDICAL TECHNOLOGY is my brainchild that has not been submitted before to any institution by me or any other person in fulfilment of the requirements for the attainment of any qualification.

24 November 2020

SIGNATURE OF STUDENT **DATE**

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SUMMARY

Geophagia, the habitual consumption of soil, is associated with iron deficiency anaemia (IDA), especially in women of childbearing age. Geophagia and IDA are very prevalent in southern Africa. The reasons for the association of geophagia with IDA are numerous but the one that has prominence is mineral supplementation. However, there is contradictory *in vitro* evidence; with studies that support supplementation versus studies that postulate removal of iron. If soil reduces the bioavailability of dietary iron, could it also interfere with the treatment of IDA? The current treatment protocol of IDA is with oral iron and if oral iron is not effective then intravenous iron therapy is undertaken. The challenge would be that the patient would suffer symptoms of anaemia while on oral iron therapy.

This study aimed to determine if oral or intravenous iron therapy will be effective for the treatment of IDA in non-pregnant geophagic Botshabelo females. A study comparing oral and IV iron therapy has not been performed before. In this randomised prospective dose-escalating intervention study a group of geophagic females with IDA were recruited and randomly assigned to two groups. Group A was expected to abstain from soil consumption and group B continued with soil consumption. Both groups received oral iron therapy for ten weeks. During this period, study-related procedures and blood analysis and questionnaires, at different time intervals to assess the effects of oral therapy, were undertaken. At the end of ten weeks, IV iron therapy was administered to those participants who did not respond to oral therapy.

Of the 320 recruited participants only 83 met the inclusion criteria. At baseline, the general health indicators pointed towards an otherwise healthy population group with hypertension, obesity and IDA as exceptions. Randomisation into two groups was effective as there was no significant difference between the two groups at baseline. There were no other

confounding factors that could have resulted in the changes observed in the study population. The absence of these confounding factors was due to non-aberrant findings in blood loss or nutrition; absence of other medication or diseases that could interfere with oral therapy; normal liver enzyme levels, minerals and normal general health indicators.

There were no significant red blood cell parameter changes for both short (week 4) and intermediate (week 7) follow up periods of oral therapy for both groups. Group A showed a statistical but clinically insignificant increase for the red blood cell parameters contrary to group B which did not show changes. While serum iron, total iron-binding capacity and transferrin saturation showed statistical but clinical insignificant changes for both groups. The expected endpoint was not achieved for both groups, signifying that oral iron was not effective in the treatment of IDA in geophagia. Soil decreased oral iron therapy bioavailability because group B did not show significant changes. The clinical insignificant increase in group A also implied that the absorptive surface of the gastrointestinal tract was affected and did not recover within the ten weeks. For IV iron therapy, both groups showed a statistical and clinically significant increase for red blood cell and iron study parameters for both short and long term follow up study periods. Moreover, the changes for red blood cell and iron study parameters achieved the desired endpoint for therapy. Therefore, IV iron therapy was effective while oral iron therapy was not. These findings are contrary to other study fields' observation where both oral and IV iron were both effective.

It can be concluded that oral iron therapy is not effective for the treatment of IDA in geophagia while IV iron is effective. Oral iron's non-effectiveness indicates that the patient will bear the symptoms of IDA while therapy is not effective. This study supports the use of IV iron for the treatment of IDA in geophagia, thus necessitating the change of iron therapy protocol for the benefit of geophagic IDA patients.

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LIST OF ABBREVIATIONS

α	Alpha
β	Beta
γ	Gamma
μ	Micro
ADE	Adverse drug event
Ca	Calcium
CHF	Chronic heart failure
CKD	Chronic kidney disease
CRP	C-reactive protein
Cu	Copper
DMT-1	Divalent metal transporter-1
EDTA	Ethylenediaminetetraacetic acid
ESA	Erythropoiesis stimulating agents
F	Fluoride
Fe	Iron
Fe(II)	Iron(II) oxide or Fe2+
Fe(III)	Iron(III) oxide or Fe3+
FBC	Full blood count
G	gram
GIT	Gastrointestinal tract
Hb	Haemoglobin
HCT	Haematocrit
IBD	Inflammatory bowel disease

ID	Iron deficiency
IDA	Iron deficiency anaemia
IRE	Iron regulatory elements
K	Potassium
kg	kilogram
MCV	Mean cell volume
MCH	Mean cell haemoglobin
MCHC	Mean cell haemoglobin concentration
Mg	Magnesium
mg	milligram
Mn	Manganese
mRNA	messenger ribonucleic acid
PLT	Platelets
RBC	Red blood cells
RDW	Red cell distribution width
TIBC	Total iron-binding capacity
TSAT	Transferrin saturation
WBC	White blood cells
WHO	World Health Organization

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

The deliberate consumption of soil, also known as geophagia, is mostly seen as a practice of marginal oddity. However, the practice is found throughout history, it is observed in many individuals and it has a peculiar multifactorial origin. Geophagia is derived from the Greek words *geo-* meaning earth and *phagia-* meaning eat (Geophagy n.d.). Consequently, geophagia can be described as the deliberate habitual consumption of soil/clay that is considered developmentally inappropriate (Hooda *et al.* 2004; Blinder & Salama 2008; Barton *et al.* 2010). For a person to be diagnosed as geophagic, a person must have consumed earthly substances continuously for more than one month (Dugan 2002). The geophagic practice has been described worldwide (Hunter 1973; Vermeer & Dennis 1979; Ziegler 1997; Boyle & Mackay 1999; Mahaney *et al.* 2000; Reilly & Henry 2000; Utara 2002; Woywodt & Kiss 2002; Luoba *et al.* 2004; Momoh *et al.* 2012; Bryant *et al.* 2013; Raphuthing *et al.* 2014). Geophagia has been reported among women and children in southern Africa (Luoba *et al.* 2004), particularly in South Africa (Momoh *et al.* 2012; de Jager *et al.* 2013; Raphuthing *et al.* 2014); There are multiple reasons for the practice of geophagia namely: cultural, medicinal,

socio-economical, psychological and religious (Eastwoord 1999, Woywodt & Kiss 2002; Crane 2004; Dreyer *et al.* 2004; Luoba *et al.* 2004, Kondo & Sokol 2006; Yao 2006; Young *et al.* 2010; Chen *et al.* 2013).

The current debate is whether geophagia is a symptom of or the cause of iron deficiency anaemia (IDA). Researchers hypothesise that one of the causes of geophagia is mineral deficiencies, in particular, iron and zinc deficiency (Eastwoord 1999; Kondo & Sokol 2006; Ghorbani 2008). Studies indicate contradictory evidence about the bioavailability of iron in the soil; hence some indicate that soil may be a source of iron (Abrahams & Parsons, 1997; Smith *et al.* 2000; Yount 2005; Abrahams *et al.* 2006; Ghorbani 2008, Odilon Kikouama *et al.* 2009) while others postulate that soil may reduce the bioavailability of the already existing dietary iron (Hooda *et al.* 2004, von Garnier *et al.* 2008; Abrahams *et al.* 2013; Pebsworth *et al*. 2013; Seim *et al.* 2013). Therefore, if soil interferes with the bioavailability of dietary iron, the possibility exists that soil may play a causative or contributory role in the development or enhancement of iron deficiency anaemia.

Geophagia is associated with anaemia (Young *et al.* 2010; Momoh *et al.* 2012), and with iron deficiency anaemia (von Garnier *et al.* 2008; Raphuthing *et al.* 2014). The association of geophagia with iron deficiency anaemia was found in research participants who were consuming sufficient dietary iron (van Onselen & Walsh 2011). Thus, suggesting the possibility that soil could interfere with iron absorption.

Iron deficiency anaemia (IDA) is the most common cause of anaemia

worldwide (Kassebaum *et al.* 2014; Hoffbrand & Moss 2016: 28; Worwood *et al.* 2017). The causes of IDA can be divided into chronic blood loss, increased demand, malabsorption and poor diet (Auerbach & Adamson 2016; Hoffbrand & Moss 2016: 32; Worwood *et al.* 2017). The most common cause of IDA amongst these four factors is bleeding, as most of the body's iron is contained in haemoglobin.

IDA is commonly associated with children, teenagers, elderly, pregnant women and women of childbearing age. IDA in association with geophagia is commonly encountered in women of childbearing age caused by menstrual blood loss and pregnancy secondary to supply of iron to the developing foetus. IDA results when the net balance of iron absorption and loss is tilted towards iron loss.

Gastro-intestinal tract iron absorption and recycling of body iron are the body's mechanisms responsible for the regulation of body iron concentration (Auerbach & Adamson 2016; Hoffbrand & Moss 2016: 30; Worwood *et al.* 2017). The two play a pivotal role because the body does not have a mechanism to excrete excess iron. For this reason, intravenous iron treatment to correct IDA is undertaken with extreme caution to avoid iron overload (Gomollo´n *et al.* 2010; Auerbach & Adamson 2016; Hoffbrand & Moss 2016: 36).

The standard treatment of IDA is with an oral iron preparation for approximately six months to correct the anaemia and replenish the iron stores (Auerbach & Adamson 2016; Hoffbrand & Moss 2016: 37; Worwood *et al.*

2017). In cases where patients do not respond to oral iron therapy, intravenous iron is then administered (Chertow *et al.* 2006, Muñoz *et al.* 2009; Gomollo´n *et al.* 2010; Hoffbrand & Moss 2016: 37; Worwood *et al.* 2017). The expected response to oral iron is usually an increase in haemoglobin by roughly 2g/dl every 3-4 weeks (Jimenez et al, 2015; Hoffbrand & Moss 2016: 36). The reasons for non-responsiveness of anaemia may include continued bleeding, failure to take tablets, mixed deficiencies and malabsorption (Hoffbrand & Moss 2016: 36). The reason for the failure to take tablets could have been attributable to the side effects of oral iron. On the other hand, clinicians are cautious about intravenous iron since it has been associated with increased risk of anaphylaxis (Muñoz *et al.* 2009; Gomollo´n *et al.* 2010; Auerbach & Adamson 2016). However, it has been proven that the administration of a low molecular weight preparation decreased the risk to 1 in 200,000 (Chertow *et al.* 2006; Auerbach & Rodgers 2007).

Several studies have shown that oral iron therapy might not be effective for the treatment of iron deficiency anaemia in geophagia (Nchito *et al.* 2004; von Garnier *et al.* 2008; Pebsworth *et al*. 2013; Seim *et al.* 2013). Iron supplementation in Zambian school children did not cause the child participants to stop consuming soil, while non-iron supplementation (micronutrient) did reduce the consumption of soil (Nchito *et al.* 2004). In another study where oral and intravenous iron therapy was undertaken; a female patient who suffered from iron deficiency anaemia was treated with oral iron without success (von Garnier *et al.* 2008). Upon treatment with

intravenous iron, the patient's iron deficiency anaemia corrected. Upon further investigation, von Garnier *et al.* (2008) discovered that the reason for the patient's lack of response to oral iron treatment was because of the patient practising geophagia. The soil that the patient was consuming contained kaolinite that was thought to bind to iron thus inhibiting its absorption (von Garnier *et al.* 2008).

1.1.2 Problem statement

It has become clear that geophagia is associated with iron deficiency anaemia and this is thought to be partly as a result of soil's effect on iron absorption. The reasons why geophagia is associated with iron deficiency are multi-faceted and have been the subject of research for the last number of years. Soil, owing to its granular composition might damage the gastrointestinal tract (GIT), leading to bleeding. Alternatively, soil attributable to its absorptive nature may lead to iron malabsorption by binding to the mucosal surface and absorbing the already available dietary iron. Instead, iron malabsorption may also be as a result of the soil changing the pH of the GIT, thus interfering with non-haem iron absorption which is dependent on pH. Another contributing factor might be soil acting as a vector for parasitic infestation, which might lead to the development of IDA. If soil consumption seems to interfere with dietary iron absorption, then it will most probably interfere with oral iron therapy. The reasons for the non-response could be either decreased bioavailability of iron or changes to the

mucosal surface of the GIT leading to malabsorption. The normal treatment protocol of iron deficiency anaemia is oral iron therapy for six months, followed by intravenous iron if the patient's anaemia does not correct (Auerbach *et al.* 2007; Auerbach & Ballard 2010). The study intended to determine if intravenous iron therapy would be the preferred method of treatment in geophagic women instead of starting with oral iron therapy.

Most of the studies on geophagia have concentrated on pregnant females (Geissler *et al.* 1998a; Kawai *et al.* 2009; Young *et al.* 2010; Njuri *et al.* 2011; Mathee *et al.* 2014; Macheka *et al.* 2016) and children (Nchito *et al.* 2004). This current study's focus was on non-pregnant females, which brought another dimension to the study of geophagia, as few studies are available (Mogongoa *et al.* 2011; Traugott *et al.* 2019). The other motivation for the study was to create critical mass concerning IDA treatment in geophagia because studies are limited in this area. The current study incorporated different variables like menstrual blood loss, occult blood, duration of consumption, amount of consumption and type of soil consumed. A study incorporating all these variables has not been undertaken, according to the authors' knowledge. Therefore, taking on an investigation from this point of view also added a different dimension to the topic.

Comparison of oral and intravenous iron therapy has been undertaken in patients with renal disease (Agarwal *et al.* 2015; Toblli & Di Gennaro 2015), heart failure (McDonagh & Macdougall 2015), inflammatory bowel disease (Reinisch *et al.* 2013; Bonovas *et al.* 2016) and pregnancy (Khalafallah *et*

al. 2010; Khalafallah & Dennis 2012), but it has not been performed in geophagia. An oral and intravenous iron therapy comparative study needed to be undertaken to address the following questions. To determine whether oral or intravenous iron treatment was more effective for the treatment of iron deficiency anaemia in geophagia; to determine whether the successful treatment of iron deficiency anaemia in geophagia would lead to the cessation of the geophagic practice.

If this proposed study confirmed that oral iron therapy was ineffective for the treatment of iron deficiency anaemia in geophagia, then a new treatment protocol would be developed. Meaning, iron deficiency anaemia patients with geophagia would not have to carry the burden of having anaemia symptoms for six months while oral iron therapy was not effective. Likewise, moving directly to intravenous iron would decrease the financial burden on the health care system and the patient, because an effective treatment would be administered from the beginning.

The question may be asked, why develop or try to develop a new treatment protocol when participants can be instructed or convinced to stop the practice? Iron-deficient participants of Bryant *et al.* (2013) described the craving as intense, disruptive and pervasive; the practice also affected their quality of life. In a cross-sectional study conducted in QwaQwa, South Africa, most participants indicated that they desired to stop consuming soil but they could not stop as a result of craving (Ekosse & Jumbam, 2010) thus causing them to revert to the habit. Similar situations were described in the

case reports presentation by Gonzalez *et al.* (1982) and Lassmann *et al.* (2007). It must also be kept in mind that the reasons for geophagia are multi-faceted. Cultural, psychological and physiological factors play a role, thus refraining from the practice is easier said than done. Therefore, the suggested geophagia treatment is multifactorial and includes the following: pharmacological, behavioural and patient education (Blinder & Salama, 2008). In addition, it was imperative to observe the time it will take for the GIT to absorb iron normally from therapy and diet. Especially after the possible negative effects of soil on the GIT absorptive surface have been eliminated.

1.1.2 Research Question

The questions that this study attempted to address were: which treatment approach would be best suited to treat iron deficiency anaemia in geophagia; oral or intravenous? The possible theories that could cause the soil to interfere with oral iron therapy were as follows: (a) is it kaolinite or soil's constituents that caused malabsorption of nutritional and treatment iron, thereby interfering with oral iron therapy? (b) Alternatively, did soil, due to its granular composition, damage the absorptive surface of the gastrointestinal tract, therefore causing bleeding that is the leading cause of iron deficiency anaemia? (c) Could soil interfere with iron absorption by coating or causing changes to the absorptive surface of the gastrointestinal tract thereby interfering with the iron transport across the intestinal wall? (d)

Finally, could soil change the pH of the gastrointestinal tract environment, which plays an important role in iron absorption?

1.1.3 Hypothesis

Hypothesis I

H0 – Oral iron therapy for iron deficiency anaemia in geophagic women was not an effective treatment, implying that population means of red blood cell indicators and iron study results before and after treatment were equal (σ before = σ after).

H1 – Oral iron therapy for iron deficiency anaemia in geophagic women was an effective treatment, meaning the population means of red blood cell indicators and iron study results before and after treatment were different (σ before ≠ σ after).

Hypothesis II

H0 – Intravenous iron therapy for iron deficiency anaemia in geophagic women was not an effective treatment, implying that population means of red blood cell indicators and iron study results before and after treatment were equal (σ before = σ after).

H1 – Intravenous iron therapy for iron deficiency anaemia in geophagic women was an effective treatment, meaning the population means of red blood cell indicators and iron study results before and after treatment were different (σ before ≠ σ after).

Hypothesis III

H0 – Neither oral nor intravenous iron therapy for iron deficiency anaemia in geophagic women was superior treatment, implying that population mean difference of red blood cell indicators and iron study results before and after each different therapy type were equal (σ oral = σ intravenous).

H1 – Either oral or intravenous iron therapy for iron deficiency anaemia in geophagic women was superior treatment, meaning the population mean difference of red blood cell indicators and iron study results before and after each therapy type were different (σ oral ≠ σ intravenous).

1.2 STUDY AIM

The study aimed to investigate the most effective treatment protocol between oral and intravenous iron therapy in geophagic female patients with IDA.

1.3 OBJECTIVES

To compare oral and intravenous iron therapy by performing an intervention (*in vivo*) study that entailed the following:

- Evaluation of geophagic practices to determine their possible impact on the development of iron deficiency anaemia.
- Evaluation of the significance of bleeding as a cause of IDA in geophagia by analysing gastrointestinal and menstrual bleeding.
- Evaluation of the efficacy of oral iron therapy for the treatment of iron

deficiency anaemia in geophagic females.

- Evaluation of the efficacy of intravenous therapy for the treatment of iron deficiency anaemia in geophagic females.
- Comparison of oral and intravenous iron treatment modes for their effectivity in the therapy of iron deficiency anaemia in geophagia.

1.4 THESIS STRUCTURE

In the subsequent chapter, an extensive literature review of geophagia and iron deficiency anaemia – manifestations, causes and therapy will be presented. The chapter will then be followed by the methodology undertaken in this study, to test the above-stated postulates. The methodology will then be followed by the results obtained from the different investigations undertaken in this study, which were organised according to the general health indicators, geophagic practice, bleeding assessment and oral versus intravenous iron deficiency therapy. In the penultimate chapter, the findings presented in the aforementioned chapter will be deduced. Finally, the thesis will be concluded with a chapter where the conclusions will be drawn about the significance of the entire collected study data.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Geophagia, a form of pica, have been linked to IDA in ancient plus modern literature. The major ideas behind this association are that iron deficiency anaemia leads to the craving of non-food (non-nutritive) substances or it is perceived as a symptom of IDA. Iron deficiency anaemia is the world's most common form of anaemia, seen mostly in females (de Benoist et al. 2008; Kassebaum *et al.* 2014). IDA's most common cause is blood loss. Therefore, the study population's menstrual and occult blood were investigated to ascertain their contribution to the development of IDA. In addition, geophagic soils may supplement or even reduce the bioavailability of certain minerals in the GIT of humans (Ghorbani 2008, Odilon Kikouama *et al.* 2009 Pebsworth *et al*. 2013; Seim *et al.* 2013). Therefore, soils may alleviate or contribute to IDA, or even affect treatment thereof. When all these factors are being considered, the intricate absorption of iron from GIT must also be borne in mind, as it plays a pivotal role in the type and amount of iron that can be absorbed because iron absorption is tightly regulated in the human body. Geophagia and IDA link will be further explored in the context of treatment of IDA in geophagic individuals. This implies that oral and intravenous iron

therapy will be compared to see which one would theoretically be more effective in cases of geophagia coupled with IDA.

2.2 GEOPHAGIA

Geophagia has an interesting place in history and has been a subject that peaks a significant amount of scientists' interest. It has a peculiar origin and it is complicated in that it has many reasons given for the practice. Geophagia involves psychological (presumptive neurobiological mechanisms e.g., iron deficiency, central nervous system neurotransmission, physiological conditioning), cultural (traditional) and social (acquired taste) aspects (Blinder & Salama 2008). In addition, there are certain advantages and disadvantages that literature captures concerning the practice.

2.2.1 Historical perspective

Geophagia is practised by many members of the animal kingdom and its existence in humans can be traced back in literature. It has been described throughout the course of history, dating as far back as the time of Hippocrates (440 – 337 BC). In 14 – 37 AD, A Cornelius Celsus implied the link with anaemia through skin colour while Pliny (Gaius Plinius Secundus, 23 – 79 AD) linked geophagia with anaemia (Woywodt & Kiss 2002). During the 6th century, Aetius linked geophagia with pregnancy. Avicenna (Ibn Sina, 980 – 1037 AD) also alluded to geophagia. In the 11th century Trotula of Salerno, a medical practitioner, postulated diet as a possible treatment of

geophagia in pregnancy (Mason-Hohl 1940). Liebault, in 1582, linked geophagia with chlorosis (Liebault 1609 referenced in Woywodt & Kiss 2002), while Veryser in Utrecht suggested a psychological link (Veryser 1694 referenced in Woywodt & Kiss 2002). Similarly, during that era, 16th & 17th century, Boetius advocated iron therapy for geophagia (Boetius 1638 referenced in Woywodt & Kiss 2002). Although iron therapy was advocated in 1638, there is a paucity of studies investigating this phenomenon. This is due to the multiplicity of possible causes. Von Humboldt (18 & 19 century) also described geophagia and postulated hunger as one of the reasons for the practice (Woywodt & Kiss 2002). However, Livingstone disputed poverty as a factor.

As can be observed from the above statements; each era of history added to the complexity of the subject known as geophagia. Some of the theories given for the practice, which add to the intricacy of the topic, are being a consequence of famine, psychiatric disorder, culturally sanctioned practice, detoxifying agent; mineral deficiency and morning sickness remedy. The question that springs to mind is: how big is the geophagia phenomenon?

2.2.2 Prevalence of geophagia

Geophagia has been described worldwide and found on all six continents; Asia (Al-Rmalli *et al.* 2010), Australia (Eastwell 1976; Bateson & Lebroy 1978), Europe (Derman *et al.* 2005), North America (Vermeer & Frate 1979, Lassmann *et al.* 2007), South America (Halsted 1968) and Africa (Luoba *et*

al. 2004; Momoh *et al.* 2012; Raphuthing *et al.* 2014). It has been postulated that geophagia is more prevalent in sub-Saharan Africa (Walker *et al.* 1997, Smith *et al.* 2010). It is also said to be more common in people of African descent (Walker *et al.* 1997).

From a plethora of studies, the percentage of people practising geophagia has been estimated to be anything from 5% to 85%, depending on the specific population that was studied. For instance; Greissler *et al.* (1998) conducted a cross-sectional study at an antenatal clinic in Kenya where 56% of 257 pregnant women consumed soil. Nchito *et al.* (2004) reported geophagia in about 75% of the 304 child participants. Young *et al.* (2010) reported a 5.4% prevalence of geophagia in a sample of 2356 pregnant women representative of the Pemba island population. Kawai *et al.* (2009) also found that 29% of the 971 participants consumed soil regularly. A literature review by Njuri *et al.* (2011) presented geophagia prevalence of 50% to 84% among pregnant women. Walker *et al.* (1997) estimated the incidence in sub-Saharan Africa at 90%. The challenge with these estimates is that individuals who practice are reluctant to admit to the practice for fear of judgement. For that reason, accurate prevalence estimate is difficult to ascertain. As observed, geophagia is more prevalent in children and women of childbearing age, it is especially more rive during pregnancy.

The challenge for the current study was the paucity of information on the prevalence of geophagia in non-pregnant females. Hence, an accurate assessment of practice in the target population was difficult to ascertain.

The reason for the lack of information may be because geophagia is seen as a problem of pregnancy, and studies conducted on non-pregnant females' primary objective was not to determine prevalence. The reason why geophagia may be rife in non-pregnant females is associated with the fact that if a person practices geophagia during pregnancy, the practice will stop during the post-partum stage, but this might not be the scenario. Another reason might be that the need for iron might be more emphasised during pregnancy due to the developing foetus but the contribution of lactation and the menstrual cycle to the development of IDA might be overlooked.

2.2.3 Reasons for practice

The reasons that are captured below are not an exhaustive list, only those that are commonly investigated and popularly mentioned in literature are presented. A complete list can be found elsewhere in literature; this study's objective was not to investigate the reasons for the practice. Thus this section is given as part of the overview of the intricate topic of geophagia. The selected ones are psychology, hunger, cultural and mineral supplementation.

2.2.3.1 Psychological

Geophagia is associated with psychological factors through a variety of ways, one way being seen as a practice that is observed in the mentally

and developmentally disabled people (Danford & Huber 1981; FisHbain & Rotondo 1983; Jawed *et al.* 1993; Luiselli 1996; Gravestock 2000; Dumanguing *et al.* 2003). On the other hand, the majority of the population that practice geophagia is not affected by severe mental aberrations, thus another way of geophagia association is via IDA. The focus of this study was on the latter population, thus the second theory of IDA will be emphasised.

Firstly, iron is needed for sensorimotor, cognitive, social-emotional functioning and development; due to the dependence of the central nervous system on iron-containing proteins and enzymes (Chen *et al.* 2013). Therefore, IDA has been associated with psychiatric disorders among children and adolescents (Chen *et al.* 2013). The reason for association centres on iron being utilised for myelination of white matter, development and functioning of dopamine, norepinephrine and serotonin neurotransmitters (Parks & Wharton 1989; Beard 2003; Beard *et al.* 2003; Burhans *et al.* 2005; Beard 2008).

The practice of pica may secondly relate to diminished postsynaptic dopamine receptor changes (Gravestock 2000; Dumaguing *et al.* 2003). A critical determinant of pica is theorised to be decreased dopamine transmission resulting from IDA, thus treatment that increases dopaminergic transmission is suggested for refractory and hazardous pica (Brahm *et al.* 2006). In addition, certain psychosocial stressors are proposed to increase the practice of pica. These include maternal

deprivation, joint family, parental neglect, pregnancy, impoverished parent-child interaction, and disorganized family structure (Barltrop 1966; Blinder & Salama 2008).

The third mechanism that is in operation (Rolls 1988; Blinder & Salama 2008) suggests that two adaptive mechanisms involved in the control of eating are malfunctioning or compromised in pica, namely; (1) *sensory-specific satiety* and (2) *neophobia*. Sensory-specific satiety postulates that the more you consume certain food its pleasantness decreases, while the pleasantness of food that has not been consumed for some time increases over time. This functions to increase variety while neophobia ensures avoidance of non-nutritive and dangerous foods. Neophobia by definition is the avoidance of food that is not usually consumed.

The three theories presented above can be summarised as two of those affecting physiological developmental and functioning of the brain through the ill-development of white matter and by malfunctioning of dopamine signal transmission, respectively. This is contrary to the third one, which entails the functional psychological level, where the aberration is in the control of the eating mechanism. These intricate and multi-layered associations might also be the reason why geophagia is complex and quitting the practice has generally not been an easy task. Consequently, the treatment of IDA is paramount when geophagia treatment is considered.

2.2.3.2 Cultural

In the absence of other reasons for the consumption of soil, then the cultural context seems to play a significant role (Vermeer & Frate 1979). Geophagia has historically been associated with fertility and childbearing or even the promotion thereof, according to general folk understanding. Thus, the practice of geophagia is not discouraged in certain cultures, especially for pregnant women. Young women in Turkey (Derman *et al.* 2005), Australian Aborigines (Eastwell 1976; Bateson & Lebroy 1978), Africa (Hunter 1993) and United States' African Americans (Vermeer & Frate 1979) believed that soil consumption promotes fertility or is a sign thereof. In South Africa, urban young women believe that geophagia will soften and improve their skin tone (Woywodt & Kiss 2002).

There is a disorder called cachexia Africana, where slaves in Tanzania and southern parts of North America would consume soil to the point of death because they are longing for home. In Brazil, slaves were fitted with face masks to prevent geophagia since addiction to geophagia would make the slave lethargic and become progressively debilitating (Mustacchi 1971).

Geophagic soils also have socio-economic implication; in certain circumstances, geophagia has been linked with religion and traditional medicine. Geophagic soils are sold in markets in Africa (more especially in South Africa), Asia, the United Kingdom, South and North America. There are street vendors in South Africa that make a living off selling soil.

In Mexico (Bick *et al.* 1993) and Guatemala (Bartas & Ekman 2001) soil was sold in the shape of the cross and cathedral designs, respectively. Soil is also utilised by many traditional healers in their remedies (Walker *et al.* 1997). In Southern Africa, about 60% of people depend on traditional medicine (van Wyk *et al.* 1997).

As observed, geophagia in certain cultures is accepted or even promoted while in others it is seen as an unacceptable practice. It should be noted that this acceptance is not blanket, in Malawi for instance, soil consumption outside of pregnancy is frowned upon (Hunter 1993). The acceptance during pregnancy creates a space where the practice can become rife and people who are not pregnant may pick up the practice from their pregnant elders. This might further increase the incidence of geophagia, since geophagia may contribute or cause IDA. However, the opposite is true in that because the practice is not sanctioned in other circles, this creates a culture of secrecy about the practice which makes it difficult to determine the prevalence. Therefore, delaying the process of obtaining a solution to the challenge that is geophagia.

2.2.3.3 Hunger

Numerous authors have postulated hunger as one of the reasons for geophagia; some are in support while others dispute the fact. Hawass *et al.* (1987) stated that when famine and hunger were implicated then soil served as a filler and appetite suppressant. In addition, as geophagia is seen as an eating disorder, it is not surprising that is has been observed

in anorexia nervosa (Hawass *et al.* 1987). Another author, von Humboldt, observed that the Potomac tribe (Native of South America) consumed a lot of soil and stored dried soil for periods of famine (von Humboldt *et al.* 1821 in Wodwodt and Kiss 2002). Ghorbani (2008) links soil consumption with poverty, especially in developing countries. While Ljung *et al.* (2006) indicated that soil acted as a food detoxifier during a famine. Soil consumption came to the limelight in the not so distant past, during times of famine especially after a devastating earthquake in Haiti (Katz 2008).

Contrary to the authors above, the below-mentioned authors dispute the theory of hunger as the reason for geophagia. Livingstone, who described *safura* in Zanzibar, refuted poverty as a factor since geophagia did not just affect the economically disadvantaged but affected the wealthy as well (Livingstone 1874). Young *et al.* (2010) also disputed the hunger theory by stating that if women can afford to eat raw rice then poverty is not a primary driver of pica. A study by van Onselen & Walsh (2011) did not find a significant different dietary nutrient composition between the geophagic and non-geophagic participants of that study. In a review by Young *et al.* (2011), only 22% (16/76) of cultural reports solely attributed geophagia to hunger, contrary to 50% that unequivocally refuted the association with hunger.

The complexity of whether hunger is a factor in geophagia's causation emanates from the fact that the practice was observed more commonly in the low-income bracket. However, it must be noted that geophagia due to

multi-faceted origin or causes, plus the practice been found in people who do not belong in low-income bracket and support from the above-mentioned studies that do not support the hunger theory. Therefore, income may play a role via diet but it is not the only aspect. More importantly, blood loss and malabsorption of iron perhaps play an even major role than hunger, thus a conclusion that geophagia is caused by hunger is not supported by the author.

2.2.3.4 Mineral supplementation

Another important postulate that is popular in literature is that the craving of soil is thought to be an adaptive mechanism to supplement mineral deficiency. This theory is firmly established in the animal sphere (Healy & Ludwig 1965; Healy 1967; Mayland *et al.* 1975; Abrahams & Thornton 1994; Smith *et al.* 2009); however contradictory evidence is available for humans (Minnich *et al.* 1968; Suttle *et al.* 1984; Tateo & Summa 2006; Young 2007). The difference might be brought about by the differences in the GIT. In animals, the following minerals and trace elements have been proven to be supplemented by soil: sodium (Holdø et al. 2002; Boggs & Dau, 2004; Hui, 2004; Monaco *et al.* 2019), calcium (Marlow & Tollestrup 1982; Penzhorn 1982; McDowell 1992), iron (Mahaney & Hancock 1990; Mahaney *et al.* 1990) and iodine (Statham & Bray 1975; Grace 2006). Furthermore, this theory is supported by animals visiting lick sites during particular times when the particular mineral might not be readily available

during that season (Kreulen & Jager 1984; Klaus *et al.* 1998). These lick sites contain other minerals that could be of benefit to the animals, namely; K, Mg, F, molybdenum, cobalt and selenium (Bowell & Ansah 1994; Mills & Milewski 2007). However, soil can remove Cu resulting in swayback, a lamb nervous disorder. This removal of Cu by soil is depended on the soil mineralogy. This disorder becomes scarce during high snow cover when soil consumption is reduced (Suttle, 1988).

In humans, especially focusing on iron deficiency which is associated with geophagia, there are varied ideas about the effectiveness of iron supplementation in geophagia. Some authors indicate that iron is not bioavailable (Hooda *et al.* 2004; Abrahams *et al.* 2013; Seim *et al.* 2013) and in some cases, the soil might even decrease the dietary bioavailable fraction (von Garnier *et al.* 2008; Pebsworth *et al*. 2013). On the other spectrum, other scholars indicate that soil can supplement dietary iron (Abrahams & Parsons; 1997; Smith *et al.* 2000; Yount 2005; Abrahams *et al.* 2006; Ghorbani 2008, Odilon Kikouama *et al.* 2009).

The contradictory human study results might be brought about by two major aspects, soil type and methodological differences of research studies. Different soil types utilised in the experiments; specifically, the different soil composition and their cation exchange capacity play a significant role. The other reason is the different methodologies that are applied and the lack of a standardised *in vivo* method. This current study might shed some indirect light on the *in vivo* human interactions. All hope

is not lost as a recently developed chicken model offered promising results for *in vivo* study model (Siem *et al.* 2013).

2.2.4 Positive health effects associated with geophagia

The hypothesised positive health effects are numerous and include among others mineral supplementation, detoxification of harmful elements in the GIT and nausea remedy in pregnancy. Mineral supplementation was postulated by van Onselen & Walsh (2011) who found a sub-optimal dietary calcium content in a geophagic study group but a normal calcium level in the blood of these individuals. *In vitro* studies also support the mineral supplementation theory as Ca, Mg and Mn are bioavailable in different GIT tract simulation compartment (Hooda *et al.* 2004).

Pliny stated that soil is “used in an enema as it arrests diarrhoea”. This detoxification of harmful elements’ effect is coupled with the absorptive nature of soil (Woywodt & Kiss 2002). Soil’s absorptive nature has been exploited in the design of modern medicine by isolating kaolinite and using it as an active ingredient for diarrhoea medicine (Florez *et al.* 2018). Kaolin is also an ingredient for face masks as it produces excellent results for the skin (Kwan *et al.* 2006). The other use of kaolin is as a drying agent when applied topically. It has been utilised to dry lesions caused by poison sumac, poison oak and poison ivy. Pregnant females from West Africa believe that soil, specifically Calabar chalk, relieves nausea related to morning sickness (Vermeer & Ferell 1985).

2.2.5 Negative health effects associated with geophagia

Some of the negative health effects of geophagia that have been noted in literature are directly and indirectly linked to the GIT. The direct effects are obstruction (constipation), stomach perforation and peritonitis, although the last two are not commonly encountered (Woywodt & Kiss 2002). Constipation is due to the absorptive nature while others are as a result of soil granularity. The indirect events are that soil acts as a vector for geohelminths. Soil may also lead to electrolyte imbalance, specifically hypokalemia (Trivedi *et al.* 2005). It may also result in heavy metal poisoning, for instance, lead (Mahaffey 1981) and arsenic (Ljung *et al.* 2006).

2.2.5.1 Parasitic infestation

In endemic areas, a quarter of the population may be infected with soil-transmitted helminths (Keiser & Utzinger, 2008). Parasitic infestation is more common in the tropics and sub-tropics. These infections are characterised by *Ascaris lumbricoides,* the hookworms (*Ancylostoma duodenale* and *Necator americanus*) and *Trichuris trichiura* (Bethony *et al.* 2006). *Ascaris* infestation is more common. However, *Trichuris* and hookworm are the ones associated with IDA (Albonico *et al.* 1998; Hotez *et al.* 2004), although hookworm that is strongly associated with IDA is not frequently encountered.

Another helminth that has been associated with geophagia is *Toxocara canis*, although not so commonly encountered (Stagno *et al.* 1980; Lassmann *et al.* 2007). Parasite's eggs are found in soil contaminated by puppy litter. The eggs can survive for months or years in soil, especially in tropical and temperate temperature. However, infection is rare at greater than 60^{o} latitude. Toxocariasis is commonly encountered in children that consume soil than adults because geophagic females in most cases seem to be selective when it comes to where they obtain their soil (Young *et al.* 2010; Perridge *et al.* 2011).

Geophagia is seen as a major vector for soil-transmitted geohelminths. However, in certain circumstances, it might not be a major factor for the causation of IDA. Young *et al.* (2010) and Perridge *et al.* (2011) collected geophagic soil material in their studies; in Zanzibar, Tanzania and QwaQwa in South Africa; did not find parasites associated with IDA. Further lending indirect support, that parasitic infestation was not a major factor, was finding normal eosinophil count in geophagic QwaQwa participants and their control group. Helminths infestation is generally associated with marked eosinophilia as parasites migrate and invade tissue (Lassmann *et al.* 2007). Likewise, Greissler *et al*. (1998) associated geophagia with IDA having ruled out hookworm infestation as a confounding factor. Another factor that may play a significant role is that QwaQwa is not in the tropics, similar to Botshabelo, therefore soil-transmitted parasites that result in IDA may not be commonly encountered

in these areas. The final factor that does not support IDA causation due to parasite infestation is that *Ascaris* is more frequently encountered however, it is not commonly associated with IDA.

2.2.5.2 Electrolyte imbalance

Many studies have postulated that geophagia may be driven by mineral deficiency but one of its negative effects is associated with hypokalaemia. Case study presentations have been described where geophagic soils were associated with the development of hypokalaemia (Mengel *et al.* 1962; Gonzalez *et al.* 1982, Severance *et al.* 1988; Chaushev *et al.* 2003; Trivedi *et al.* 2005). Selected soil samples tested confirmed that soil was responsible for the absorption of potassium (Dreyer *et al*. 2004).

Putting hypokalaemia into context, even though individual cases have been presented it does not seem to be a common occurrence but it should be borne in mind that the type of soil also plays a pivotal role on whether the soil will remove potassium. The question might arise as to the reason for finding a few studies that have measured potassium level. This could be attributed to the absence of clinical features, due to the subclinical presentation of hypokalaemia, leading to the absence of potassium level investigation.

2.2.5.3 Heavy metal poisoning

Soil ingestion has been linked to heavy metal poisoning which has serious health consequences for the individual. These commonly implicated heavy metals are lead (Filippelli et al. 2005), arsenic (Ljung *et al.* 2006), less frequently mercury (Ljung *et al.* 2006a); chromium (Broadway *et al.* 2010) and cadmium (Ljung *et al.* 2006). On the one hand, scholars have postulated that low concentrations are found (Ekosse & Jumbam 2010; Abrahams *et al.* 2013) and these heavy metals are not bioavailable thus are not absorbed (Abrahams *et al.* 2013). Contradictory findings are based on the source of the soil, its mineralogy, cation exchange capacity and the form in which the heavy metal is found. Although heavy metal poisoning from soil may not always play a significant role in geophagia, one of the major associations of geophagia with heavy metal is commonly encountered in iron deficiency. However, it is not the poisoning of it but rather the removal of iron.

2.3 IRON DEFICIENCY ANAEMIA

The widely known and investigated effect of geophagia on haematology is the development of IDA (Greissler *et al.* 1998; Luoba *et al.* 2004; Nchito *et al.* 2004; Kawai *et al.* 2009; Young *et al.* 2010; Mogongoa *et al.* 2011; Pebsworth *et al.* 2013; Seim *et al.* 2013; Raphuthing *et al.* 2014). Iron is an important constituent of haemoglobin, which is found in red blood cells, whose primary function is oxygen transport. When iron is deficient, it can cause problems in

the human body, resulting in iron deficiency anaemia which affects the delivery of oxygen to tissue, restless legs syndrome, infertility, cognitive impairment and retarded growth development in children (Bruner *et al.* 1996; Chavaro *et al.* 2006; Lozoff *et al.* 2006; Wells *et al.* 2006; Falkingham *et al.* 2010; Allen *et al.* 2013; Seid *et al.* 2014). It can also affect the functioning of enzymes that contain iron; causing cell abnormalities like hair thinning and diminished cell-mediated immunity (Hoffbrand *et al.* 2016: 28).

Iron is a transitional metal that can exist in both ferrous and ferric form; it is insoluble, abundant in diet and earth's crust (about 4%). The ability to exist in two forms makes it ideal to play a critical role in enzyme reactions. The insolubility of iron affects its absorption; the fact that the body has no physiological mechanism to get rid of iron; while iron overload causes damage to endocrine glands, liver and heart. Body iron is also regulated to prevent damage to biomolecules like nucleotide basis that may result in DNA abnormalities via the Fenton reaction (Koskenkorva-Frank *et al.* 2013). The reaction generates highly reactive hydroxyl radicals when hydrogen peroxide reacts with Fe(II). The above-mentioned reasons necessitate the regulation of iron concentration in the body.

In the body, iron is transported bound to special proteins called transferrin and it is stored as ferritin and haemosiderin. These proteins, together with haematologic findings of hypochromic (less haemoglobin) microcytic (small) red blood cells, can be employed to assess the iron status of the individual (Hoffbrand *et al.* 2016: 34; Worwood *et al.* 2017). However, the diagnosis of

iron deficiency anaemia is complicated by the inflammatory response that causes a change in the iron study parameters that are usually measured to assess the iron status.

2.3.1 The link between geophagia and IDA

The association of geophagia and iron deficiency anaemia has been the subject of research; cross-sectional (Greissel *et al.* 1998; Nchito et al. 2004; Kawai *et al.* 2009; Young *et al.* 2010; Mogongoa *et al.* 2011; Raphuthing *et al.* 2014) and case reports (Yao 2006; von Garnier *et al.* 2008) that illustrate this phenomenon has been published. Greissler *et al.* (1998) found that the mean haemoglobin and ferritin level of the geophagic group was lower than that of the non-geophagic group. This difference was statistically significant even when gestational age, malaria and hookworm infection was taken into consideration. Nchito *et al.* (2004) reported a significant association of lower ferritin level in the geophagic group than the non-geophagic group. Contrary to ferritin, the haemoglobin level was not associated with geophagic status. This finding indicated that some of the geophagic children had not developed iron deficiency anaemia but were iron deficient as indicated by the ferritin level.

It is noted that differentiation should be made between IDA and iron deficiency (ID). ID indicates a state where the body's iron stores are depleted and IDA signifies that iron deficiency is now coupled with a decreased haemoglobin concentration. Thus, iron deficiency is

characterised by decreased iron study parameters and normal haemoglobin concentration. Conversely, with IDA both measurements are decreased. From a clinical background, ID is associated with fewer symptoms than IDA; however, both are associated with restless leg syndrome. Therefore, treatment of both ID and IDA is advocated (Allen 2013).

Young *et al.* (2010) found a strong association of geophagia and amylophagy with low haemoglobin concentration and iron deficiency anaemia. Kawai *et al.* (2009) also found an association between iron deficiency anaemia amongst human immunodeficiency virus-positive pregnant Tanzanian women. In a pilot (Mogongoa *et al.*, 2011) and an extended (Raphuthing *et al.*, 2014) study with more participants where geophagic (12 participants in pilot & 47 in extended) and non-geophagic (5 in pilot & 36 in the extended) women who were not pregnant; a statistically significant association between geophagia and iron deficiency was obtained.

Yao (2006) described a 60-year-old woman who rediscovered her childhood habit of geophagia and presented with iron deficiency anaemia at routine follow-up visit. While von Garnier *et al.* (2008) presented a 34-year-old woman who had refractory iron deficiency anaemia for 10 years, who on questioning revealed practising geophagia. From these cross-sectional studies and case study reports it can be observed that geophagia is associated with iron deficiency anaemia, but it should be emphasised that

causality has not been completely established. However, an aspect that may play a role is bleeding which is the most common cause of IDA.

Does bleeding play a role in the development of IDA in geophagia? Khan & Tisman (2010) presented three pica case studies whereby heavy bleeding from menses and colon cancer were the cause of IDA. Furthermore, an argument can be made that soil might damage the lining of the GIT leading to leakage although it is said to be rare (Woydolt & Kiss 2002) and bleeding, thus creating another challenge. Contrary to bleeding causing IDA in geophagia, subsequent studies that refute the theory should also be taken into account. In a case study presentation by von Garnier *et al.* (2008) the patient had a normal gastric mucosa with *Helicobacter pylori* infection and no sign of bleeding. In addition, endoscopy revealed non-specific erythematous mucosal changes in the bulbus duodeni. Likewise, cross-sectional studies by Mogongoa *et al.* (2011) and Raphuthing *et al.* (2014) did not find a significantly higher platelet count in the geophagic group, in comparison to the control group. Although there was no direct determination of bleeding in these studies, platelet count usually increases secondary to bleeding.

Nchito *et al.* (2006) did not find any difference in the permeability of the intestine as measured by lactose: mannitol ratio in geophagic and non-geophagic Lusaka school children. Nevertheless, the finding by Nchito *et al.* (2006) does not exclude the possibility that iron absorption might be impaired by changes to the absorptive surface. Noting that iron and lactose

or mannitol absorption occur through different mechanisms. More studies are needed to confirm that iron absorption is not impaired by changes on the absorptive surface of the gastrointestinal tract.

The aforesaid studies illustrated an array of findings that both supported and disputed bleeding as a cause of iron deficiency anaemia in geophagia, but more studies are needed to clarify the role and effect of bleeding. Due to this reason, it was imperative that menstrual bleeding assessment and occult blood testing be incorporated in the current study. It should be noted that these measurements have not been researched enough in geophagia cases, or even investigated simultaneously.

2.3.2 Prevalence of iron deficiency anaemia

Over two billion people worldwide are affected by iron deficiency, thus the World Health Organization declared iron deficiency as the most prevalent nutritional deficiency (de Benoist *et al.* 2008). The sector of the population that is mostly affected is infants, pre-school aged children, women of childbearing age and strict vegetarians. This is a consequence of increased demand due to growth, blood volume that needs to increase to adult level, menstrual blood loss plus pregnancy and non-effective absorption of inorganic iron from the diet, respectively (Hoffbrand *et al.* 2016: 32). Due to iron's non-excretion from the body and recycling of iron from senescent red blood cells, males are not commonly affected by IDA.

In the high-risk group, a prevalence of 30% for IDA is noted for non-industrialized countries, while in industrialized countries iron deficiency is the most prevalent nutritional deficiency (de Benoist *et al.* 2008). The IDA disease burden is more severe in sub-Saharan Africa and Asia (de Benoist et al. 2008). In 1994, an anaemia prevalence of 10% was reported by Kruger *et al.* (1994) in non-pregnant coloured Cape Peninsula females with 7% for IDA. Almost similar to the 1994 study, Lawrie *et al.* (2008) found 15% anaemia prevalence with 10% of women showing signs of IDA. In addition, the South African National Health and Nutrition Examination Survey performed in 2012 found a prevalence of 15.3% and 9.7% for anaemia and IDA, respectively (Shisana et al. 2013). However, Phatlhane *et al.* (2016) found a prevalence of 18.3% for anaemia with 15.1% being IDA. Females of African descent were severely affected than coloured and Caucasians counterparts (Phatlhane *et al.* 2016).

Differences were found in studies presented above and WHO estimate seems to be higher than the actual study reported prevalence. An overall estimate of between 15–20% for anaemia and 7–15% for IDA in non-pregnant women were reported. Whereas WHO estimated 30% for non-industrialised countries, which is higher than the reported cases. The phenomenon of prevalence differences could be attributed to different selection criteria, diverse categorization of iron deficiency, the geographical distribution of participants that signify different altitudes and differences in age. Furthermore, the WHO estimate was for the entire population which

included other groups where IDA's prevalence might be even higher, thus increasing the prevalence. In addition, South Africa has both industrialised and non-industrialised areas, thus the estimate can be adjusted slightly upward when non-industrialised areas are included. Furthermore, most of the above-mentioned studies where performed in industrialised areas. The disadvantage of the studies was that the mentioned estimates were based solely on the study population. However, if they were to be extrapolated to the general population, parity of the estimates could result.

2.3.3 Aetiology of iron deficiency anaemia

There are many causes for iron deficiency anaemia, but these can be grouped under four headings namely: diet, increased physiological iron requirements, malabsorption and blood loss (Hoffbrand *et al.* 2016: 32). Diet is important in strict vegetarians especially when it is associated with increased physiological demand for iron. Increased iron requirements are associated with the following: infancy, adolescence, females and pregnancy. Although during pregnancy the menses stop; the requirements of the foetus, placental loss and increased erythropoiesis in the maternal circulation in preparation for blood loss at birth, puts significant pressure on the mother's iron requirements.

Malabsorption, another major cause of IDA, can be linked to gluten-induced enteropathy, geophagia and gastrectomy. Gastrectomy results in decreased stomach hydrochloric acid concentration which leads to

decreased iron absorption. The GIT can be associated with *Helicobacter pylori* and hookworm infestation (Worwood *et al.* 2017). Hence, the need to investigate the geophagic soil for parasite was identified as indicated by studies in the literature.

Most of the body's iron is found in the haemoglobin, it is not surprising that blood loss is the most common cause of iron deficiency anaemia. Bleeding can result from the uterus, respiratory, pulmonary, renal and gastrointestinal tract. In association with geophagia, the question needs to be asked: soil being abrasive, could it lead to GIT bleeding, further contributing to the body's bleeding avenues? Literature captures studies that are for and against the bleeding theory in geophagia, thus further studies need to be undertaken to clarify the role of bleeding.

2.3.3.1 Menstrual blood loss and measurement

Heavy menstrual blood flow can result in a loss of 5% of the female's red blood cell mass, meaning 4% of the body iron content, and if the replacement of cells is not at the rate of loss then a negative balance results causing iron deficiency. There is a dearth of studies in geophagia where menstrual blood loss has been assessed.

Various menstrual blood loss assessment tools have been utilised in non-geophagia related studies, like counting of sanitary pads (Warner et al. 2004), questionnaire (Warner *et al.* 2004), pictogram (Ismail *et al.* 2004) and blood pad weight assessment / dissolving of blood followed by

spectrophotometric measurement. Counting of sanitary pads does not correlate with the amount of menstrual blood because the limit for changing is not depended on the amount of blood on the pad but on individual preference. The questionnaire is based on recollection and is heavily influenced by the subjectivity of the interviewee. Pictogram removes most of the subjectivity as the sanitary pad is compared with a picture and it not based on recollection as the tool is utilised when sanitary pads are at hand. The most accurate method currently, is the weighing of sanitary pads to estimate the exact amount of blood loss (Schumacher *et al.* 2012)

2.3.3.2 Occult blood assessment

Occult blood assessment has not been undertaken in geophagia, according to the author's knowledge. Therefore, the contribution and extent of GIT bleeding to geophagia have not been assessed. Occult blood analysis is employed to screen for gastrointestinal blood loss, it is positive especially for intestinal blood loss but may be negative for upper GIT blood loss. The other challenge with occult blood testing is that depending on the method used, a false positive reaction can be obtained. This particular method, causing false positive results, depended on dietary restriction to increase the specificity. The new generation method of ELISA does not depend on the dietary restrictions since it utilises antibody against the

haemoglobin or combination of haemoglobin/haptoglobin. The method utilised in the current study was ELISA.

2.3.4 Body iron distribution and loss

The amount of iron contained in the male body is 50 mg/kg while a female has 40 mg/kg. Two-thirds (66%) of the body's functional iron is within circulating red blood cells as part of haemoglobin which is responsible for gaseous exchange. The other 12% is in iron-containing enzymes, myoglobulin and bound to transferrin in plasma. The remainder, approximately 22%, is in bone marrow erythroblasts, liver and macrophages, as storage (Hoffbrand *et al.* 2016: 28).

The primary form of storage iron is in proteins called ferritin which is soluble and haemosiderin which is the insoluble form, both storage forms are available for the production of newly synthesised erythroblasts. Under normal circumstances, most storage iron is in ferritin form while haemosiderin is mostly found in macrophages. Ferritin is directly proportional to the body's iron stores, except in cases of inflammation or infections where it may be falsely elevated (Worwood *et al.* 2017).

The loss of iron is approximately 1 mg/day through the loss of epithelial cells of the gastrointestinal, urinary tract, skin, hair and nails that contain enzymes with iron (Worwood *et al.* 2017). Iron absorption from a well-balanced diet is also 1 mg (Hoffbrand & Moss 2016: 29; Worwood *et al.* 2017). This is true for adult males; while for the sector of the population

where IDA is common, due to increased demand or loss, this situation does not hold ground.

2.3.5 Iron absorption, transport and recycling

Iron absorption optimally takes place in the duodenum because of the acid environment of the stomach that changes the charge on iron; and the proteolytic enzymes that lead to the release of haem from protein complexes. Iron from meat and fish, in haem form, is easily absorbed; unlike iron from vegetables and inorganic sources, non-haem – Fe3+. Iron forms insoluble ferric chloride complexes in the jejunum because of the alkaline environment. Only 3mg to 4mg per day of dietary iron is absorbed, most of which is haem iron (Worwood *et al.* 2017).

During iron deficiency states, the absorption of non-haem iron is increased by expression of divalent metal transporter 1 (DMT-1) on the intestinal cell surface and transferrin receptor 1 on body cells that require iron. This process is regulated by the binding of iron response element that detects iron concentration within the cell, to iron regulatory protein on mRNA. DMT-1 is expressed in the duodenum and promotes absorption of inorganic iron. The role of transferrin receptor 1 is to transport iron into the body cells that require iron.

After intestinal absorption, approximately 1mg of iron per day is transported to plasma where it is bound to transferrin. This process is governed by ferroportin that is expressed on the intestinal cell. Ferroportin's expression

is governed by hepcidin, which is produced by the liver. During inflammation, infection and/or increased plasma iron saturation, hepcidin is produced – which degrades ferroportin. However, in IDA, hepcidin production is suppressed, thus iron can be transported into circulation and also mobilised from storage in macrophages which also possess ferroportin. Transferrin-bound with iron in blood will adhere to the target cell's transferrin receptor 1. The transferrin-iron-transferrin receptor complex will be transported into the cell where iron is stored or used; while transferrin is released to recirculate (Hoffbrand *et al.* 2016: 30).

The body does not have a physiological mechanism to get rid of excess iron; consequently, iron is conserved and recycled. Iron from senescent red blood cells is returned to the bone marrow for the formation of new red blood cells (Robertson & Roper 2017). When senile red blood cells are broken down, the released haem iron binds with transferrin for transportation to macrophages for erythroblasts production in the bone marrow or for storage in ferritin and haemosiderin (Hoffbrand & Moss 2016: 61; Robertson & Roper 2017).

Iron absorption is regulated at two levels; absorption plus mobilization phase mediated by IRE – DMT-1 and hepcidin – ferroportin, respectively. These mechanisms play a vital role in body iron concentration regulation. Hepcidin is also important in inflammatory states where it decreases iron absorption and transport in the body. The mechanism is postulated as a means to keep iron away from the infective microorganisms. Some of the

proteins used for transport and storage of iron in the body are utilised as indicators for diagnosis of iron deficiency anaemia.

2.3.6 Laboratory findings

IDA results in decreased haemoglobin concentration because iron is an important constituent of haemoglobin. In iron absence, there is defective haemoglobin synthesis which results in decreased formation of red blood cells (anaemia), red blood cell size (mean corpuscular volume – MCV), red blood cell haemoglobin content (mean corpuscular haemoglobin – MCH), absent erythroblast iron and depleted bone marrow iron stores (Hoffbrand & Moss 2016: 35; Worwood *et al.* 2017). This hypochromic (decreased red blood cell haemoglobin content) microcytic (decreased red blood cell size) anaemia picture is coupled with the characteristic iron study findings.

The iron study results associated with IDA are decreased serum iron, ferritin level, transferrin saturation (TSAT), hepcidin, an increase in total iron binding capacity (TIBC) and serum transferrin receptor 1 concentration (Hoffbrand & Moss 2016: 38; Worwood *et al.* 2017). Confounding factors that need to be taken into consideration when interpreting iron studies are: (1) although serum ferritin reflects the iron stores, it may be increased due to infection or inflammation as it is an acute phase protein. Inflammation causes an increase in hepcidin which leads to decreased iron transport into circulation from the gastrointestinal tract and release of iron from macrophages, mediated by ferroportin. (2) Damage to hepatocytes, which

are rich in iron, will also increase the ferritin level. (3) Serum iron and TSAT can sometimes be normal on the short term as values may not reflect supply over a long term. (4) Serum transferrin receptor reflects the erythroid activity and is inversely related to the cells' iron supply (Hoffbrand & Moss 2016: 29). Consequently, it becomes imperative to evaluate haematologic findings, inflammatory markers and iron study results when accurately diagnosing iron deficiency anaemia. Accurate diagnosis ensures effective treatment of the disorder.

2.4 TREATMENT OF IRON DEFICIENCY ANAEMIA

The holistic management of IDA comprises of identifying the cause, treating the underlying cause, correction of anaemia and iron deficiency (Whittaker 2011, Jimenez *et al.* 2015). The last two factors have historically been addressed by blood transfusion and by inorganic iron therapy, respectively. There are attempts to decrease the practice of blood transfusion owing to the associated risk; transfusion-transmitted infection, alloimmunisation, transfusion reactions, circulatory overload and temporary effects of therapy (Argarwal 2010, Reudiger & Lopez-Plaza 2012). Transfusion was historically reserved for cases of severe anaemia, patients who are haemodynamically unstable and those with active bleeding (Jimenez *et al.* 2015).

In current practice, the recommended first line of treatment for iron deficiency anaemia is oral iron supplementation, if non-responsive, then

parenteral iron is administered (Hoffbrand *et al.* 2016: 36). However, both inorganic iron therapy forms have advantages and are not without their challenges. These include adverse drug events (ADE).

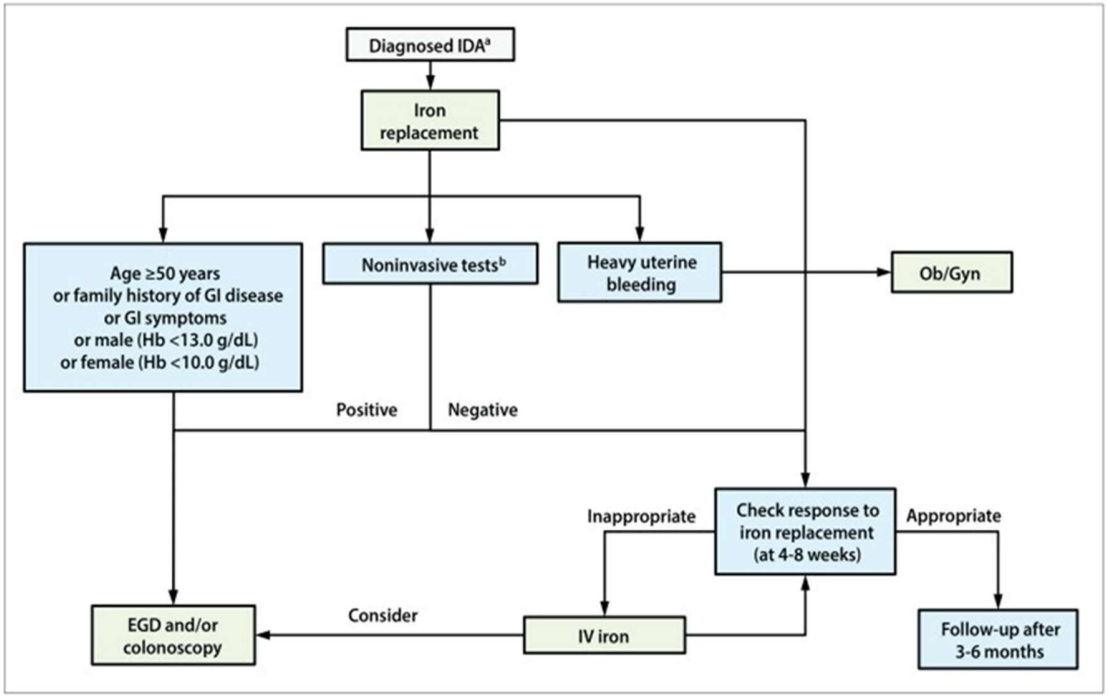


Figure 2.1: IDA treatment algorithm (Jimenez *et al.* 2015)
[a] Endurance athletes and pregnant women should be treated without further diagnostic testing.
[b] Celiac serology, anti–parietal cell antibody, Helicobacter pylori (stool), and fecal occult blood test.
EGD, esophagogastroduodenoscopy; GI, gastrointestinal; Hb, hemoglobin; IV, intravenous; Ob/Gyn, obstetrics/gynecology.

The first and most critical step in the management of IDA is the identification of the cause. The major causes of IDA for pre-menopausal women is menno(metro)rhagia. However, for males plus post-menopausal females, it is gastrointestinal bleeding. Identifying the cause can be expensive and may involve invasive procedures, that carry certain risks for the patient.

Therefore, Jimenez *et al.* (2015) compiled a treatment algorithm that also identifies circumstances where invasive tests are warranted, see Figure 2.1. As depicted in Figure 2.1, the algorithm suggested that pregnant female and endurance athletes should be treated without further testing. While those patients with increased risk for GI disease should undergo GI tract investigation. When non-invasive tests, which include celiac serology, anti-parietal cell antibody, *Helicobacter pylori* and faecal occult blood, are positive then invasive GI tract investigation should be undertaken. On the other hand, during cases of heavy uterine bleeding, patients should be referred to the obstetric gynaecologist. The last scenario when GI tract investigation should be considered is when oral iron is not effective.

2.4.1 Oral iron therapy

Oral iron has for many years and continues to be the mainstay of IDA therapy as it has advantages. However, the effectiveness of therapy is dependent upon an intact GI tract. The response to therapy may be stifled when the rate of uptake is below the rate of need, or the loss is heavy or there is a concomitant inflammatory disease.

2.4.1.1 Dosing and effective therapy outcome

Oral iron is administered at a concentration of 100 mg to 200 mg elementary iron, even though levels as low as of 15 mg to 30 mg may suffice (Makrides *et al.* 2003; Rimon *et al.* 2005; Zhou *et al.* 2009). During

the late stage of iron deficiency, as much as 20% to 25% can be absorbed from 100 mg iron. However, during latent iron deficiency and IDA, the rate of absorption is 10% and 13%, respectively. This is contrary to 5% and 5.6% for healthy male and females, respectively (Werner *et al.*1976 referenced in Jimenez *et al.* 2015).

Effective treatment outcome will be observed by an increase of 1–2g/dL in haemoglobin concentration over a period of 3–4 weeks (Jimenez *et al.* 2015; Bonovas *et al.* 2016). However, if anaemia is severe then the response may take as much as 8 weeks and even up to 3 months for the correction of the anaemia (Jimenez *et al.,* 2015). It may take another 3 months or more to replenish the iron stores to ferritin level of >100 µg/L. Therapy effectiveness depends on the various types of oral preparations.

2.4.1.2 Different oral iron preparations

The efficacy of oral iron therapy depends on the type of preparation. Iron salts exist in two forms, ferrous and ferric (Whittaker 2011). The preferred iron salt is ferrous because iron has to be converted to ferrous form before absorption (Camaschella 2015; Bates, 2017). Different forms of soluble ferrous salts are ferrous sulphate, ferrous gluconate and ferrous fumarate (Whittaker 2011; Camaschella 2015; Bates, 2017). In spite of ferrous being preferred, it has been proven that ferric polymaltose is equally effective as ferrous salts and it is well tolerated (Whittaker 2011; Bates 2017). A major issue that negatively affects the efficacy of oral therapy is inflammation.

2.4.1.3 Effect of inflammation on iron utilization

The effectiveness of oral iron therapy is limited during inflammatory diseases. This results in functional iron deficiency (Jimenez *et al.* 2015). Iron is available but is not utilised for haemoglobin synthesis, due to impaired iron release into the circulation. Inflammatory conditions lead to hepcidin secretion which breakdown ferroportin, a transporter of iron out of enterocytes, hepatocytes and macrophages. Therefore, oral iron therapy in cases of inflammation is not effective, hence parenteral iron is advocated (Jimenez *et al.* 2015).

2.4.1.4 Advantages and disadvantages of oral iron

The advantage of oral iron is the cost-effectiveness, easy access, availability of different forms and ease of use. It is cheaper than intravenous iron (Whittaker 2011). It is available at pharmacies without a need for doctor's prescription. It exists in pill, capsule and liquid form; which can be used by both adults and children (liquid). Since it is administered orally, it is easy to self-administer (Whittaker 2011).

However, its negative effect is decreased compliance (67%) due to adverse drug events (Anker & von Haehling 2012). Oral iron therapy may result in side effects like constipation and stomach pains. These side effects can be reduced by taking iron therapy after eating, but this decreases the absorption of iron (Worwood *et al.* 2017). If there is no

response following iron treatment or the patient does not respond because of malabsorption, then intravenous iron can be administered.

2.4.2 Intravenous iron therapy

Intravenous iron should be administered in cases of oral iron non-responsiveness resulting from malabsorption or iron loss exceeding uptake. Intravenous iron is associated with side effects like headache, skin rashes, urticaria, general aches and pains; depending on the type of preparation. The major complication of intravenous iron can be anaphylaxis resulting in iron overload and even death, thus precautionary measures should be taken to avoid these.

2.4.2.1 Different parenteral iron preparations

The preparation that seems to be responsible for most of the ADE is the high-molecular-weight iron dextran (InFed [Watson]). Mamula *et al.* (2002) indicated an estimate of >25%. Ever since the introduction of low-molecular-weight iron dextran (INFed® / Cosmofer® in SA), sodium ferric gluconate (Ferrlecit®); ferric carboxymaltose (Ferinject®); ferumoxytol (Feraheme®), iron isomaltoside 1000 (Monofer®) and iron sucrose (Venofer®) the number of ADE has decreased significantly (Martin-Malo *et al.* 2019).

Different preparation's advantage is the total dose that can be administered. Two preparations can be utilised for maximum dose while

another two needs repeated sessions, the difference is related to the side effects. With iron polymaltose (Ferrosig) an increased iron concentration can be administered, but iron sucrose (Venofer) is given as a series of small doses over a period of days or weeks. While iron carboxymaltose (Ferinject) can administer medium dose over 15 minutes and be repeated at another session (Martin-Malo *et al.* 2019).

2.4.2.2 Effective treatment outcome and dosing

The haemoglobin level following IV therapy increases at the same rate as the oral iron, which is an increase of 2 g/dL over 2 to 4 weeks (Bonovas *et al.* 2016; Martin-Malo *et al.* 2019). It should also be noted that the ferritin level increase excessively, thus within 8 weeks of administration ferritin level does not correlate with body stores. Iron balance in the body is controlled by absorption instead of excretion hence iron overload that can cause serious health consequences like heart and liver failure should be avoided. In the laboratory, iron overload is described by TSAT >50%.

The total IV iron dose is calculated by use of Ganzoni formula, in which total iron deficit in mg = [body weight in kg x (target Hb – actual Hb in g/dl) x 0.24] + 500. The 500 mg is added to replenish the store. However, in cases of severe anaemia (<7 g/L) it is advocated that an additional 500 mg should be added to the treatment regime. Some scholars suggest that the Ganzoni formula underestimates iron requirements, thus its use is not

advocated. Evstatiev *et al.* (2011) came up with a simpler dosing scheme that showed better efficacy and compliance.

2.4.2.3 Advantages and disadvantages of parenteral iron therapy

The main advantage of IV iron is bypassing of the absorption pathway and the ability to correct anaemia over a short space of time. The disadvantage is the perceived association with serious ADE, the cost since it involves nursing staff due to administration in hospital and the actual cost of medicine.

2.4.3 Oral and intravenous iron therapy in geophagia

There is a serious lack of studies relating to iron deficiency anaemia treatment in geophagia. Furthermore, the only oral versus parenteral iron therapy study that was undertaken, according to the authors' knowledge, was a case study (von Garnier *et al*. 2006). This is an interesting phenomenon considering that IDA is strongly associated with geophagia. Moreover, geophagia is considered a sign of IDA in haematology circles. However, the soil could interfere with iron bioavailability because there is uncertainty over the soil's effect on iron absorption. The reasons for no interest might be due to geophagia not being recognised as a major role player in IDA as its prevalence is underestimated and its impact in haematology is only considered a symptom of IDA, not as a possible contributor. Lack of studies on IDA therapy in geophagia necessitated a

review of other disciplines to elucidate the intricacy of comparing the two major forms of therapy.

2.4.4 Oral versus intravenous iron therapy in other diseases

Many lessons could be learned from other disciplines about comparing oral and IV iron therapies, since there a serious deficiency of such studies in geophagia. The disciplines where these studies have been undertaken are gastroenterology via inflammatory bowel diseases (IBD), cardiology due to chronic heart failure (CHF), urology because of chronic kidney disease (CKD), obstetrics and gynaecology due to pregnancy and the postpartum period. IDA prevalence in the above-mentioned diseases is high (Munoz & Martin-Montanez 2012; Beguin & Jaspers 2014). The reasons why these disorders are associated with IDA vary from malabsorption, iron loss and increased iron demand. The lessons include among others; the side effects that are associated with different therapy types, improvement of quality of life, reduction of mortality rate, advantages of therapy, the best mode of therapy based on efficacy in correcting haemoglobin and ferritin levels.

2.4.4.1 Oral versus IV iron therapy in inflammatory bowel disorder (IBD)

IBD involves two major diseases ulcerative colitis and Crohn disease which are associated with anaemia. Anaemia is a frequent complication of IBD at a reported prevalence of 75% (Gasche *et al.* 1994; Gisbert *et al.* 2008). A Scandinavian study reported 20% for IDA and 30% for anaemia

(Bager *et al.* 2011). Similarly, 70% anaemia prevalence was reported in hospitalised patients and only 20% was found in outpatients (Gisbert *et al.* 2008; Bager *et al.* 2013; Abitbol *et al.* 2015). The different prevalence is linked to the severity of IBD and whether the patient is hospitalised or an outpatient. Furthermore, the type of anaemia also plays a role in the difference of prevalence, whether it is IDA or anaemia of chronic disorders (ACD) or combination of both anaemias or a minor contributor – megaloblastic anaemia (Gomollón & Gisbert 2009; Dignass *et al.* 2015). Reasons for the development of IDA in IBD are chronic blood loss, impaired iron intake and uptake (Hodges *et al.* 1984; de Vizia *et al.* 1992; Lomer *et al.* 2004). In addition, the toxicity of the medication is also implicated (Gomollón & Gisbert 2009; Dignass *et al.* 2015).

Varied and multidimensional reasons exist for the probing of oral versus IV iron studies. The reasons included the uncertainty of some gastroenterologists about safety of IV iron in IBD (Stein *et al.* 2013), the variable iron absorption rate in IBD due to hepcidin levels (Semrin *et al.* 2006), oral iron that seems to worsen the disease symptoms (Uritski *et al.* 2004; Werner *et al.* 2011) and the potential side effects of oral therapy leading to non-compliance or termination of therapy (Rasul & Kandel 2001; de Silva *et al.* 2005; Dignass *et al.* 2015). In animal studies it has been suggested that mucosal damage is mediated by oxidative radicals and cancer development may be promoted by oral iron (Carrier *et al.* 1989; Tompkins *et al.* 2001; Seril *et al.* 2002). Plus, historically no standard

therapy protocol existed pertaining to oral and IV therapy with some suggesting oral for Hb>10g/dL and IV for Hb<10g/dL (Gisbert *et al.* 2009). Furthermore, the treatment protocol was complicated by the addition of erythropoiesis-stimulating agents (ESA) for patients who had refractory anaemia (Schreiber *et al.* 1996). The recent therapy protocol advocates that IV should be first-line therapy in patients with clinically active IBD, with Hb < 10g/dL, oral iron intolerance and in patients who require ESA, while oral is reserved for mild anaemia, clinically inactive disease and oral iron tolerant patients (Dignass *et al.* 2015). The protocol is based on the fact that IV iron is thought to be more effective, shows a faster response and is better tolerated than oral iron.

A summary of comparison study revealed that IV iron Hb improvement was statistical significantly higher than oral (Lee *et al.* 2012), although it may not be deemed as clinically significant (0.68 g/dl). However, iron storage was significantly improved by IV contrary to oral iron. IV iron also resulted in fewer side effects than oral. However, there was no difference in the quality of life in this meta-analysis (Lee *et al.* 2012). Another meta-analysis showed that IV iron was superior to oral as 67% of IV treated patients contrary to 52% of oral achieved >2 g/dL Hb increase (Bonovas *et al.* 2016). It should also be noted that oral iron achieved correction of anaemia. Bonovas *et al.* (2016) further supported the idea of oral having more side-effects than IV. The difference relating to Hb level was influenced by the different endpoint calculation strategies, the different

selection strategies for inclusion of studies and the different statistical analysis used by both meta-analyses. The peculiar finding is the fact that IBD is associated with hepcidin release, that should suppress iron absorption but oral therapy resulted in the correction of anaemia. However, the key seems to lie in the severity of the disease, thus whether severe inflammation is involved or the concomitant medication was taken into consideration. Inflammation and medication were not considered in the meta-analyses (Lee *et al.* 2012; Bonovas *et al.* 2016). Another important aspect is that in cases of IDA, the iron absorption regulatory system suppresses the production of hepcidin.

2.4.4.2 Oral versus IV iron therapy in chronic heart failure

CHF is associated with ID and anaemia, plus this combination negatively affects patient health status. Prevalence of ID is reported at 50%, with severe disease (New York Heart Association - NYHA class III & IV) being even higher – 73% (Nanas *et al.* 2006) while class II and I have >30% (Jankowska *et al.* 2010; Klip *et al.* 2013). Coexistence of CHF and ID is associated with decreased quality of life, increased hospitalization risk, decreased exercise tolerance, worse NHYA class and could contribute to muscle dysfunction (Haas & Brownie 2001; Jankowska *et al.* 2010; Okonko *et al.* 2011; Anvi *et al*. 2012). Therefore, it has been recognised that treatment of ID in CHF is essential for the improvement of quality of life and not just IDA (Anker *et al*. 2009; Guazzi *et al*. 2012). This stems

from the other roles played by iron in spite of anaemia absence. ID leads to decreased oxygen storage in the muscles due to the absence of iron in myoglobin (Clark *et al.* 1996; Dunn *et al*. 2007; Guazzi *et al*. 2012). In addition, iron plays a role in oxidative phosphorylation, citric acid cycle and cellular respiration as part of the enzyme (Maguire *et al*. 1982; Dunn *et al*. 2007). Finally, decreased myocardial iron content caused decreased left ventricular function mediated by abnormal mitochondrial / sarcomere dysfunction (Davies *et al*. 1982, Guazzi *et al*. 2012).

The reasons for the association of CHD with ID are numerous and slightly different from those of other disorders (van Veldhuisen *et al.* 2011, Pfister *et al.* 2012; Jankowska *et al.* 2013). These include low iron intake as a result of poor nutrition and concomitant renal failure associated with advocating a low protein diet (Schena 2011; van Veldhuisen *et al.* 2011). The second reason is iron loss because of proteinuria secondary to chronic renal disease, plus ulceration or gastritis as a result of pharmacotherapy (Sica 2003; Okonko & Anker 2004; Schena 2011; Ather *et al.* 2012). Thirdly, decreased iron absorption precipitated by increased hepcidin level, GIT oedema formation and decreased GIT perfusion rate (Jankowska *et al.* 2012). Finally, haemorrhage attributable to treatment with antiplatelet drugs and anticoagulants (Scarf 2009).

Few articles have compared oral and IV because suggested therapy protocol was addressed in the not so distant past. Previously there was no consensus on whether to treat ID or anaemia, with an emphasis mostly on

anaemia treatment (Pfiser *et al.* 2012; von Haehling *et al.* 2012). Moreover, the importance of treating ID has recently been advocated (Jankowska *et al.* 2013; McDonagh & Macdougall 2015). Earlier comparisons were based on oral iron and oral plus ESA (van Veldhuizen *et al.* 2007; Ghali *et al.* 2008; Swedberg *et al.* 2013). The findings indicate that ESA improves the symptomatic burden and corrects the anaemia, contrary to oral alone (Zhang *et al.* 2016). However, the improvement was not associated with improved clinical outcomes.

The comparison of IV therapy was mostly with placebo and only a single pilot study was found comparing IV with oral. In spite of the different study designs, single-arm or randomised, the outcome of IV therapy was positive (Bolger *et al.* 2006; Toblli *et al*. 2007; Onkonko *et al.* 2008; Usmanor *et al.* 2008; Anker *et al.* 2009a; Gaber *et al.* 2012; Toblli & Gennaro 2012). With improved Hb levels, iron stores, quality of life, decreased symptomatic burden and hospitalization frequency. This effect was observed across different iron preparations and maintained throughout the follow-up periods. However, it is worth noting the studies did not show a mortality benefit, as supported by meta-analyses (Anvi *et al.* 2012, Kapoor *et al.* 2013). The IV vs oral pilot study showed improved Hb and ferritin levels for both groups (Beck-da-Silva *et al.* 2013). The increase in mean TSAT was more pronounced for the IV than the oral group. In addition, the mean peak VO_2 was increased in IV while oral showed a decrease (Beck-da-Silva *et al.* 2013).

2.4.4.3 Oral versus IV iron therapy in chronic kidney disease

The prevalence of chronic kidney disease is >10% in adults of developed countries and one of its complications is anaemia (de Jong *et al.* 2006; Hallan *et al.* 2006; Coresh *et al.* 2007). Anaemia may be encountered at any stage of kidney disease. Anaemia is observed in 25% of patients with mild disease, non-dialysis chronic kidney disease (ND-CKD) Stages 1-2. The prevalence increases to more than 50% for severe disease, ND-CKD Stage 4 (McClellan *et al.* 2004). The reasons for the development of anaemia are decreased absorption as a result of chronic inflammatory diseases mediated by hepcidin release, lack of erythropoietin and blood loss during dialysis, approximately 2.5 litres per annum (Locatelli *et al*. 2004; Hörl 2009; Babitt & Lin 2010).

There are several reasons why oral vs IV iron therapy has been undertaken. Reasons include the non-standardization of therapy protocol, the use of erythropoietin stimulating agents (ESA) and the associated decline in the quality of life of CKD patients (Coresh *et al.* 2001; FisHbane *et al.* 2009; Macdougall *et al.* 2014). The treatment protocol states that IV iron should be reserved for dialysis patients while non-dialysis patient should use oral therapy, plus the degree of anaemia plays a role (KIDGO 2012). The use of high concentration ESA is reserved for refractory anaemia. However, the use of a combination of high concentration ESA and higher haemoglobin level target is associated with cardiovascular events, increased risk of thrombosis and stroke (Besarab *et al.* 1998;

Drueke *et al.* 2006; Singh *et al.* 2006; Pfeffer *et al.* 2009; Palmer *et al.* 2010).

The lessons learned from oral vs IV iron are that decreasing the concentration of ESA leads to a decreased complication rate. IV iron can also be employed for non-dialysis patients (Covic & Mircescu 2010; Macdougall *et al.* 2014a; Toblli & Genaro 2015). Oral and IV are equally efficient in correcting the anaemia while in case of severe anaemia IV corrects the anaemia rapidly (Covic & Mircescu 2010; Macdougall *et al.* 2014a; Toblli & Genaro 2015). The quality of life and the risk of complication are reduced when the anaemia is corrected (Macdougall *et al.* 2014a; Toblli & Genaro 2015).

2.4.4.4 Oral versus IV iron therapy during pregnancy and postpartum

IDA is common among pregnant females with a prevalence range of 35% to 75% (WHO 1992; ACC/SCN 2004; Barroso *et al.* 2011) and postpartum females with an incidence range of 12.7% to 30% (Bodnar *et al.* 2002; Bodnar *et al.* 2005; Barroso *et al.* 2011), in spite of the public health measures advocating iron supplementation (Maberly *et al.* 1994; Beard 2000). The reasons why iron deficiency should be treated during pregnancy and post-partum are attributable to their association with premature labour, foetal developmental abnormalities, decreased lactation periods and increased rate of postpartum depression (WHO 1992; Bodnar *et al.* 2002; Corwin *et al.* 2003; ACC/SCN 2004; Bodnar *et al.* 2005;

Zimmerman & Hurrell 2007). The association of IDA with pregnancy and lactation is based on the fact that as much as 1000mg iron is needed for pregnancy. That translates to 27 mg of iron per day contrary to 8mg in non-pregnant adults, while 10 mg of iron is required for lactation (Institute of Medicine (US) Panel on Micronutrients 2001).

The main reason why the obstetric professionals investigated if IV iron could be implemented to treat the IDA was the increased demand for iron and the prevalence of IDA. Furthermore, for pregnant women other reasons for investigating oral versus intravenous therapy were indigestion that leads to decreased compliance to oral iron, to decrease the rate of blood transfusion, cost implication of different therapies, safety and side effects of IV therapy for both foetus and mother (Singh *et al.* 1998; Beard 2000; Makrides 2003; Al *et al.* 2005; Auerbach *et al.* 2007; Auerbach *et al.* 2008). IV therapy has historically been associated with severe allergic but the introduction of newer preparation changed the treatment landscape (Al *et al.* 2005; Auerbach & Rodgers 2007). IV iron complications were dramatically decreased and a higher concentration of iron could be administered as a single dose. IV therapy was well tolerated with no significant complications plus the efficacy was similar to that of oral, except in one study were IV produced a rapid increase in haemoglobin level (Khalafallah *et al.* 2010; Khalafallah & Dennis 2012). However, it should be noted that in the pregnancy field the combination therapy is more

common than oral vs IV because oral supplementation is already advocated during pregnancy.

IV versus oral studies in postpartum patients were undertaken because of the increased prevalence of anaemia, clarifying the treatment protocol, in an attempt to improve current and subsequent clinical outcomes of pregnancies. The treatment protocol of postpartum anaemia involves treating mild anaemia with oral iron and moderate to severe anaemia with intravenous iron (Beris & Maniatia 2007; Breymann *et al.* 2010). The major findings of these studies were that oral and IV therapies were equivalent in correcting the anaemia as measured by haemoglobin level (Bhandal & Russell 2006; Van Wyck et al. 2007; Westad *et al.* 2008; Giannoulis *et al.* 2009; Becuzzi *et al.* 2014). However, intravenous was superior in replenishing the iron stores as measured by ferritin concentration (Bhandal & Russell 2006; Van Wyck et al. 2007; Westad *et al.* 2008; Giannoulis *et al.* 2009; Becuzzi *et al.* 2014). Likewise, IV iron improved clinical outcomes by decreasing depression rate, increasing breast milk iron concentration and improving post-partum fatigue (Holm *et al.* 2015, 2017, 2017a).

2.5 SUMMARY

Geophagia, a practice that has been in existence for a long time seems to be associated with many causalities. Geophagia is also not seen as a great threat to human health, since it does not cause mortality and its prevalence is not accurately estimated. However, geophagia is not a topic to be ignored because it is associated with abnormalities that can affect human health. The link between geophagia and IDA clearly exist, no matter the theory underpinning such an association. There are varied plausible reasons for the link; mineral deficiency, parasitic infestation, soil decreasing the bioavailability of iron, regulation of iron concentration by absorption, malabsorption as a result of iron insolubility, soil-damaging the intestinal lining or resulting in bleeding. Therefore, a multidisciplinary approach and a strategic study design are needed to enlighten the principles underpinning which therapy protocol will be best suited for the treatment of IDA in geophagia, notwithstanding the lessons learned from other disciplines' comparative studies of oral and IV iron therapy. The results are varied depending on the purpose of the investigation and the emphasis is placed on improving patient quality of life in a cost-effective manner. All the above necessitated the investigation into evaluating which therapy will be best suited for IDA in cases of geophagia. By undertaking this study, the novelty of this project will be underpinned by developing a new treatment protocol, that will decrease the negative effects associated with geophagia.

CHAPTER 3

MATERIALS AND METHODS

3.1 INTRODUCTION

This study was conducted in Botshabelo which is situated 45 kilometres East of Bloemfontein, the capital city of Free State province, South Africa. The geographical coordinates of Botshabelo are 29° 14' 0" South, 26° 44' 0" East, with an average elevation of 1451 meters (Anonymous 2016; Anonymous A 2016). Botshabelo's climate is warm and temperate, with an average annual temperature of 14.8 °C (Anonymous 2017, Climate-Data.org 2017).

According to Statistics South Africa (Stats SA) census of 2011, the population of Botshabelo was estimated at 181 712, composed of 50 593 households and 99% of residents being people of African descent. The average annual household income of 77% of the population was below R38 201; with 12.6% of the population having no income at all (Stats SA 2012). The latest data from the community survey (2016) were difficult to compare with those of census 2011 since they were summarised by a municipality, not a town. Nonetheless, it can be noted that intercensal total population growth rate for the municipality was 0.004 (Stats SA, 2016).

The current study was divided into a pilot and main study; the pilot was conducted from May till November 2015, while the main study was conducted

from January till November 2016. The pilot research participants were recruited from May till July 2015 and oral therapy was initiated in August 2015. The main study was divided into six groups, with the first group enrolled in January 2016 and the final group enrolled in August 2016. Multiple trips were undertaken between Bloemfontein and Botshabelo. These trips were undertaken to bring participants to the Central University of Technology, Free State (CUT) for follow-up visits. During these visits questionnaires were completed; blood specimens were obtained; body composition, blood pressure measurements and different iron therapy protocols were undertaken.

3.1.1 Ethical consideration

This study's proposal was submitted to and approved by the Ethics Committee of the University of the Free State, reference number: ETOVS 17/2014 (Appendix A). The study was conducted following the International Good Clinical Practice Guidelines and adhered to the principles of the Declaration of Helsinki (WMA General Assembly). Three members of the research team were trained for good clinical practice.

Participants' names were only utilized by the research team when telephonic contact was made with participants. Each participant was allocated a unique number, which was utilized for all research-related documents so that confidentiality could be maintained. Even with data capturing and statistical analysis, unique numbers were utilised. Published

results were presented as a group and no participant names were mentioned.

Study aim, rationale and procedures were explained in an understandable language to the participants. During that initial encounter, a screening questionnaire (Appendix B) was completed to ascertain the geophagic status of the potential participant. Information document (Appendix C) was provided for further reading and consultation with the family. After a week had passed, an appointment was made where the participants were asked questions about the study and allowed to ask questions as well. This process was followed so that informed consent and family support can be ensured, before signing the informed consent document (Appendix D).

At the end of the study, participants were given iron therapy to supplement iron stores. In addition, participants were advised to monitor their iron level, while those that did not respond to both forms of treatment were referred to a health practitioner for further investigation. During the study, when other study-unrelated health abnormalities were encountered or a participant withdrew from the study, a referral was emphasised. Participants were advised to visit their nearest clinic, district hospital or medical practitioner for further medical attention. The participants also completed an exit questionnaire to assess their experiences of the study (Appendix E).

3.1.1.1 Patient safety and risk

Standard preparation used for IDA treatment were utilised thus no major safety concerns were expected. For oral iron, the generally expected side effects included stomach pain and constipation which was addressed by a change in oral preparation as per standard protocol. For IV iron therapy the risk was of anaphylactic reaction which was prevented by an initial test dose administration of IV iron followed by observation by a medical doctor. Furthermore, antihistamine was also administered as an extra precautionary measure.

IV iron therapy poses a risk for iron overload. This was not a significant risk as enrolled participants suffered from IDA. In addition, their ferritin levels were determined at every visit. Furthermore, the dose was calculated based on haemoglobin level and subject weight, thus iron overload was averted.

3.1.2 Study design

The design was a prospective dose escalation intervention study with the intention to treat IDA. The primary outcome of this study was the correction of anaemia and iron deficiency. The study composed of geophagic female participants with IDA divided into two groups. One group was expected to abstain (Group A) from consuming soil while another continued (Group B) with the geophagic practice. The rationale for utilising two groups was to determine whether abstinence from geophagia would cause correction of anaemia by oral iron. If it did then there was no absorptive surface

malabsorption detected. However, if it did not correct the anaemia, then it meant soil affected or damaged the gastrointestinal tract absorptive surface. Participants of both groups were treated with oral iron for ten weeks. Intravenous iron was administered to those participants that did not respond to oral iron, based on full blood count and iron study test results. However, those that responded continued with oral iron therapy for the remainder of the study, i.e. another four weeks.

3.1.3 Study population

Eighty-six non-remunerated voluntary geophagic women participants, from Botshabelo, with haemoglobin and ferritin concentration of 10.5 g/dL and 15 µg/L or less, respectively, were enrolled for the study. The enrolled participants were selected from 320 recruited participants. Participants were divided into two groups of approximately 40 participants each. Participants were recruited by a combination of purposive (a non-randomised door to door approach), coupled with a snowballing technique (referrals from geophagic individuals) (Heckathorn 2002). The door to door method was chosen so that the participants could be comfortable in their territory and the strategy enabled easy follow-up. The bias that is created by the purposive technique was counteracted by recruiting a maximum of 50 participants in one residential area. While bias for snowballing was counteracted by the fact that only those research participants that met the inclusion criteria were enrolled in the study.

3.1.3.1 Randomisation

Assignment of the participants to the two groups was performed randomly, by each participant selecting three papers, from 26 numbers and 26 letter combination. If two numbers and one letter were drawn by the participant, then she continued with soil consumption. Contrary to one number and two letters which meant abstain from the practice for the duration of the oral therapy study phase.

3.1.3.2 Inclusion and exclusion criteria

- *Soil consumption should be for more than one month*; this is part of the definition of geophagia
- *Ferritin lower than the reference range (15–200 µg/L)*; this is the lowest level that indicates depletion of the iron store according to the reference range utilised for this study (Bates, 2017). Although other scholars use less than 30 µg/L to designate iron deficiency (Mast *et al.* 1998, Phatlhane *et al.* 2016). Another school of thought advocates the use of less than 12 µg/L as an indicator for IDA (Ali *et al*. 1978). In the current study, the reference range that was utilised was what the haematology fraternity currently subscribe to (<15 µg/L). However, in cases of inflammation and or infection, the recommended ferritin cut-off level for iron deficiency is 100 µg/L (Knovich *et al.* 2009; Camaschella 2015, Dignass *et al.* 2015).

- *Permanent residents of the district for at least 6 months*; the participants would have consumed soil found in the district and their diet might be similar to that of local people.
- *No pregnant and lactating women were included*; pregnancy and lactation exert pressure on the mother's iron level as the developing foetus and nursing baby needs to obtain iron from the mother's circulation. Hence, the iron demand would mask treatment effectiveness. Most importantly, certain IV iron therapy has not been approved for administration during pregnancy.
- *Age ranged from 18 to 55 years*; no differentiation was made between pre- and post-menopausal females because menstrual blood loss was determined in this study. 18 years was preferred so that consent would not be an issue. While 55 years was selected as the highest point as the risk for IDA becomes equivalent to that of males.
- *Haemoglobin of 10.5 g/dL or less for inclusion into both groups*; anaemia is defined as a haemoglobin level below 11.5 g/dl for women of African descent. Therefore, a level of 10.5 g/dl was preferred, which is classified as mild anaemia. In so doing, the effect of therapy can be easily observed. Moreover, IDA associated with geophagia is mild and moderate in most cases.

3.1.3.3 The rationale of sample size

Standard sample size calculation was not undertaken due to the exploratory purpose of the current study. However, the rationale for 80

participants was for statistical analysis and to contain the financial implications of the study. Starting with 80 meant enough statistical power to analyse the results. Even when 20% dropped out, 64 participants would remain. So ideally, at least 30 participants would be maintained in each group. Also, a significant number was maintained in cases where some participants responded to oral iron because those that responded to oral iron therapy (with corrected IDA and iron stores) did not continue with the intravenous iron therapy stage. In retrospect, once the results were available, the sample size was calculated with Epi Info ™ version 3. The average number of participants based on an effect of 30%, as revealed by the Hb results, was 77 participants for the three sample size calculation methods (Appendix F).

Other reasons for participants' number included: decreasing drop-out rate and to mitigate the financial burden. It was concluded that oral therapy with the least side effects would be utilised, to mitigate the drop-out rate thus maintaining statistical power. The challenge was that oral therapy utilised in the study was expensive. Other contributing factors that further increased study financial implication were: travelling, the number of visits and laboratory tests that were undertaken as part of the study protocol. For cost containment, 80 participants were a feasible option.

3.1.4 Work schedule

The intervention study was divided into two stages namely; pilot and main. The study lasted 14 to 16 weeks for each participant. The first phase, pilot study, entailed recruiting of at least ten participants – five for each treatment group. The pilot study aimed to identify and resolve problems that would be encountered when the main study was undertaken. In so doing, effective use of time and resources was achieved. The second stage was the main study, where the rest of the participants (76) were enrolled. Six groups were enrolled at different times throughout the study. The results from both stages were combined during the final analysis stage.

Figure 3.1 lists the work breakdown structure, which describes the timing of different procedures that were undertaken for the study. Title of the phase plus procedures carried out at each stage are listed in the work plan. The recruitment and selection of study participants according to the inclusion criteria were handled first. At different intervals of the intervention study, different questionnaires were administered. Blood was obtained to assess short term, intermediate and long term effects of different iron therapy. Sample collection for occult blood and data collection for menstrual blood loss analysis were embarked on at different time intervals throughout the intervention study. Finally, all the collected data was analysed to determine which iron treatment protocol achieved the best efficacy.

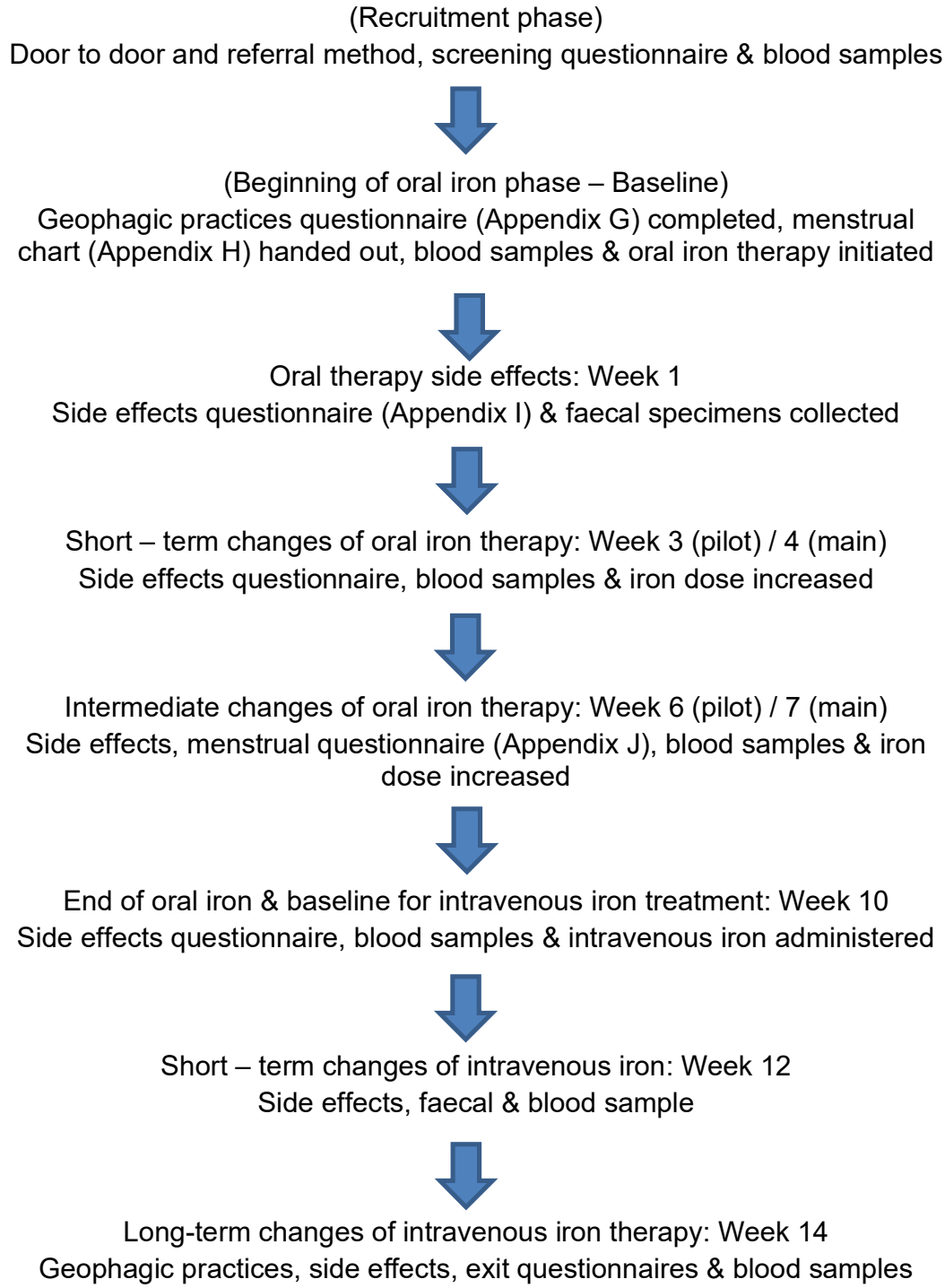


Figure 3.1 Work breakdown structure of the intervention (*in vivo)* study

3.1.5 Workload breakdown and assignment

The recruitment of participants; selection of participants; drawing of blood; laboratory analysis; completion of questionnaires on geophagic practices, side effects and sources of bleeding were performed by the research team, under the leadership and supervision of the primary researcher. The oral iron intervention was the responsibility of the primary researcher, under the supervision of the medical doctor. While the intravenous iron intervention was managed by the medical doctor with the assistance of the primary researcher and research team. Collation of research results and data capturing was the responsibility of the primary researcher with assistance from some members of the research team. For standardization purposes, training was undertaken before the start of the project.

3.1.5.1 Research team

Primary researcher, post-graduate student	LF Mogongoa
Promoter, medical scientist	Prof SS Mashele
Collaborator, haematology pathologist	Dr AD Jafta
Field worker, student medical technologist	MV Raphuthing
Field worker, student medical technologist	LB Shashane
B Tech student	STM Ncoko
B Tech student	KD Mogakabe
Final year undergraduate student	MM Segalo

3.2 MATERIALS

A list and description of mostly specialised materials utilised to achieve the aim of the study are presented in the sections below. It is composed of intervention study medication, standards, controls, specialised equipment and consumables materials utilised for laboratory analysis. This is by no means an exhaustive list of all materials that were needed.

3.2.1 Iron therapy medication

Ferrimed® D.S. Chewable Tablets and Ferrimed® Capsules manufactured by Takeda, on behalf of Vifor, were utilised for oral iron therapy. The chewable tablet contained 100 mg elementary iron as iron (III)-hydroxide polymaltose complex. Contrary to the chewable tablet, each capsule was composed of 50 mg elementary iron as iron (III)-hydroxide polymaltose complex plus 150 µg folic acid. The reason for product selection was because it had fewer side-effects and palatable taste, thus decreasing the odds of non-compliance or drop-out.

The oral iron was administered at a concentration of between 100 and 200 mg elementary iron a day. At baseline, all participants consumed 1 chewable tablet a day. At week 4, 1 capsule was added to the regime – resulting in a total of 150 mg elementary iron. At week 7, second capsules were added to the regime giving a total of 200 mg elementary iron. Therapy of IDA is recommended at 100 to 200 mg of elementary iron (Jimenez *et al.* 2015). Owing to side effects of oral therapy being dose-dependent, plus it

can result in noncompliance in 50% of patients (Tolkien *et al.* 2015). Therefore, the first 4 weeks were also deemed as a run in phase to acclimatise participants in taking study medication and to manage the side effects that may have been encountered secondary to medication. The total elementary dose for ten weeks as 10.15 g while 14 weeks was 15.75 g.

Intravenous iron preparation utilised was Cosmofer® by Pharmaplan (Pty) Ltd. Each millilitre contained iron (III)-hydroxide low molecular weight dextran complex equivalent to 50 mg iron (III). At each IV administration visit, 200 mg was administered. The pathologist decided that she is comfortable with that form of therapy as the originally suggested single dose was not available or licenced in the country at the time of the study. Furthermore, a study by Chertow *et al.* (2006) did not find a statistically significant difference for ADE between Cosmofer® and the originally suggested sodium ferric gluconate complex or iron sucrose. However, the ADEs were higher for Cosmofer® than the other two preparations. For prevention of anaphylaxis, a major side effect of IV therapy, another drug, Phenergran®, by Sanofi-Aventis was utilised. The injectable form was employed, it contained 25 mg per ml Promethazine HCl. A single dose was administered per IV therapy visit, before IV iron administration.

3.2.2 Apparatus

The apparatus operated during the laboratory procedures were in good working condition and calibrated to give accurate results (Table 3.1). ABX Pentra 60 was employed for full blood count analysis, while the Siemens Dimension was used for clinical chemistry analysis and bleeding abnormality screen was performed on the PFA-200. These three instruments were available at the CUT laboratories.

TABLE 3.1 The apparatus utilised during the study procedures

Apparatus	Description	Brand name	Supplier
Haemostasis analyser	Platelet function	PFA-200	Siemens
Blood cell counter	Full blood count	ABX Pentra 60	The Scientific Group
Chemical analyzer	Clinical chemistry	Dimension	Siemens
Microplate reader	EL 312e	Biotek	Biotek instruments
Personal electronic body stat scale	Model 3201	Elektra ™ Care	S & P Africa

3.2.3 Standards and controls

All the automated instruments were calibrated and quality control specimens were run to ensure the accuracy of laboratory results. This was performed according to accepted good laboratory practice. All ELISA and automated analysers' test runs were accompanied by all quality control levels that were provided with the kit. For standardised automated instrument tests, calibrators and quality control materials (Table 3.2) were obtained from the instrument supplier, while ELISA kits were obtained for occult blood analysis.

TABLE 3.2 Standards and controls used during variables' determinations

Standard or control	Catalogue no	Supplier
FBC control#	PX406	The Scientific Group
MAS® ChemTRAK L	CHA-1	Thermo SCIENTIFIC
MAS® ChemTRAK N	CHA-2	Thermo SCIENTIFIC
MAS® ChemTRAK H	CHA-3	Thermo SCIENTIFIC
Liquimmune® L	LIA16031	Thermo SCIENTIFIC
Liquimmune® N	LIA16032	Thermo SCIENTIFIC
Liquimmune® H	LIA16033	Thermo SCIENTIFIC
HCG CAL	RC430	Siemens
ALPI CAL	DC150	Siemens
IRON CAL	DC85	Siemens
CHEM I CAL	DC188	Siemens
ENZ II CAL	DC143	Siemens
Enzyme Verifier	DC19	Siemens
FERR CAL	RC440	Siemens
Special Protein CAL	DC51	Siemens
ICBT CAL	DC84	Siemens
CRP CAL	DC34	Siemens
CHEM II CAL	DC20	Siemens
TP/ALB CAL	DC31	Siemens
Standard A Quicklyte	S625	Siemens
Standard B Quicklyte	S630	Siemens

3.3 METHODS

Methods that are briefly described in this section were the ones that were exploited to address the stated study objectives. These include among others, a measure of compliance for oral iron, specimen collection and preparation. Also indicated are questionnaires used for evaluation of geophagic practices and menstrual information acquisition. Finally, the laboratory tests employed to assess gastrointestinal bleeding, general health indicators and changes associated with the efficacy of iron therapy in the human body.

3.3.1 Oral study medication measure of compliance

Compliance could not be guaranteed, therefore two methods were utilised to assess it; pill count and questionnaire. The data from both methods were combined to obtain a close to an accurate estimate of compliance. Compliance was calculated for each participant at all oral iron visitation periods, namely; week 1, 4, 5, 7, and 10. Those participants that continued with oral therapy had week 12 and 14 as well.

For pill count, participants were given a different number of pills and they were required to present the excess number at subsequent visit for a count. In so doing, an indication of compliance was 'semi'-obtained if the pills added up to the count that was issued at the beginning of the treatment period under review (Bregman *et al.* 2013).

For the questionnaire method of assessing compliance (Khalafallah *et al.* 2010), data was captured on the side effects questionnaire. The participants were asked to indicate if they have missed doses and the research team emphasised that missing a dose did not disqualify the participant but would assist the team in understanding anomalous results. Over time a good trust relationship was built with the participants and they became truthful about all aspects of the study.

3.3.2 The measure of compliance for abstaining from soil consumption

If a geophagic participant was supposed to abstain from the consumption of soil and indicated that she did not, she was transferred to the group that was not supposed to abstain. It was difficult to ensure compliance with non-consumption of soil, thus this depended on the integrity of the participants and the reassurance to participants that would not be obligated to leave the study. Those participants that consumed more than 5% of what they normally consumed were moved to the other group. The amount of soil consumed was calculated from the declared recollection of participants as captured on the side effects questionnaire.

3.3.3 Specimen collection

The samples were collected by qualified medical technologists experienced in phlebotomy utilizing the standard protocol for median cephalic or basilic vein (McNamara *et al*. 2017). Five millilitres ethylenediamine tetra-acetic acid (EDTA) blood was obtained at each visit, on which full blood count analysis was performed. While 10ml clotted blood was utilised for clinical chemistry analyses namely; beta HCG, serum liver enzyme tests, iron studies, C reactive protein (CRP), selected minerals, urea and electrolytes. Finally, five millilitres tri-sodium citrate was obtained to screen for bleeding abnormalities using Platelet Function Analyser 200 (PFA). Faecal specimens were obtained for occult blood analysis, at week one and either week 12 or 14 of the intervention study period.

3.3.4 Blood specimen preparation

The anticoagulant free specimens were centrifuged at 1 000 g for 8 minutes to separate the serum, within four hours of collection. Serum was split into three separate containers and stored at –70 °C for those clinical chemistry analyses that were not performed immediately. The serum was thawed once and utilised for clinical chemistry analyses.

3.3.5 Geophagic practices

Geophagic practices were determined by a questionnaire which was developed during an earlier cross-sectional study; where bibliographic, anthropic, soil consumption habits, knowledge and attitudes about geophagia were obtained. Data from this questionnaire was utilised to link the type of soil, duration of consumption and quantity of soil with the different markers of iron deficiency anaemia. The type of soil consumed seems to play a role in whether soil supplements or removes dietary iron as presented by the contrasting results of *in vitro* studies. The duration of consumption and quantity of soil might have had an effect on the degree of iron deficiency anaemia.

3.3.6 Bleeding abnormality assessments and screenings

A major cause of IDA is bleeding; thus it was imperative that the contribution of bleeding to IDA be assessed. Most common sites of blood loss are the female genital tract attributable to the menstrual cycle and the

gastrointestinal tract. For the menstrual cycle, a pictogram and menstrual assessment questionnaire were employed, while occult blood was utilised to assess GIT bleeding. In addition, screening tests for bleeding abnormalities were also included.

3.3.6.1 Menstrual blood flow assessment

Menstrual blood loss is a major contributor to the development of IDA, that is why females are prone to IDA than males. Pictogram (Ismail *et al.*, 2004) and questionnaire (Warner *et al.*, 2004) methods were used to estimate the amount of blood that was lost through menses. The pictogram was utilised because it removed subjectivity that is associated with questionnaires. However, the questionnaire offered more in terms of menstrual cycle history. Three pictograms were given during the three months of the study, while the questionnaire was administered once. The most accurate method, that involves sanitary pads weighing, was not employed as the research participants would have to store the sanitary pads until the next visit and they were not comfortable with providing their sanitary pads.

3.3.6.2 Occult blood analysis

GIT bleeding is another major contributor to the development of IDA since it can carry on without detection. The only time it would be detected may be due to a fact-finding mission for an IDA cause. The methods that have

been previously employed required a change in diet before testing, due to possible interferences by vitamin C and meat (Thomas *et al.* 1992), so that accurate test results may be obtained. The change in diet would have been a great inconvenience to the participants, thus another method was sought. An immunological method that was utilised did not require a change in diet. The ELISA kits utilised for analysis, RIDASCREEN® Haemoglobin (catalogue number: G09030) and RIDASCREEN® Haemo- / Haptoglobin Complex (catalogue number: G09031) were sourced from R-Biopharm AG. Occult blood analysis was performed at different time intervals of the oral and intravenous iron therapy namely; week 1 and 12 or 14.

3.3.6.3 Screening for bleeding abnormalities

Bleeding abnormalities were screened for using PFA-200 with a method as described in Luddington (2005). The value of this screen was to determine if participants have an increased propensity to bleeding, which may be the cause of IDA. Obtaining a holistic picture of bleeding was a priority to give a new dimension to the study of geophagia.

PFA-200 analysis was not performed on all participants as phlebotomy for these test was performed during the participants' menstrual cycle. This procedure was followed because the blood was also utilised for von Willebrand disease screening that is more sensitive during the menstrual cycle. Most participants did not inform the research team when they had

their monthly period, this decreased the number of specimens that could be obtained. PFA-200 was performed at CUT utilizing Dade® PFA Collagen/EPI (catalogue number: B4170-20) and Dade® PFA Collagen/ADP (catalogue number: B4170-21) consumables from Siemens.

3.3.7 Laboratory analyses

To correctly identify IDA, both full blood count and iron studies were required. On the other hand, inflammatory indicators were needed for differential diagnosis. Other tests that were included in this section were those that assessed the general health of the research participants.

3.3.7.1 Full blood count analysis

Full blood count was performed at all visits, within four hours of blood collection; using the ABX Pentra 60. The instrument employs current impedance changes; spectrophotometry; double hydrodynamic sleeving coupled with cytochemistry, and measuring of transmitted light; to measure the different parameters of the full blood count (Nakamine 2004). Red blood cell parameters were employed to assess the type of anaemia, while the platelet count was utilised for indirect bleeding assessment and white blood cell parameters gave an indication of the immune system status.

3.3.7.2 Clinical chemistry analysis

All clinical chemistry analyses were performed using the Siemens Dimension auto analyser, utilising methods listed in **Table 3.3**. The principles of the different tests that were undertaken ranged from spectrophotometry, turbidity, nephelometry and ion-selective electrodes (Wu 2005). The iron studies, beta HCG and CRP analysis were performed at all visits, while liver enzyme tests, selected minerals, albumin, blood urea nitrogen and serum creatinine were performed at the beginning and end of each treatment cycle (baseline, week 10 & 14).

TABLE 3.3 Methods utilised for clinical chemistry testing

Measured variable	Catalogue no	Test grouping	Principle
B HCG	RC430	Pregnancy testing	CMIA
Iron	DF85	Iron studies	Ferene
Ferritin	RF440	Iron studies	Immunoturbimetric
Transferrin	DF103	Iron studies	Turbimetric
ICBT	DF84	Iron studies	Ferene, Direct
Transferrin saturation	N/A	Iron studies	Calculation
CRP	DF34	Inflammation screen	
ALP	DF150	Liver enzyme test	PNP 37^{O}C
AST	DF41A	Liver enzyme test	IFCC/Standard
ALT	DF143	Liver enzyme test	IFCC/Standard
Total protein	DF43	General liver health	Modified Buret
Albumin	DF13	General liver health	BCP
BUN	DF21	Kidney function	Urease/GLD
CRE	DF33B	Kidney function	Modified Jaffe
PHOS	DF61A	Mineral	Phosphomolybdate
CA	DF23A	Mineral	o-Cresolphalein Complexone
MG	DF57	Mineral	Methylthymol Blue
CL	S600	Electrolytes	IMT Indirect
Sodium	S600	Electrolytes	IMT Indirect
Potassium	S600	Electrolytes	IMT Indirect

The iron studies were performed to evaluate the efficacy of the two iron therapy modes. HCG was performed to exclude pregnancy, that can affect the response to therapy, plus treatment can pose a danger to the foetus. CRP was performed to exclude inflammation that may cause an increase in ferritin level. The rest of the clinical chemistry analyses were performed to assess the general health status of participants and to observe the effects on geophagia on minerals.

3.4 STATISTICAL ANALYSES

The statistical analyses were performed by the Department of Information Technology, Central University of Technology, using the SPSS® programme. Data were summarised as means and standard deviation for each measured variable, with a normal distribution. Normality was ascertained by performing the Shapiro-Wilk test and combined with histogram evaluation. In addition, Levene's test for equality of variances was also undertaken to ascertain the *p*-value that should be reported. Paired sample T-test analysis was utilised to compare two study periods of the same group. Independent T-test was employed when comparing different groups. The differences among the data sets within a treatment (baseline, short-term, intermediate-term and end of treatment) group were analysed by repeated measures analysis of variance (ANOVA). To ascertain sphericity, the Mauchly's test was performed. If the sphericity assumption was not met, then a Greenhouse-Geisser correction was determined.

However, data from the variables that did not produce a Gaussian distribution were summarised as median and interquartile range. The difference among follow-up time periods and the different treatment groups were compared utilizing the Friedman test and Wilcoxon signed ranked test, respectively. The post hoc test employed for the Friedman test was the paired Wilcoxon signed ranked test.

Post hoc test analysis was undertaken to determine which group differed significantly. For both parametric and non-parametric analysis, a Bonferroni adjustment was undertaken where p <0.05 was divided by the number of different comparisons undertaken. Thus for comparison of four (oral iron therapy) or three (IV iron therapy) different time periods, the adjusted significant level was p <0.0083 (0.05/6) or p <0.0167 (0.05/3), respectively.

CHAPTER 4

RESULTS

4.1 INTRODUCTION

The methods described in the preceding chapter were exploited to interrogate the study aim; which was to assess the effectiveness of different iron therapies in geophagia. Geophagic practices of participants were considered to illuminate their contribution to IDA development. The bleeding assessment was also undertaken to assess its contribution because bleeding is a major cause of iron deficiency anaemia. Laboratory investigations were undertaken to assess the health of participants and more importantly to monitor response to therapy. Physical and laboratory results were divided into three sections: (1) anthropometric (age, weight, height, waist, hip & bust), body composition (percentage of fat, muscle & water) and general health (pulse & blood pressure) variables. (2) The red blood cell, platelet and white blood cell parameters, including an eosinophil count, of a full blood count, were utilised to ascertain the type of anaemia, link with bleeding and for a general assessment of immune status, plus parasitic infestation, respectively. (3) Clinical chemistry general health (kidney, liver and mineral variables); iron study for monitoring treatment response; inflammatory and pregnancy assessments were also undertaken.

The geophagic practices and bleeding assessments of the participants were presented first. Subsequently, the baseline results were presented followed by the intervention study. The intervention study results were divided into; oral iron therapy, IV iron therapy, comparison of oral and IV iron therapy. The results of each of the subsections were evaluated based on three sections, namely; the entire study group, the group that abstained from soil consumption (Group A) and the group that continued with soil consumption (Group B) throughout the study.

4.2 GEOPHAGIC PRACTICE RESULTS

The results were summarised on the bases of years of practice, the amount of soil consumed and colour of soil consumed as described by participants. These parameters were selected for their possible effect of GIT damage due to prolonged exposure, repeated exposure and colour which can reflect the soil content that may reduce iron bioavailability, respectively. Presented in Table 4.1 are the median, interquartile range and population range for years of consumption and the amount of soil consumed per day. Followed by a presentation of the frequency of consumption and colours that were consumed, as illustrated in Figure 4.1 and 4.2, respectively.

The participants consumed a substantial amount of soil per day and the majority has been involved in the practice for many years. The years of soil consumption ranged from 0.66 years until 30 years with a median of 8 years. No significant difference was found for years of consumption between the

groups that abstained and continued with soil consumption. Half of the participants consumed more than 250 g of soil, with half of these consuming greater than 500 g per day, as depicted in Table 4.1. Although group B seemed to consume less soil and have fewer years of consumption than the abstain group, there was no statistically significant difference between the two groups.

Table 4.1 Years of soil consumption and amount of soil consumed per day

	Group	Median	IQR	Min-max	*p* value
Years of soil consumption	Entire	8	4-17	0.67-30	
	Abstain	9	5-18	1-30	0.1547
	Continue	7	3-12	0.67-27	
Amount consumed per day (gm)	Entire	250	117-500	6-1500	
	Abstain	250	131-500	25-1500	0.064
	Continue	161	69-339	6-1250	

gm = gram; IQR = interquartile range; max = maximum; min = minimum

Furthermore, there was no direct correlation between the amount of soil and years of consumption versus red blood cell parameters (Hb, RBC, HCT, red blood cell indices) and iron study parameters.

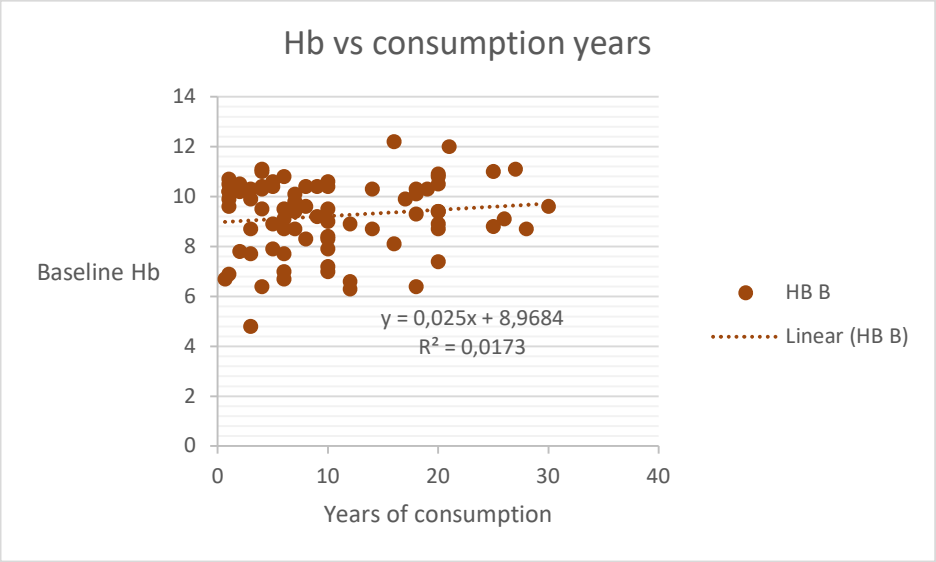


Figure 4.1 Correlation of years of soil consumption and haemoglobin level

Only Hb (Figure 4.1) and ferritin (Figure 4.2) correlation graphic presentations are captured, the other parameters' correlation data are not presented because they were non-significant.

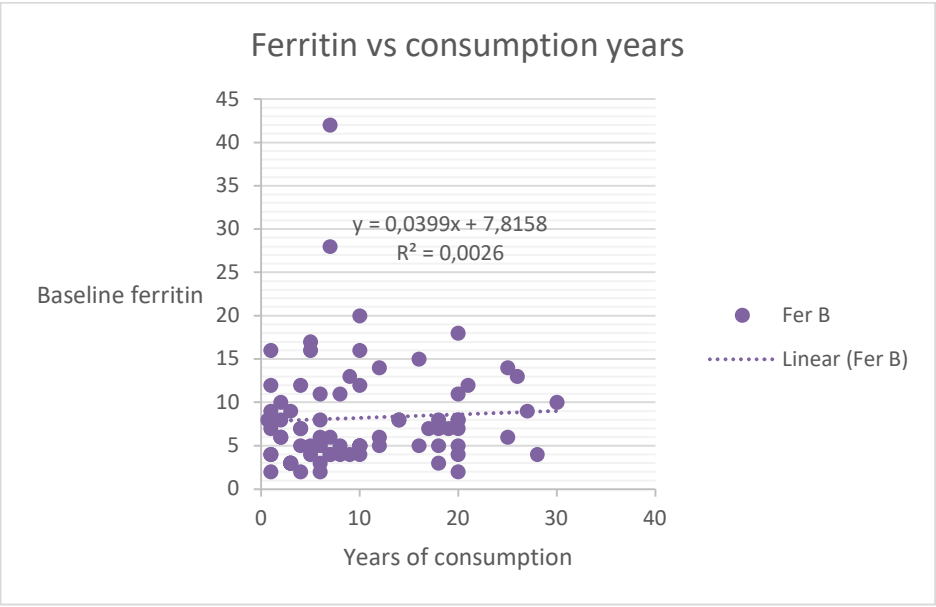


Figure 4.2 Correlation of years of soil consumption and ferritin concentration

Besides the years and quantity of soil consumed, the frequency revealed that more than 93% (75/81) of participants in the entire, 91% (39/43) in abstain group and 95% (36/38) of continue group consumed soil daily. Based on more than once daily and once per day consumption of soil, the entire population had 77% (62/81) and 16% (13/81); abstain group had 79% (34/43) and 12% (5/43), and continue group had 74% (28/38) and 21% (8/38) participants, respectively. Figure 4.3 gave a presentation of the comparison of group A and B which are represented by the outer and inner rings, respectively.

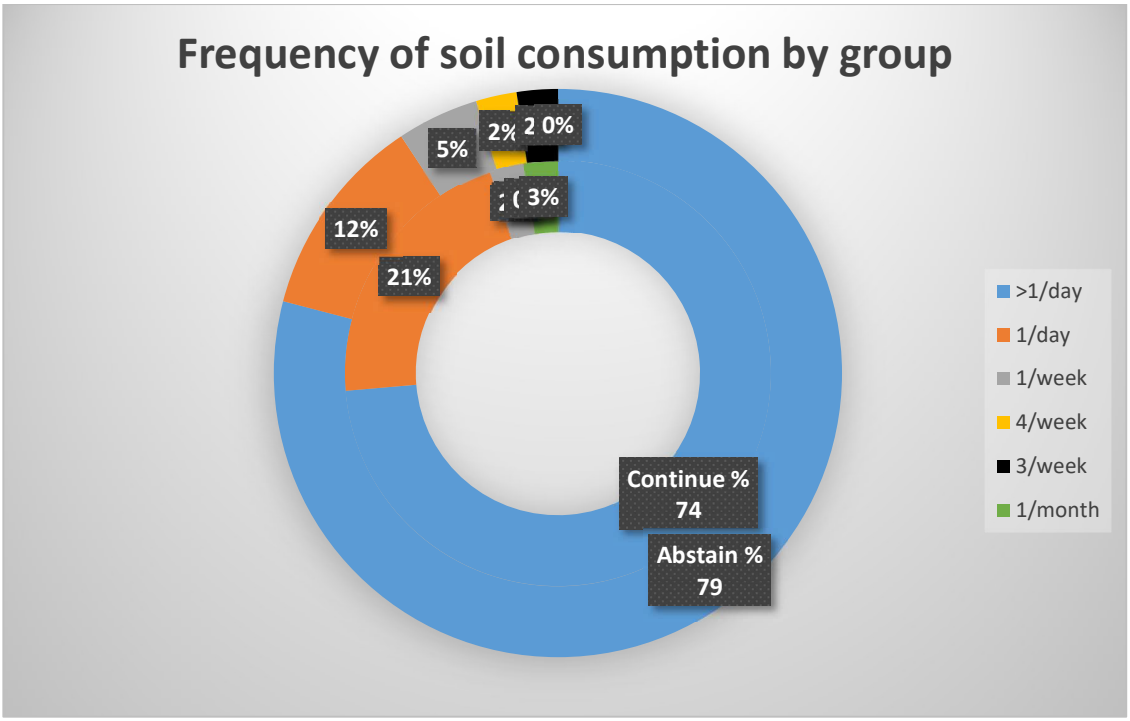


Figure 4.3 Frequency of soil consumption of group A versus B

The most prevalent soil colour was black with 57% (33/58) and 44% (29/66) of participants in group A and B, respectively. The next most prevalent soil colours

were khaki and red with 10%(6/58) each for group A, contrary to khaki at 17% (11/66) and yellow at 15% (10/66) for group B. 67% (29/43) consumed one type and 30% (13/43) consumed two types of soil in group A. While 63% (24/38) and 29% (11/38) was observed for group B.

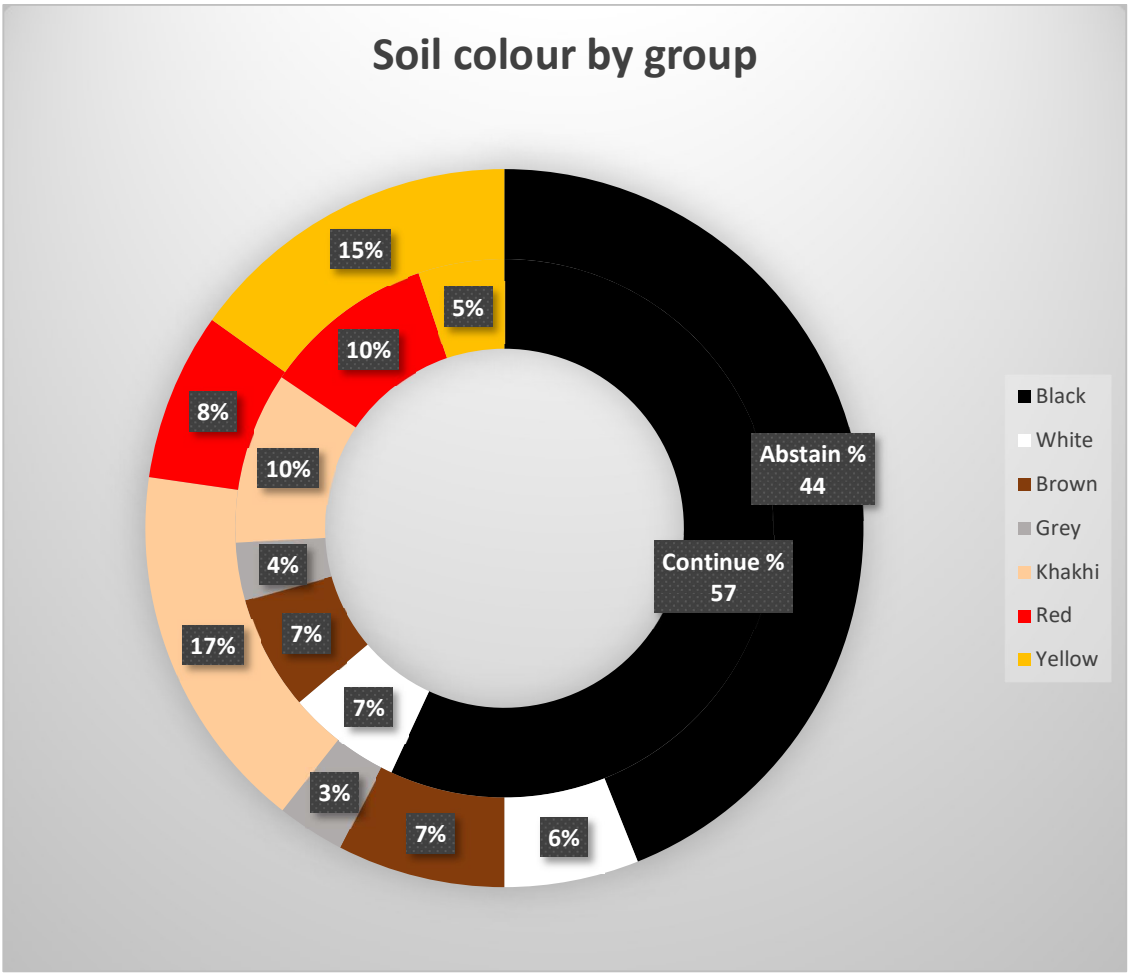


Figure 4.4 Soil colour consumed by group A versus B, as described by participants

4.3 BLEEDING ASSESSMENT RESULTS

Bleeding is a cardinal cause of iron deficiency anaemia thus different assessments were undertaken to ascertain the contribution of bleeding to IDA development in geophagic participants. Platelet function analysis was embarked on to confirm that there was no problem with the platelets. In addition to it, menstrual and gastrointestinal bleeding was evaluated utilising pictogram and occult blood analysis, respectively.

Other undertaken bleeding assessments were thromboelastography (TEG) that screens the entire haemostatic process for abnormalities, plus screening for von Willebrand's disease (vWD). The requirement for vWD screening was that the specimen should be obtained during the menstrual period because testing is most sensitive at that time. TEG and vWD screenings were performed at another site, thus it was decided that when participants were on their periods they should make contact, to enable phlebotomy. Few participants called, therefore the available results are insufficient to represent the study population. Therefore, TEG and vWD results were not presented in this section.

4.3.1 Platelet function analysis result

Of the 20 PFA collected samples 65% (13/20) were from group A and 35% (7/20) from group B. Only one participant (5%) from group A had a positive platelet function abnormality.

4.3.2 Menstrual bleeding assessment

The results presented here are a summary of 54 participants, 29 from group A and 25 from group B. Some participants submitted more than one pictogram (26) and their results were averaged before incorporation into the analysis. The median coefficient of variation of those with more than one tool was 19.5% with IQR of 12%-37.7% with a range of 5.2%-87% (n=26). The other participants that did not submit their tools were mostly on contraceptives, especially Depo-Provera, which caused most of the participants not to have menstrual periods at all. A small proportion of participants were post-menopausal.

The median blood loss per menstrual cycle was 51 ml with IQR of 33.8 ml-81.8 ml and a range of 2 ml-337 ml. There was no statistically significant difference between the groups (*p* 0.781); with a median of 52 ml vs 43 ml with IQR of 37 ml-84 ml vs 25 ml-78.5 ml and a range of 2 ml-161.5 ml vs 5.5 ml-337 ml, respectively.

The average menstrual blood loss amount was not directly correlated with baseline red blood cell parameters. Menstrual blood loss was not correlated with the baseline haemoglobin concentration because of a horizontal line of best fit and R^2 of less than 0.7, as depicted in Figure 4.5. The other red blood cell parameters' correlation data are not presented because they were non-significant.

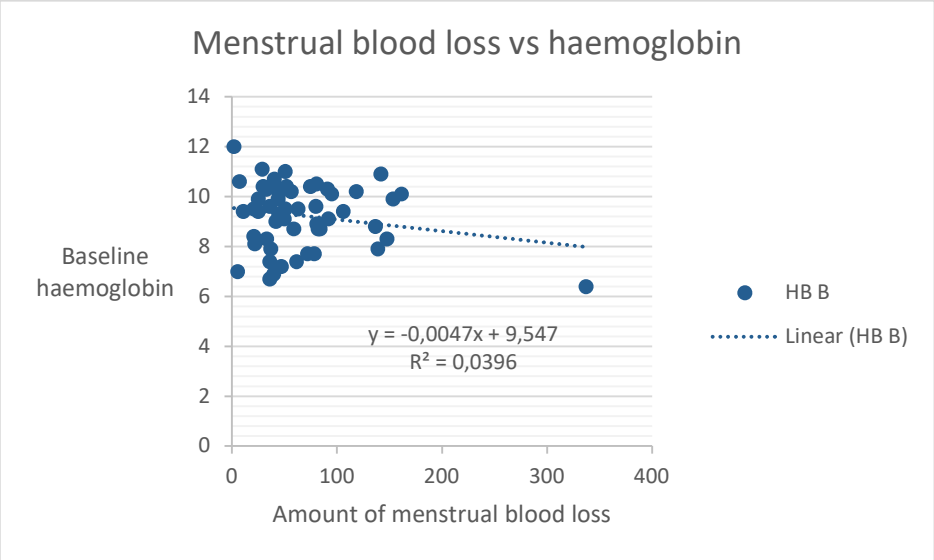


Figure 4.5 Correlation of menstrual blood loss and baseline haemoglobin of the entire study group

Similar to the red blood cell parameters, the baseline iron study parameters were not directly correlated with the amount of menstrual blood loss. Shown in Figure 4.6 is the correlation with ferritin concentration while the other parameters' correlation data are not presented.

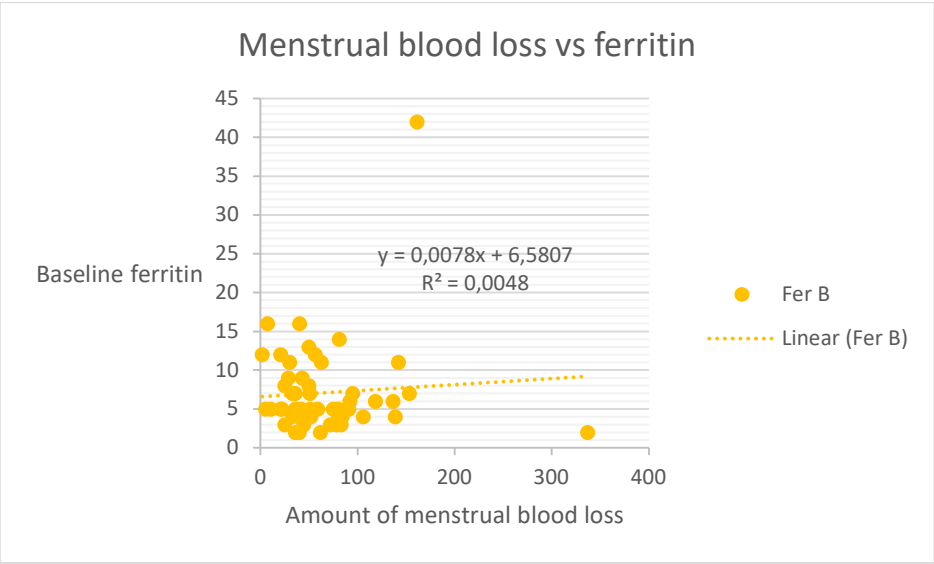


Figure 4.6 Correlation of menstrual blood loss and baseline ferritin concentration of the entire study group

4.3.3 Gastrointestinal bleeding assessment

Of the 32 collected stool samples, 44% (15/32) were from group A and 54% (17/32) from group B. Only three participants (9%) had two samples that were obtained at the beginning and the end of the study. These three repeat samples were negative for both periods. Seven participants samples were positive (22%) for occult blood, with three from group A (3/15 = 20%) and four were from group B (4/17 = 24%).

4.4 BASELINE STUDY RESULTS

The baseline results were presented to provide an overview of the study population and the two groups were compared to evaluate the effectiveness of randomisation. Presented first were the anthropometric, body composition and general health profile; followed by the full blood count parameters; finally ending with general health profile based on clinical chemistry and iron studies results. The baseline comparison of study groups and the intervention study results were also presented employing the above-mentioned sub-division.

Of the 320 people screened, a total of 84 participants met the inclusion criteria and participated in the intervention study, as illustrated in Figure 4.5. Randomization resulted in a 45:39 split for Group A: Group B. The participants were exposed to oral and IV iron therapy while being followed up at different time intervals. In total eight participants were lost to follow-up throughout the study, two from Group A and six from Group B.

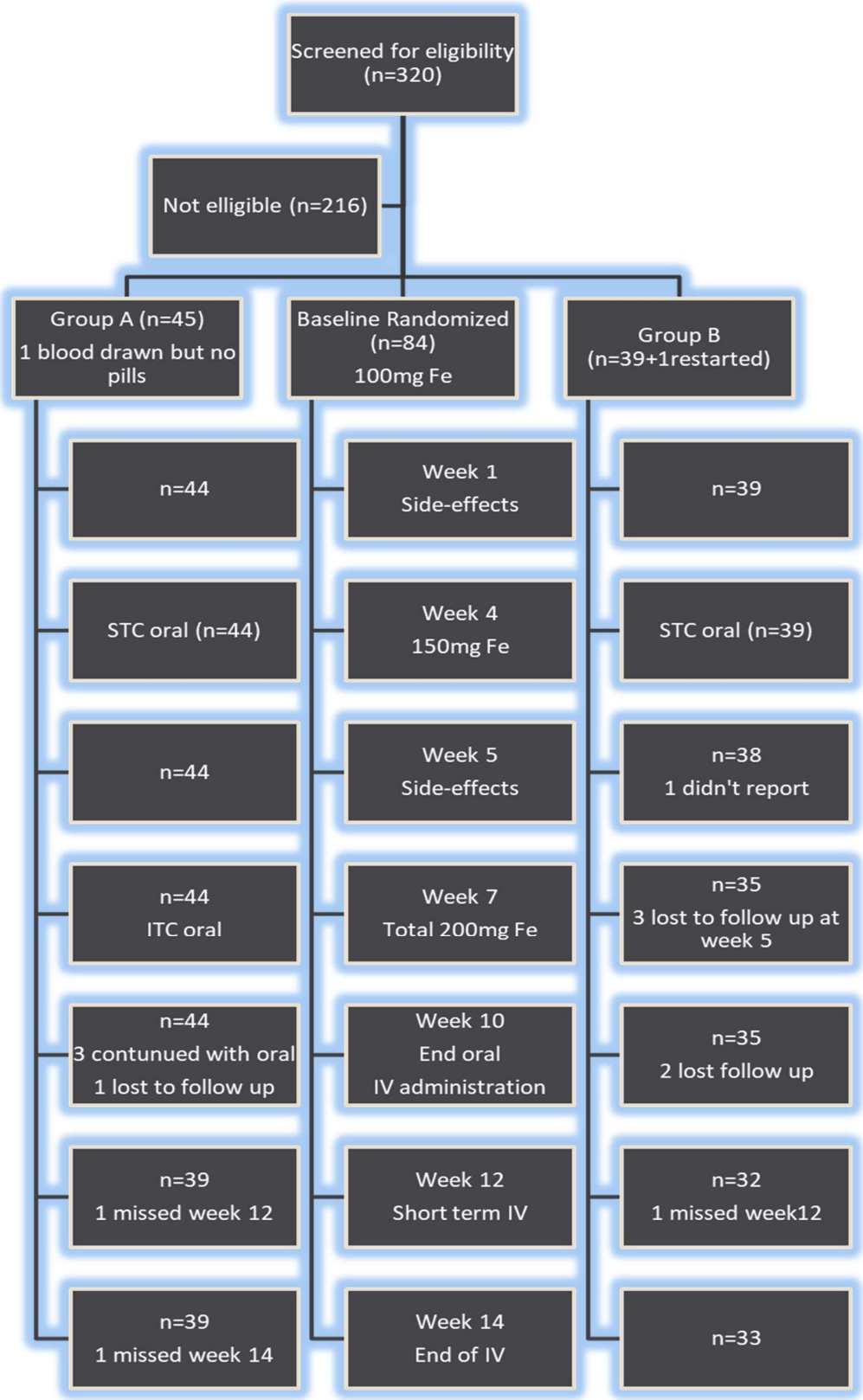


Figure 4.7 Flow chart of different stages of the intervention study

Three participants from the abstain group continued with oral iron for the entire 14 weeks of the study, because they responded to oral iron and/or did not consent to IV. In should be noted that the majority in both groups continued to IV iron. The results of those participants that continued with oral were excluded from the IV results analysis.

4.4.1 General health profile of the entire study group

The mean age was 32 years with a range of 15 – 51 years, with 25% of participants being below 24 years and 25% being above 40 years, as shown by the interquartile range in Table 4.2. The age criterion was met, except for the 15-year-old who was underage. Mean body mass index (BMI) was just above the reference range; this indicated that approximately half of the participants were overweight (25 – 29.9 kg/m^2). Less than 16% of participants were considered underweight (<18.5 kg/m^2) based on the minimum (17.3 kg/m^2) and mean minus SD (19.33 kg/m^2). Whereas just shy of 25% fell in the obese category (>30 kg/m^2) based on the 75^{th} percentile (29.5 kg/m^2). This data was supported by the waist-hip ratio (WHR), fat and muscle body percentage which showed similar trends. WHR of more than 50% fell within the moderate risk category (0.81 – 0.85) based on the mean of 0.83. Furthermore, more than 25% were within the high-risk category (>0.85) based on the 75^{th} percentile of 0.88.

Table 4.2 illustrates that the mean fat % was in the high range (23 – 32 %) while 75th percentile was within the over range (33 – 38 %). This indicated that greater than 50% and 25% of participants have increased and excessive body fat percentage, respectively. The 75th percentile (32.8 %) and mean plus SD (33.6 %) of muscle % were below the ideal range (>34 %), signifying that just below 16% participants had recommended muscle mass.

The blood pressure measurements of approximately 25% of participants were above the reference range, as witnessed by the 75th percentile of both systolic (142.3 mmHg) and diastolic (89.3 mmHg) measurements. Furthermore, less than 16% of participants had systolic and diastolic blood pressure of more 156 mmHg and 104 mmHg based on mean plus SD, respectively.

Table 4.2 Baseline anthropometric, body composition and general health indicators of the entire study population

VARIABLE		Descriptive statistics study results					
	RR	N	X / Med	SD	Min	IQR	Max
Age (years)		85	32.3 / 36.0	9.3	15.0	24.0-40.0	51.0
Height (m)		84	1.61 / 1.62	0.06	1.46	1.57-1.65	1.77
Weight (kg)		84	65.5 / 60.7	15.5	42.4	53.5-76.7	109.1
BMI (kg/m^2)	18.5-24.9	84	25.4 / 24.2	6.1	17.3	20.3-29.5	44.3
Fat %	21-26	84	29.2 / 28.2	7.3	18.8	23.1-35.0	49.4
Water %	42-57	84	47.7 / 48.0	6.3	30.4	42.8-52.6	56.8
Muscle %	>34%	84	29.8 / 30.0	3.8	20	26.8-32.8	35.5
Cal. req./day		84	2176 / 2177	232	1529	1998-2340	2590
Waist (cm)	<80	82	86.0 / 82.0	14.5	61.0	75.0-99.0	128.0
Hip (cm)		82	104.0 / 102.0	12.0	85.0	95.3-112.0	139.0
WHR	≤ 0.80	82	0.83 / 0.83	0.07	0.69	0.76-0.88	1.11
Bust (cm)		82	93.1 / 88.0	13.7	74.0	82.5-103.5	134.0
SBP (mmHg)	125±13.6[a]	84	131.4 / 122.0	24.9	82.0	116.0-142.3	205.0
DBP (mmHg)	78±9.9 [a]	84	85.0 / 84.0	18.7	55.0	71.8-89.3	144.0
Pulse (b/min)	60-100	76	79.6 / 80.5	11.0	55.0	72.0-89.0	111.0

(b/min = beats per minute; BMI = body mass index; Cal. req./day = calories required per day; DBP = diastolic blood pressure; IQR = interquartile range; Max = maximum; Med = median; Min = minimum; n = sample population; RR = reference range; SBP = systolic blood pressure; SD = standard deviation;WHR = waist:hip ratio; X = mean)

4.4.2 Full blood count profile of the entire study group

Greater than 75% of participants presented with hypochromic microcytic anaemia based on the 75th percentile of MCH, MCV and Hb, as expressed in Table 4.3. Hb 75th percentile (10.3 g/dL) also attested that more than 75% of participants officially qualified for the study according to the 10.5 g/dl or less haemoglobin level criteria. Strongly supported by red blood cell indices

means, MCH (23.4 pg) and MCV (72.7 fl), which were both below their respective reference ranges. Contrary to the median and mean of MCHC which were within the reference range. A decreased red blood cell count and haematocrit could be found in 25% and more than 95% of participants, respectively. This was based on 25th percentile (3.77 x 10^{12} /L) and means plus 2SD (36.7%). Moreover, it should be noted that more than 84% of participants had increased RDW (>14%) based on mean minus SD (15.2%) and sturdily supported by 25% percentile (16.1%).

Table 4.3 Baseline blood cell counts of the entire study population

VARIABLE		Descriptive statistics study results					
	RR	n	X / Med	SD	Min	IQR	Max
RBC (x10^{12} /L)	3.8-4.8 [a]	85	4.00 / 4.02	0.42	3.13	3.77-4.25	5.13
Hb (g/dL)	12.0-15.0 [a]	85	9.2 / 9.5	1.5	4.8	8.4-10.3	12.2
HCT (%)	36-46 [a]	85	29.1 / 29.2	3.8	17.0	26.9-31.4	37.3
MCV (fl)	83-101 [a]	85	72.9 / 74.0	8.6	52.0	67.0-79.0	93.3
MCH (pg)	27.0-32.0 [a]	85	23.2 / 23.5	3.5	14.4	20.7-26.2	30.0
MCHC (g/dl)	31.5-34.5 [a]	85	31.8 / 32.1	1.7	26.0	30.9-33.0	34.3
RDW (%)	11.6-14.0 [a]	85	17.7 / 17.5	2.5	12.7	16.1-19.2	28.9
PLT (x10^{9} /L)	150-410 [a]	85	344 / 332	79	166	298-382	590
WBC (x10^{9} /L)	4.0-10.0 [a]	85	5.16 / 4.30	1.51	2.50	3.93-6.20	9.30
Neutrophil [#]	2.0-7.0 [a]	85	2.35 / 2.16	0.92	0.60	1.69-2.92	4.87
Lymphocyte [#]	1.0-3.0 [a]	85	2.32 / 2.16	0.77	0.73	1.74-2.83	4.24
Monocyte [#]	0.2-1.0 [a]	85	0.31 / 0.26	0.15	0.06	0.21-0.37	0.72
Eosinophil [#]	0.02-0.5 [a]	85	0.12 / 0.06	0.17	0.01	0.05-0.12	1.19
Basophil [#]	0.02-0.1 [a]	85	0.05 / 0.03	0.09	0.01	0.02-0.05	0.78

(RR = reference range; n = sample population; X = mean; Med = median; SD = standard deviation; Min = minimum; IQR = interquartile range; Max = maximum; RBC = red blood cell; Hb = haemoglobin; HCT = haematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; RDW = red cell distribution width; PLT = platelet; WBC =white blood cell; [#] = x10^{9} /L; [a] = Bates (2017).

Platelet count was normal in between 75% and 84% of participants, based on 75th percentile (382 x 10^9 /L) and means plus SD (423 x 10^9 /L). It must be noted that all the white blood cell variables' means, medians, IQR, minimums and maximums were within their respective reference ranges. The exception was observed with the maximums of basophil and eosinophil which were above the reference range, as presented in Table 4.3.

4.4.3 Clinical chemistry general health and iron studies profile of the entire study group

Serum creatinine and BUN were utilised to assess kidney function, their means, medians and interquartile range results were within their respective reference ranges, as observed in Table 4.4. Means plus 2SD for both parameters were within range, 15.31 mg/dL and 1.12 mg/dL, signifying that only 2.5% of the study population (which translates to two participants) was above the reference range as depicted by the maximums. Additionally, the minimums of both were below the range while 25th percentile of creatinine was slightly above the lower limit of the reference range, signifying that <25% of participants' results were below the range.

Albumin and liver enzymes tests were embarked on to assess liver function, as depicted in Table 4.4. A similar pattern as kidney function tests were revealed. In addition, the minimums of ALP, AST and ALT were below the range, with the 25th percentile of AST being just below the range. Indicating >25% of participants had low AST level.

Table 4.4 Baseline general health clinical chemistry indicators of the entire study population

VARIABLE		Baseline study results					
	RR	n	X / Med	SD	Min	IQR	Max
BUN (mg/dL)	6-18 [b]	84	9.67 / 9.19	2.72	4.33	7.93-11.25	19.29
S Creat. (mg/dl)	0.6-1.2 [b]	74	0.76 / 0.73	0.18	0.51	0.62-0.86	1.32
Albumin (g/L)	35-50 [b]	83	42.6 / 42	6.1	31	39-46	61
ALP (U/L)	50-120 [b]	74	76.5 / 69.5	27.7	36	55.3-94.5	165
AST (U/L)	20-48 [b]	74	24.3 / 23.0	7.8	8	19-28	47
ALT (U/L)	10-40 [b]	85	15.9 / 15.0	5.9	7	12-19	42
K (mmol/L)	3.5-5.0 [b]	84	3.52 / 3.50	0.52	1.8	3.3-3.83	4.7
Na (mmol/L)	136-142 [b]	84	129.6 / 130.0	3.5	119	128-132	142
Phos (mmol/L)	0.74-1.52 [b]	83	1.18 / 1.14	0.23	0.65	1.04-1.31	1.89
Mg (mg/dL)	1.5 – 2.5 [b]	85	2.21 / 2.20	0.36	1.6	2-2.4	3.5
Ca (mg/dL)	8.2-10.2 [b]	85	9.44 / 9.30	1.04	7.2	8.9-10	12.5
Zinc (umol/L)	7.7-23.0 [b]	83	13.47 / 12.40	4.99	5.9	9.85-16.65	30.9

(RR = reference range; n = sample population; X = mean; Med = median; SD = standard deviation; Min = minimum; IQR = interquartile range; Max = maximum; BUN = blood urea nitrogen; S Creat. = serum creatinine; ALP = alkaline phosphatase; AST = aspartate aminotransferase; ALT = alanine aminotransferase; K = potassium; Na = sodium; Phos = phosphate; Mg = magnesium; Ca = Calcium; **[b] =** Agrawal *et al.* (2014)

Some of the essential minerals followed a similar pattern as kidney and liver function tests, namely: inorganic phosphate, calcium and zinc. It was however noted that the minimums and maximums of phosphate, calcium and zinc were outside their respective reference ranges, as captured in Table 4.4. However, the 75th percentile of magnesium was just below the upper limit of the range while mean plus SD (2.57 mg/dl) was above the range. Signifying that between 16% and 25% of participants had increased levels of magnesium. Divergent to the pattern on above-mentioned minerals was sodium, where the 75th percentile was below the reference range.

However, the mean potassium level was on the lower end of the range. Thus more than 75% and 50% of participants had sodium and potassium levels below the reference range, respectively.

The serum iron, ferritin and TSAT level of way less than 16%, mean plus SD (45.1 µg/dL, 14 µg/dL & 10%, respectively), had concentrations below the reference ranges as captured in Table 4.5. Ferritin level signified that those participants with levels below range met the inclusion criteria of the study which was ferritin <15 µg/L. For TSAT it was further noted that less than 2.5% (mean plus 2SD = 14.2%) were within the reference range. This signifies that only the maximum was within the range because 2.5% of 85 amounts to 1.7.

Table 4.5 Baseline iron study, pregnancy and inflammatory results of the entire study population

VARIABLE		Baseline study results					
	RR	n	X / Med	SD	Min	IQR	Max
S Fe (µg/dL)	56-168 [a]	85	26.1 / 22.0	19.0	9	16 – 27	150
Trans. (g/L)	2.0-3.8 [b]	83	4.21 / 4.03	1.29	1.95	3.48 – 4.72	12.6
Ferritin (µg/L)	15-200 [a]	84	8.0 / 6.5	6.0	4	4 – 10	42
TIBC (µg/L)	250-400 [b]	85	483 / 478	77.6	261	432 – 531	652
TSAT (%)	16-50 [a]	85	5.6 / 4.5	4.3	1.6	3.4 – 6.1	31.4
hCG (IU/L)	<3 [b]	83	0.7 / 0.0	2.9	0.0	0.0 – 1.0	25.0
cRP (mg/L)	0-10 [b]	84	0.58 / 0.50	0.36	0.0	0.38 – 0.6	1.9

(RR = reference range; n = sample population; X = mean; Med = median; SD = standard deviation; Min = minimum; IQR = interquartile range; Max = maximum; S Fe= serum iron; Trans. = transferrin; TIBC = total iron binding capacity; TSAT = transferrin saturation; hCG = human chorionic gonadotropin; cRP = c-reactive protein; **[a] =** Bates (2017) **[b] =** Laposata *et al.* (2014)

More than 50% but less than 75% of participants had increased transferrin levels as shown by mean, median and 25th percentile. Furthermore, the maximum transferrin concentration was 12.6 g/L. Increased levels of TIBC were observed in 86% of participants established by mean minus SD (405 µg/L). The means, medians, IQR, minimums and maximums of HCG and CRP were within the reference range, as seen in Table 4.5. The only exception was the maximum of HCG.

4.4.4 General health profile of both groups for baseline visits

The group that was supposed to abstain from soil consumption was composed of 46 participants (Group A), while the group that continued with soil consumption had 39 participants (Group B). The purpose of the exercise was to compare the two groups in so doing determine the effectiveness of randomization. The descriptive statistics of anthropometric variables, body composition and general health indicators were similar for both groups. In addition, independent samples t-test *p* values of >0.05 were obtained for all variables measured under this heading, as demonstrated in Table 4.6.

Table 4.6 Baseline anthropometric, body composition and general health indicators of the two study groups

VARIABLE	n	Group A	n	Group B	*p*-value
		X ± SD (min-max)		X ± SD (min-max)	
Age (years)	46	32.2 ± 9.1 (15.0-51.0)	39	33.5 ± 9.6 (17.0-51.0)	**0,518**
Height (m)	45	1.61 ± 0.06 (1.46-1.71)	39	1.61 ± 0.06 (1.48-1.77)	**0,950**
Weight (kg)	45	66.0 ± 14.7 (42.4-103.6)	39	64.8 ± 16.5 (44.2-109.1)	**0734**
BMI (kg/m^2)	45	25.6 ± 5.9 (17.5-44.3)	39	25.1 ± 6.3 (17.3-41.6)	**0,732**
Fat %	45	29.4 ± 7.0 (18.8-48.5)	39	28.9 ± 7.7 (19.0-49.4)	**0,746**
Water %	45	47.4 ± 6.1 (31.3-56.8)	39	47.7 ± 6.6 (30.4-56.6)	**0,879**
Muscle %	45	29.7 ± 3.7 (20.0-35.3)	39	29.8 ± 4.01 (20.0-35.5)	**0,791**
Cal. req./day	45	2180 ± 221 (1628-2590)	39	2171 ± 247 (1529-2588)	**0,862**
Waist (cm)	43	86.4 ± 14.1 (61.0-128.0)	39	85.6 ± 15.0 (85.0-139.0)	**0,810**
Hip (cm)	43	104.5 ± 11.3 (88.0-128.0)	39	103.4 ± 28.0 (56.0-144.0)	**0,701**
WHR	43	0.83 ± 0.08 (0.69-1.11)	39	0.82 ± 0.07 (0.69-0.99)	**0,990**
Bust (cm)	43	93.7± 13.4 (74.0-134.0)	39	92.5 ± 14.2 (74.0-134.0)	**0,693**
SBP (mmHg)	45	132.3 ± 22.2 (94.0-201.0)	39	130.4 ± 28.0 (82.0-205.0)	**0,726**
DBP (mmHg)	45	85.0 ± 17.6 (55.0-137.0)	39	85.0 ± 20.2 (56.0-144.0)	**1,000**
Pulse (b/min)	40	78.2 ± 10.2 (55.0-103.0)	36	81.5 ± 11.7 (62.0-111.0)	**0,202**

(n = sample population; X = mean; SD = standard deviation; min = minimum; max = maximum; Cal. req./day = calorie requirement per day SBP = systolic blood pressure; DBP = diastolic blood pressure; BMI = body mass index; WHR = waist:hip ratio; b/min = beats per minute) Group A = abstained from soil consumption, Group B = continued with soil consumption

4.4.5 Full blood count profile of both groups for baseline visits

Table 4.7 Baseline blood cell counts of the two study groups

VARIABLE	n	Group A	n	Group B	*p* – value
		X ± SD (min-max)		X ± SD (min-max)	
RBC (x10^3/µL)	46	3.99 ± 0.42 (3.13-5.13)	39	4.01 ± 0.42 (3.20-4.89)	**0,727**
Hb (g/dL)	46	9.4 ± 1.5 (4.8-12.2)	39	9.01 ± 1.42 (6.30-11.1)	**0,233**
HCT (%)	46	29.3 ± 4.1 (17.0-37.3)	39	28.8 ± 3.5 (22.5-36.4)	**0,544**
MCV (fl)	46	73.6 ± 8.6 (54.0-93.3)	39	72.0 ± 8.7 (52.0-89.0)	**0,395**
MCH (pg)	46	23.7 ± 3.4 (15.4-29.7)	39	22.7 ± 3.6 (14.4-30.0)	**0,198**
MCHC (g/dl)	46	32.1 ± 1.4 (28.4-34.3)	39	31.4 ± 2.0 (26.0-34.0)	**0,065**
RDW (%)	46	17.5 ± 2.7 (12.7-28.9)	39	17.8 ± 2.3 (13.0-23.7)	**0,623**
PLT (x10^9/L)	46	348 ± 81 (193-590)	39	338 ± 78 (166-494)	**0,564**
WBC (x10^9/L)	46	5.29 ± 1.40 (2.50-8.80)	39	5.00 ± 1.63 (2.70-9.30)	**0,382**
Neutrophil #	46	2.39 ± 0.96 (0.60-4.87)	39	2.31 ± 0.89 (0.93-4.39)	**0,710**
Lymphocyte #	46	2.43 ± 0.68 (0.96-3.94)	39	2.20 ± 0.87 (0.73-4.24)	**0,188**
Monocyte #	46	0.30 ± 0.13 (0.07-0.63)	39	0.31 ± 0.17 (0.06-0.72)	**0,608**
Eosinophil #	46	0.11 ± 0.14 (0.02-0.95)	39	0.12 ± 0.19 (0.01-1.19)	**0,928**
Basophil #	46	0.06 ± 0.12 (0.01-0.78)	39	0.03 ± 0.02 (0.01-0.09)	**0,197**

(n = sample population; X = mean; SD = standard deviation; Min = minimum; Max = maximum; RBC = red blood cell; Hb = haemoglobin; HCT = haematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; RDW = red cell distribution width; PLT = platelet; WBC =white blood cell; # = x10^9 /L) Group A = abstained from soil consumption, Group B = continued with soil consumption

Similar to the general health indicators, none of full blood count parameters showed statistically significant differences as illustrated in Table 4.7. The *p*-values were all greater than 0.05. By comparing the means, standard deviations and ranges, it was also observed that the two groups' composition were very similar. The exception to the rule was MCHC, where the mean of Group B was on the lower limit of the reference range while Group A was within the range, although the difference was not statistically significant.

4.4.6 Clinical chemistry general health and iron studies profile of both groups for baseline visits

The clinical chemistry general health indicators and iron study profile were not significantly different as indicated by *p* values greater than 0.05 for all the parameters, as captured in Table 4.8 and Table 4.9, respectively. Summarised data of the clinical chemistry general health variables for both groups mean; SD and ranges were akin for all parameters in both groups. The discourse was found in some of the iron study results, as depicted in Table 4.9. Serum iron, ferritin, TSAT and HCG had higher means for Group A, while transferrin had a higher mean for Group B. It must also be noted that the maximums of the groups with higher means were highly elevated as compared to the group with lower means. However, the medians and interquartile ranges for the above-mentioned iron variables and HCG were akin.

Table 4.8 Baseline general health clinical chemistry indicators of the two study groups

VARIABLE	N	Group A	n	Group B	*p*-value
		X ± SD (min-max)		X ± SD (min-max)	
BUN (mg/dL)	44	9.63 ± 2.89 (4.33-19.29)	38	9.66 ± 2.47 (6.1-15.67)	**0,972**
S Creat. (mg/dl)	38	0.76 ± 0.18 (0.51-1.32)	34	0.76 ± 0.18 (0.51-1.12)	**0,887**
Albumin (g/L)	43	42.7 ± 6.1 (31-61)	38	42.9 ± 5.8 (31-56)	**0,910**
ALP (U/L)	38	79.5 ± 30.9 (41-165)	34	73.7 ± 24.6 (36-143)	**0,387**
AST (U/L)	38	24.1 ± 8.6 (8-47)	34	24.7 ± 7.2 (16-46)	**0,760**
ALT (U/L)	44	15.7 ± 6.7 (7-42)	39	16.3 ± 4.9 (8-28)	**0,647**
K (mmol/L)	44	3.54 ± 0.46 (2.5-4.7)	39	3.53 ± 0.59 (1.8-4.6)	**0,921**
Na (mmol/L)	43	130.0 ± 3.7 (119-142)	39	129.3 ± 3.2 (121-136)	**0,373**
Phos (mmol/L)	44	1.21 ± 0.22 (0.79-1.89)	37	1.14 ± 0.23 (0.65-1.63)	**0,195**
Mg (mg/dL)	44	2.19 ± 0.41 (1.6-3.5)	39	2.25 ± 0.28 (1.6-3)	**0,467**
Ca (mg/dL)	44	9.51 ± 1.10 (7.5-12.5)	39	9.41 ± 0.98 (7.2-11.1)	**0,677**
Zinc (umol/L)	43	14.05 ± 5.03 (7-30.9)	38	12.75± 4.78 (5.9-30.9)	**0,237**

(n = sample population; X = mean; SD = standard deviation; min = minimum; max = maximum; BUN = blood urea nitrogen; S Creat. = serum creatinine; TP = total protein; ALP = alkaline phosphatase; AST = aspartate aminotransferase; ALT = alanine aminotransferase; K = potassium; Na = sodium; Phos = phosphate; Mg = magnesium; Ca = Calcium)
Group A = abstained from soil consumption, Group B = continued with soil consumption

Table 4.9 Baseline iron study, pregnancy and inflammatory results of the two study groups

VARIABLE	N	Group A	n	Group B	*p*-value
		X ± SD (min-max) Med [IQR]		X ± SD (min-max) Med [IQR]	
S Fe (µg/dL)	44	28.1 ± 22.3 (12-150) 22.5 [17-28.3]	39	24.1 ± 14.8 (9-85) 21 [14-27]	**0,409**
Trans. (g/L)	44	4.02 ± 0.92 (1.95-7.45) 3.83 [3.44-4.45]	37	4.41 ± 1.60 (2.4-12.6) 4.1 [3.6-5.0]	**0,146**
Ferritin (µg/L)	43	9.3 ± 7.7 (2-42) 7 [4-12.5]	39	6.7 ± 3.1 (2-14) 6 [5-8]	**0.057**
TIBC (µg/L)	44	474.4 ± 80.0 (261-652) 467 [431.8-515.5]	39	495.3 ± 75.3 (316-613) 496 [439-554]	**0,225**
TSAT (%)	44	6.2 ± 5.4 (2.3-31.4) 4.8 [3.5-6.3]	39	4.9 ± 2.8 (1.6-14.4) 4.3 [3.1-6.1]	**0.211**
hCG (IU/L)	43	1.1 ± 4.0 (0-25) 0 [0-1]	38	0.4 ± 0.6 (0-2) 0 [0-1]	**0,250**
cRP (mg/L)	43	0.60 ± 0.38 (0.2-1.9) 0.5 [0.4-0.6]	39	0.54 ± 0.34 (0-1.6) 0.5 [0.3-0.6]	**0,451**

(n = sample population; X = mean; SD = standard deviation; med = median; IQR = interquartile range; Min = minimum; Max = maximum; S Fe= serum iron; Trans. = transferrin; TIBC = total iron binding capacity; TSAT = transferrin saturation; hCG = human chorionic gonadotropin; cRP = c-reactive protein)

Group A = abstained from soil consumption, Group B = continued with soil consumption

4.5 RESULTS OF THE INTERVENTION STUDY

The intervention results study section was divided into three sections, namely: oral, IV, comparison of oral and IV iron therapies. Oral therapy consisted of 10-week therapy with baseline, short-term change (week 4), intermediate-term change (week 7) and end of oral evaluation (week 10) as follow-up periods for phlebotomy. It must be noted that week 10's data also served as IV baseline. IV therapy was composed of a 4-week follow-up with, short-term (week 12) and end of IV (week 14) evaluations. The comparison studies were undertaken by evaluating the effect of both therapy forms when considering baseline and end of therapy for both therapy forms. These procedures and evaluation were undertaken to evaluate the effectiveness of each treatment protocol and in comparison to each other. For example, whether consumption of soil during therapy or increasing dose of oral iron or duration of therapy have an effect on the efficacy of oral therapy, and most importantly which therapy protocol could be best suited for geophagia.

4.5.1 Oral iron therapy results

Oral iron therapy was undertaken for over 10 weeks. First 4 weeks was where treatment combined with run-in phase and side effects evaluations were undertaken. Therapy iron concentration was increased twice with 50mg, at weeks 4 & 7, for the subsequent 6 weeks. In the short term (4 weeks), intermediate (week 4 to 7) and end of oral therapy (week 7 to 10)

phases the participants consumed 100 mg, 150 mg and 200 mg elementary iron daily, respectively.

Oral iron's effect was evaluated by first determining compliance with oral iron therapy. In addition, the side effects experienced by the participants were also captured and presented in the subsequent subsection. The mean changes in the physical and laboratory measurements for the entire study group were presented first. The summary of the group that abstained from soil consumption was presented penultimately. Ultimately, the group that continued with soil consumption was presented. The different variables were grouped according to previously presented sections.

4.5.1.1 Compliance with oral medication

Compliance was measured utilising two systems: firstly, the medication given to participants were counted at the beginning of therapy and at the end of visitation period, so as to try to confirm compliance via pill count. The second system was by asking participants to recall if they had missed a dose. Compliance was calculated for all follow-up visits, but in this section only the chewable capsule and both forms for the entire oral therapy period were presented, based on calculated (pill count) and declared summaries (questionnaire). Comparison of the two compliance methods of measurement was summarised based on the entire study population, abstain and continue groups by looking at chewables and

capsules. The combination of the two therapy forms was compared only utilizing the calculated compliance based on the two groups.

The two methods for measuring compliance were compared based on the entire study group, abstain group and continue group. Presented in Figure 4.8 are error charts for the entire study group's calculated vs declared based on chewable and capsules. Results revealed medians (interquartile range) that were not significantly different from one another; 94% (89%-100%) vs 96% (94%-99%) *p* 0.261 and 96% (89%-103%) vs 97% (92%-100%) *p* 0.734, respectively.

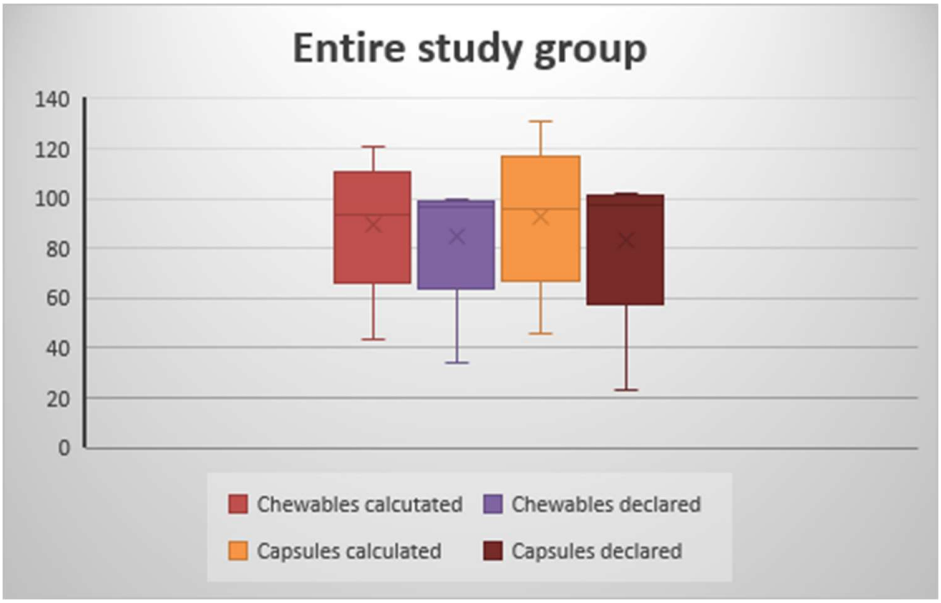


Figure 4.8 Compliance based on calculated and declared for the entire study

Depicted in Figure 4.9 for abstain group the data was also not significantly different for both chewables and capsules; 95% (91%-101%) vs 96%

(93%-99%) *p* 0.565 and 97% (93%-102%) vs 97% (90%-100%) *p* 0.209 based on calculated and declared compliance, respectively.

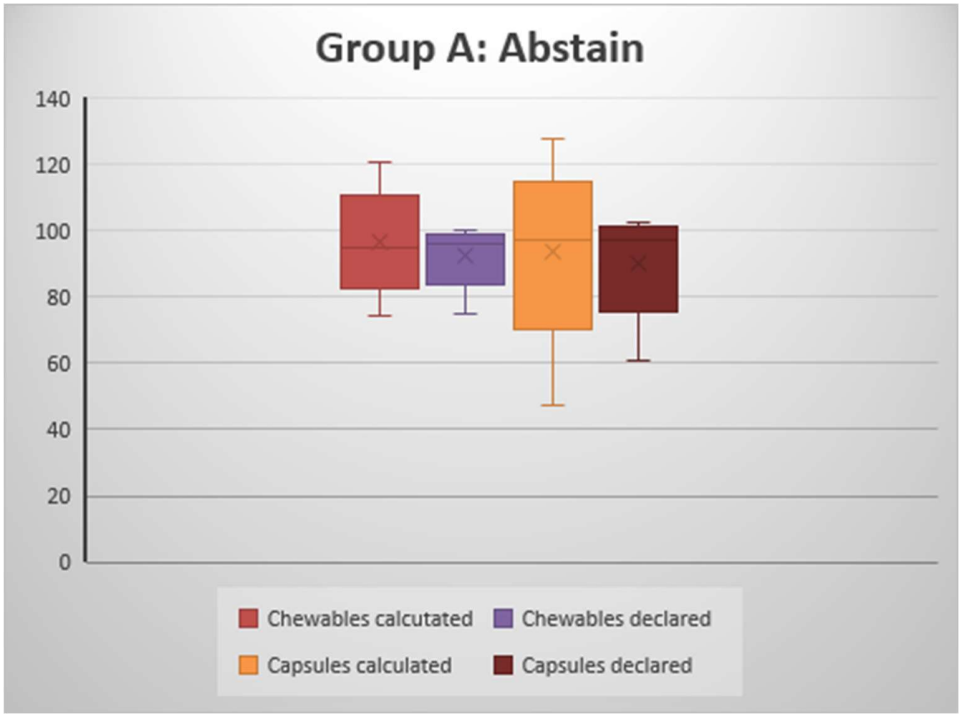


Figure 4.9 Compliance based on calculated and declared for Group A

The findings reveal that the continue group maintained the same trend with the following results presented in Figure 4.10: 92% (87%-99%) vs 96% (94%-99%) *p* 0.698 and 92% (83%-106%) vs 97% (93%-98%) *p* 0.249, for calculated versus declared.

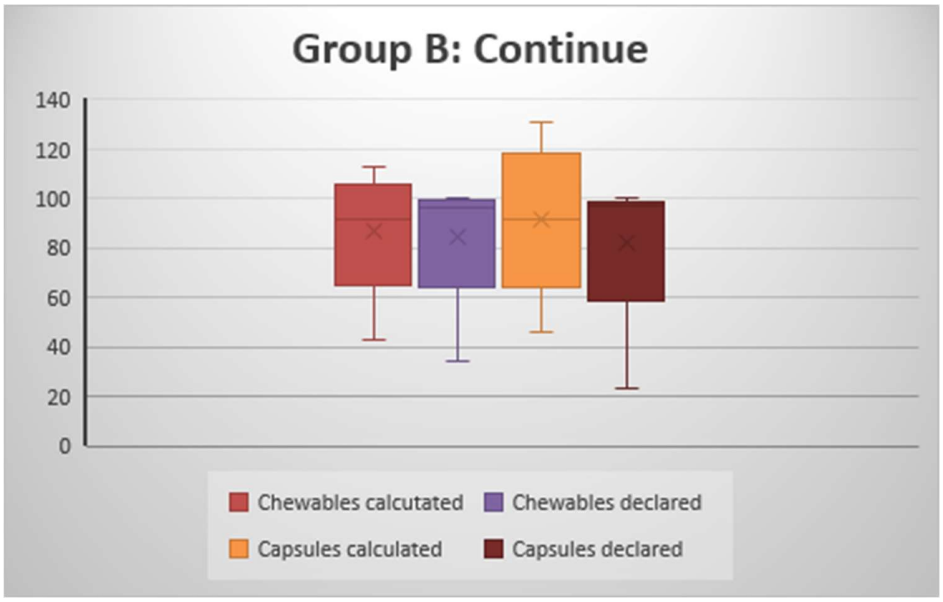
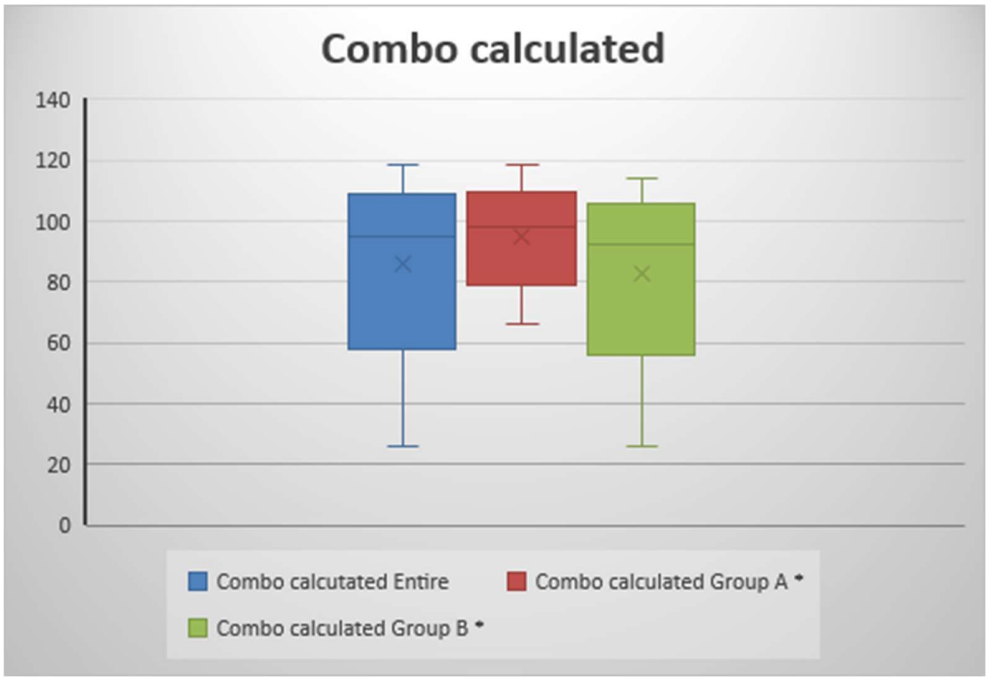


Figure 4.10 Compliance based on calculated and declared for Group B

In Figure 4.11, the total calculated compliance which was for the combination of chewables and capsules presented with the following data for the entire study population, abstain and continue group: 95% (90%-99%); 98% (92%-100%) and 92% (85%-98%), respectively.



* = colour with the same symbol indicate a statistically significant difference

Figure 4.11 Overall compliance based on the combination of two oral therapies for all groups concerned

From all the group's data, it can be noted that there was no statistically significant difference between calculated and declared descriptive statistics, although the calculated leaned towards the lower end as compared to the declared. It was also noted that the minimums of group B ranged from 23% to 43% while those of abstain group were from 47% to 77%, for both calculated and declared. The maximum of the calculated was above 100% for all types of therapy. The calculated compliance of chewables and capsules for group A versus B were not statistically different; *p* 0.062 and *p* 0.404, respectively. The exception was the calculated compliance of a combination of both therapy forms, *p* 0.031, where the median of group A (98%) was higher than that of group B (92%).

4.5.1.2 Mean changes in the entire group at different visits of oral iron

The mean changes of anthropometric, body composition and general health indicators were not statistically or clinically different for all the indicators as shown in Table 4.10. This was evidenced by repeated measures ANOVA with Greenhouse-Geisser correction that determined no statistically significant differences in all variables before treatment, at week 4, week 7 or week 10 as established by ANOVA *p*-values > 0,05. Signifying that there were no differences observed during the different study periods in all the measured variables. It should be noted that the F statistics ((df, df) = F) for all the non-significant findings were captured in Appendix K, for ease of reading considering that numerous variables were presented in each section. The correct format of reporting can be obtained later in the section, where serum creatinine results are presented.

Table 4.10 Mean changes in anthropometric, body composition and general health indicators of the entire study group during oral iron therapy

VARIABLE		Baseline	STC (W4)	ITC (W7)	End oral	ANOVA *p*-value
Weight (kg)	**X**	64.7	65.4	66.8	66.7	0,682
	SD	15.0	17.1	15.0	20.0	
BMI (kg/m^2)	**X**	25.1	25.3	26.0	25.8	0.675
	SD	6.0	6.8	6.3	7.5	
Fat %	**X**	28.8	29.7	29.7	29.1	0,541
	SD	7.1	7.3	7.3	7.3	
Water %	**X**	47.7	47.4	47.1	47.8	0,656
	SD	6.1	6.6	6.3	6.5	
Muscle %	**X**	29.9	29.5	29.4	30.2	0,401
	SD	3.8	3.8	3.8	4.7	
Cal. req./day	**X**	2185	2161	2175	2181	0,678
	SD	223	213	239	227	
Bust (cm)	**X**	93.0	92.4	92.2	91.7	0,731
	SD	14.1	13.0	14.0	14.3	
Waist (cm)	**X**	85.3	86.0	85.7	84.5	0,590
	SD	14.4	15.0	16.0	15.9	
Hip (cm)	**X**	102.5	103.6	104.3	100.8	0,235
	SD	11.0	11.7	12.0	11.1	
WHR	**X**	0.83	0.83	0.82	0.83	0,887
	SD	0.08	0.12	0.11	0.14	
SBP (mmHg)	**X**	131.5	127.33	129.4	123.9	0,209
	SD	26.3	19.6	20.3	19.6	
DBP (mmHg)	**X**	85.0	82.6	84.2	80.4	0,360
	SD	20.0	17.2	14.4	14.3	
Pulse (beats/min)	**X**	79.6	81.1	84.6	81.0	0,215
	SD	11.0	10.8	14.2	13.7	

(X = mean; SD = standard deviation; Cal. req/day = calorie requirement per day; SBP = systolic blood pressure; DBP = diastolic blood pressure; BMI = body mass index; WHR = waist:hip ratio, STC = short-term change; ITC = Intermediate-term changes, ANOVA = analysis of variance; W4 = week 4; W7 = week 7)

Mean changes of haemoglobin concentration, haematocrit, MCV and MCH indicated an increasing pattern from baseline to the end of oral therapy, as illustrated in Table 4.11. However, this increase was small and not clinically significant since rise did not increase the means of different time periods into the reference ranges. Although the repeated measures ANOVA did not show significant values ($p > 0.05$), but the Post Hoc test showed a significant change between baseline and end of the study for all four above-mentioned red blood cell parameters.

The platelet count's mean dropped by 6% (343 – 323 = 25/343) from baseline to the end of the study, although the change was not statistically or clinically significant. All other full blood count parameters did not show any pattern and the differences were not significant as presented by *p*-values of repeated measures ANOVA with a Greenhouse-Geisser correction in Table 4.11.

Table 4.11 Mean changes for blood cell counts of the entire study group at different study visits

VARIABLE		Baseline	STC (W4)	ITC (W7)	End oral	*p*-value
RBC (x10^3/µL)	**X**	4.00	4.10	4.09	4.08	0,369
	SD	0.43	0.49	0.50	0.47	
Hb (g/dL)	**X**	9.2*	9.5	9.6	9.7*	0,129
	SD	1.5	1.6	1.8	1.8	
HCT (%)	**X**	29.0*	29.7	29.9	30.2*	0,190
	SD	3.9	4.1	4.5	4.4	
MCV (fl)	**X**	72.8*	72.7	73.6	74.4*	0,307
	SD	8.9	8.7	9.1	9.4	
MCH (pg)	**X**	23.2*	23.3	23.6	23.9*	0,323
	SD	3.6	3.7	3.8	4.0	
MCHC (g/dl)	**X**	31.7	31.9	32.0	32.0	0,497
	SD	1.8	1.6	1.6	1.8	
RDW (%)	**X**	17.7	17.2	17.4	17.5	0,487
	SD	2.6	2.3	2.3	2.4	
PLT (x10^9/L)	**X**	343	361	340	323	0,059
	SD	78	92	94	87	
WBC (x10^9/L)	**X**	5.10	5.28	5.02	5.29	0,451
	SD	1.51	1.46	1.33	1.54	
Neutrophil #	**X**	2.31	2.47	2.34	2.43	0,809
	SD	0.91	1.04	0.87	1.04	
Lymphocyte #	**X**	2.27	2.41	2.31	2.34	0,584
	SD	0.78	0.74	0.67	0.74	
Monocyte #	**X**	0.31	0.34	0.33	0.35	0,313
	SD	0.15	0.16	0.16	0.19	
Eosinophil #	**X**	0.12	0.12	0.11	0.12	0,570
	SD	0.18	0.16	0.09	0.11	
Basophil #	**X**	0.05	0.04	0.03	0.04	0.238
	SD	0.09	0.03	0.01	0.04	

(X = mean; SD = standard deviation; RBC = red blood cell; Hb = haemoglobin; HCT = haematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; RDW = red cell distribution width; STC = short-term changes; ITC = Intermediate-term changes PLT = platelet; WBC =white blood cell; # = x10^9/L; * = means / medians with same special symbol signify statistical significant change within that variable; W4 = week 4; W7 = week 7)

The median of BUN and mean of serum creatinine showed statistically significant increases from baseline to end of oral iron study, as indicated by Z = -3.397, p 0.001 and (68, -2.088) p 0.041, respectively. The median and mean for both variables were still within the reference range for both study periods as depicted in Table 4.12.

The medians of albumin, ALP and ALT indicated statistically significant changes from baseline to end of oral iron study, as indicated by Z = -2.871, p 0.004; Z = -5.143, p <0.001 and Z = -3.177, p 0.001, respectively. Three of these variables showed a decrease while ALT had an increase, as captured in Table 4.12. The changes did not alter the results so that they were above the reference ranges.

The medians and mean of sodium, phosphate and zinc showed statistically significant changes from baseline to end of oral therapy as seen with Z = -4.541, p <0.001; Z = -2.354, p 0.019 and (77, 5.116) p <0.001, respectively. Sodium and zinc showed a decrease from baseline, dissimilar to phosphate that increased. The median and mean results of both study periods were still within their respective reference ranges. The exception was sodium which was below the range for both baseline and end of oral therapy. Magnesium did not show a statistically significant change as presented in Table 4.12. It should be noted that the Z scores of Wilcoxon test and (df, t) of T-test of the non-significant variables were captured in Appendix K. This was to facilitate ease of reading and decreasing the clutter within the thesis.

Table 4.12 Mean changes in the general health clinical chemistry of the entire study group during different study visits

VARIABLE		Baseline	End oral	*p*-value
BUN (mg/dL)	**Med**	9.19	11.01	0,001
	IQR	7.93-11.25	8.92-13.2	
S Creat. (mg/dl)	**X**	0.76	0.80	0,041
	SD	0.18	0.16	
TP (g/L)	**Med**	92	85.5	<0,001
	IQR	85-99	79-92.75	
Albumin (g/L)	**Med**	42	40	0.004
	IQR	39-46	38-43	
ALP (U/L)	**Med**	69.5	57.5	<0,001
	IQR	55.3-94.5	42-73.5	
AST (U/L)	**Med**	23	23	0,538
	IQR	19-28	19-29	
ALT (U/L)	**Med**	15	16	0,001
	IQR	12-19	13-20	
K (mmol/L)	**Med**	3.5	3.5	0,117
	IQR	3.3-3.8	3.3-3.7	
Na (mmol/L)	**Med**	130	127	<0,001
	IQR	128-132	123-131	
Phos (mmol/L)	**Med**	1.14	1.23	0,019
	IQR	1.04-1.31	1.12-1.38	
Mg (mg/dL)	**Med**	2.2	2.2	0,821
	IQR	2.0-2.4	2.0-2.3	
Ca (mg/dL)	**X**	9.4	9.3	0,127
	SD	1.0	0.7	
Zinc (umol/L)	**X**	13.5	11.2	<0,001
	SD	5.0	2.7	

(X = mean; Med = median; SD = standard deviation; IQR = interquartile range; BUN = blood urea nitrogen; S Creat. = serum creatinine; TP = total protein; ALP = alkaline phosphatase; AST = aspartate aminotransferase; ALT = alanine aminotransferase; K = potassium; Na = sodium; Phos = phosphate; Mg = magnesium; Ca = Calcium)

Repeated measures ANOVA with Greenhouse-Geisser correction determined that there were statistically significant decreases for transferrin and TIBC between different time periods of oral iron therapy $F(1.31, 82.68) = 16.33$, p <0.001 and $F(2.31, 168.74) = 2.96$, p 0.047. However, transferrin's significant change of post hoc test (0.045) did not meet the Bonferroni adjusted significant level of p 0.008. Thus, it did not reveal a significant change between baseline and end of oral iron study, as presented in Table 4.13. The means for both variables at all follow-up visits remained above the reference range, except for transferrin where the end of oral mean was on the upper limit of the reference range.

There were statistically significant differences for serum iron, ferritin, TSAT and HCG between the different oral iron therapy follow-up periods, $\chi^2 = 23.132$, $p < 0.001$; $\chi^2 = 9.109$, p 0.028; $\chi^2 = 15.580$, p = <0.001 & $\chi^2 = 84.348$, $p < 0.001$, respectively. Post hoc analysis with Wilcoxon signed-rank tests was conducted with a Bonferroni correction applied, resulting in a significance level set at $p < 0.008$. Even though the medians of iron, ferritin and TIBC increased from baseline, none increased into the reference range, as illuminated in Table 4.13. Median CRP values did not produce a statistical and clinically significant change, as no pattern was revealed and all median were within the reference range.

Table 4.13 Mean changes in iron study, pregnancy and inflammatory results of the entire study group at different study visits

VARIABLE		Baseline	STC (W4)	ITC (W7)	End oral	*p*-value
S Fe (µg/dL)	**Med**	22.0*	22.0	28.5*	28.0*	<0,001
	IQR	16-27	17-31	19-41	19.3-39.8	
Trans. (g/L)	**X**	4.21	3.95	3.86	3.81	0,047
	SD	1.29	0.97	0.94	0.68	
Ferritin (µg/L)	**Med**	6.5*$	7	7*	8.0$	0,028
	IQR	4-10	4-11	5-13	5-12	
TIBC (µg/L)	**X**	483.0*$	469.7	436*	436.5$	<0,001
	SD	77.6	96.4	86.3	65.4	
TSAT (%)	**Med**	4.8$&	4.9*!	6.2&!	6.9*&	<0,001
	IQR	3.4-6.1	3.4-6.9	4.4-9.3	4.3-9.6	
hCG (IU/L)	**Med**	0*	1*	1*!	0*!	<0,001
	IQR	0-1	0-1	0-1	0-0	
cRP (mg/L)	**Med**	0.5	0.5	0.5	0.5	0,336
	IQR	0.4-0.6	0.4-0.8	0.3-0.8	0.3-0.6	

(X = mean; SD = standard deviation; Med = median; IQR = interquartile range; S Fe= serum iron; Trans. = transferrin; TIBC = total iron binding capacity; TSAT = transferrin saturation; hCG = human chorionic gonadotropin; cRP = c-reactive protein, STC = short-term change, ITC = intermediate-term change; *$&! = means / medians with same special symbol signify statistical significant change within that variable; W4 = week 4; W7 = week 7)

4.5.1.3 Mean changes of the group that abstained from soil consumption

The mean changes of anthropometric, body composition and general health indicators were not statistically or clinically different for all the variables at different time intervals, as publicised in Table 4.14.

Table 4.14 Mean anthropometric, body composition and general health indicators of the group that abstained from soil consumption at different visits

VARIABLE		Baseline	STC (W4)	ITC (W7)	End oral	*p*-value
Weight (kg)	**X**	66.0	67.6	67.3	66.6	0,625
	SD	14.7	15.4	14.8	14.6	
BMI (kg/m^2)	**X**	25.6	26.1	26.2	25.7	0,672
	SD	5.9	6.2	6.3	6.0	
Fat %	**X**	29.4	29.9	29.5	29.6	0,769
	SD	7.0	7.6	7.3	7.2	
Water %	**X**	47.4	47.0	47.4	47.3	0,829
	SD	6.1	6.5	6.2	6.3	
Muscle %	**X**	29.7	29.4	29.5	30.1	0,561
	SD	3.7	3.8	3.8	5.0	
Cal. req./day	**X**	2180	2160	2190	2181	0,677
	SD	221	225	232	229	
Waist (cm)	**X**	86.4	87.5	87.6	85.7	0,577
	SD	14.1	16.7	16.5	13.8	
Hip (cm)	**X**	104.5	103.5	104.8	103.2	0,366
	SD	11.3	11.7	11.3	12.0	
WHR	**X**	0.83	0.85	0.83	0.83	0,928
	SD	0.08	0.14	0.12	0.13	
Bust (cm)	**X**	93.7	93.4	94.6	93.6	0,882
	SD	13.4	13.2	14.2	17.5	
SBP (mmHg)	**X**	132.3	127.6	127.4	126.0	0,332
	SD	22.2	16.5	18.2	18.6	
DBP (mmHg)	**X**	85.0	81.7	83.2	80.1	0,480
	SD	17.6	12.9	14.2	14.1	
Pulse (beats/min)	**X**	79.8	81.9	83.0	81.8	0,002
	SD	11.0	11.0	13.3	12.3	

(X = mean; SD = standard deviation; SBP = systolic blood pressure; Cal. Reg/day = calorie requirement per day DBP = diastolic blood pressure; BMI = body mass index; WHR = waist:hip ratio; STC = short-term change, ITC = intermediate-term change; W4 = week 4; W7 = week 7)

However, a repeated measures ANOVA with a Greenhouse-Geisser correction determined that there was statistically significant differences in pulse at week 7 versus baseline, week 4 and week 10 $F(1.96, 56.93)= 6.80$, p 0.002. The three time periods comparisons were <0.05 (p 0.017, p 0.030 & p 0.011, respectively). However, when the Bonferroni adjustment for multiple comparison calculation was undertaken, all the comparisons were above the revised significant value of 0.008.

Repeated measures ANOVA with Greenhouse-Geisser correction determined that there were statistically significant differences in haemoglobin, haematocrit, MCV, MCH, and RDW from baseline to week 10; $F(1.42, 62.56) = 4.31$, p 0.029; $F(1.45, 63.68) = 3.84$, p 0. 039; $F(1.12, 49.23) = 8.19$, p 0. 005; $F(1.23, 49.65)= 6.12$, p 0. 014 and $F(2.20, 99.70)= 4.76$, p 0. 009, respectively. Although the overall ANOVA for RDW revealed p 0.009, however when the Bonferroni adjustment for multiple comparison calculation was undertaken, the comparison was above the revised significant value of 0.008 (which was 0.038). The haemoglobin concentration, haematocrit, MCV and MCH increased slightly from baseline until the end of oral iron therapy phase, as presented in Table 4.15. It must be noted that the changes were not clinically significant as the increase did not result in parameters' means increasing into the reference ranges.

Table 4.15 Mean blood cell count changes of the group that abstained from soil consumption

VARIABLE		Baseline	STC (W4)	ITC (W7)	End oral	*p* value
RBC ($x10^3/\mu L$)	**X**	3.99	4.07	4.13	4.01	0,383
	SD	0.42	0.50	0.56	0.50	
Hb (g/dL)	**X**	9.4*	9.7	9.9	10.0*	0,029
	SD	1.5	1.6	1.6	1.7	
HCT (%)	**X**	29.3*	30.1	30.9	30.9*	0,039
	SD	4.1	4.3	4.9	4.4	
MCV (fl)	**X**	73.6*	74.2$	75.3	75.7*$	0,005
	SD	8.6	8.4	8.9	8.5	
MCH (pg)	**X**	23.7*	24.0	24.3	24.6*	0,014
	SD	3.4	3.5	3.5	3.7	
MCHC (g/dl)	**X**	32.1	32.2	32.3	32.4	0,233
	SD	1.4	1.4	1.3	1.5	
RDW (%)	**X**	17.5	17.2	17.4	17.3	0,009
	SD	2.7	2.6	2.5	2.4	
PLT ($x10^9/L$)	**X**	348*	365	327	314*	0,094
	SD	81	98	81	79	
WBC ($x10^9/L$)	**X**	5.29	5.31	5.16	5.34	0,504
	SD	1.40	1.41	1.32	1.64	
Neutrophil #	**X**	2.39	2.48	2.34	2.49	0,532
	SD	0.96	1.14	0.88	1.16	
Lymphocyte #	**X**	2.43	2.48	2.37	2.39	0,601
	SD	0.68	0.75	0.56	0.65	
Monocyte #	**X**	0.30	0.34	0.34	0.33	0,160
	SD	0.13	0.18	0.18	0.19	
Eosinophil #	**Med**	0.11	0.08	0.08	0.08	0,463
	IQR	0.04-0.11	0.04-0.14	0.05-0.11	0.04-0.14	
Basophil #	**Med**	0.05	0.03	0.03	0.03	0,312
	IQR	0.03-0.05	0.02-0.05	0.02-0.04	0.03-0.05	

(X = mean; SD = standard deviation; Med = median; IQR = interquartile range; RBC = red blood cell; Hb = haemoglobin; HCT = haematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; RDW = red cell distribution width; PLT = platelet; WBC =white blood cell; # = $x10^9/L$; STC = short-term change, ITC = intermediate-term change; *$&! = means / medians with same special symbol signify statistical significant change within that variable; W4 = week 4; W7 = week 7)

Platelet count decreased by 10% from baseline to the end of oral iron therapy (348 – 314 = 34/348). Although the difference was not statistically based on repeated measure ANOVA of *p* 0.094 and clinically significant (both means were within the reference range), it must be noted that the Post Hoc test revealed a statistically significant change between baseline and end of oral therapy (*p* <0.001). All the other blood cell counts did not show a pattern, were not statistically and clinically significantly different during the different oral iron therapy study visits, as shown in Table 4.15.

The mean of BUN showed a statistically significant increase from baseline to end of oral iron study, as indicated by (43, -2.343) *p* 0.024. Serum creatinine did not show a statistically significant change. The means for both variables were still within the reference range for both study periods as presented in Table 4.16.

The difference in means and median of albumin, ALP and ALT indicated statistically significant changes from baseline to end of oral iron study, as indicated by (42, 2.821) *p* 0.007; (37, 4.974) *p* <0.001 and *Z* = -2.495, *p* 0.013, respectively. ALT increased from baseline whereas the other three variables decreased, as illustrated in Table 4.16. The changes did not alter the results so that they outside of reference ranges.

Table 4.16 Mean general health clinical chemistry indicators changes of the group that abstained from soil consumption

VARIABLE		Baseline	End oral	*p*-value
BUN (mg/dL)	**X**	9.63	10.97	0,024
	SD	2.89	3.40	
S Creat. (mg/dl)	**X**	0.76	0.80	0,094
	SD	0.18	0.13	
Albumin (g/L)	**X**	42.7	40.7	0,007
	SD	6.1	3.7	
ALP (U/L)	**X**	79.5	64.3	<0,001
	SD	30.9	27.1	
AST (U/L)	**Med**	23	24	0,683
	IQR	18.3-28	18-29	
ALT (U/L)	**Med**	14	15	0,013
	IQR	11.8-17	13-20	
K (mmol/L)	**Med**	3.5	3.5	0,487
	IQR	3.3-3.8	3.3-3.7	
Na (mmol/L)	**Med**	131	127	0,002
	IQR	129-132	123-133	
Phos (mmol/L)	**X**	1.21	1.24	0,470
	SD	0.22	0.23	
Mg (mg/dL)	**Med**	2.1	2.1	0,754
	IQR	2.0-2.3	2.0-2.3	
Ca (mg/dL)	**X**	9.51	9.32	0,288
	SD	1.10	0.65	
Zinc (umol/L)	**X**	14.1	11.6	0,001
	SD	5.0	2.4	

(X = mean; Med = median; SD = standard deviation; IQR = interquartile range; BUN = blood urea nitrogen; S Creat. = serum creatinine; TP = total protein; ALP = alkaline phosphatase; AST = aspartate aminotransferase; ALT = alanine aminotransferase; K = potassium; Na = sodium; Phos = phosphate; Mg = magnesium; Ca = Calcium)

The change in sodium's median and zinc's mean showed statistically significant decreases from baseline to end of oral therapy as seen with Z = -3.030, p 0.002 and (42, 3.656) p 0.001, respectively. Divergent to potassium, phosphate, magnesium and calcium that did not show a statistically significant change, as demonstrated in Table 4.16. The medians and means results of both study periods were still within their respective reference ranges for all variables. The only exception was sodium which was below the range for both baseline and end of oral therapy.

There were statistically significant differences for serum iron, ferritin, TIBC, TSAT and HCG between the different follow-up periods, χ^2 = 81.707, p < 0.001; χ^2 = 8.402, p 0.038; χ^2 = 15.642, p 0.001; χ^2 = 21.900, p = <0.001 & χ^2 = 43.467, p < 0.001, respectively. Post Hoc analysis with Wilcoxon signed-rank tests was conducted of ferritin, the difference between baseline and week 7 did not meet (p 0.024) the Bonferroni correction significance level of p < 0.008, as represented in Table 4.17. The medians for ferritin were below the reference range for all time intervals. The medians of serum iron and TSAT increased from baseline to end of oral iron therapy. However, none corrected into the reference range. For TIBC, the medians dropped from baseline till ITC but increased at the end of oral iron therapy. The medians were still above the reference ranges. Although transferrin did not show a statistically significant change, the medians decreased slightly from baseline to end of oral iron therapy, where the latter was on the highest limit of the reference range.

The medians of HCG and CRP were within the reference ranges. Median CRP levels did not reveal a pattern and a statistically significant change, as illuminated in Table 4.17. HCG on the other hand, had higher median levels for STC and ITC while baseline and end of oral therapy were similar.

Table 4.17 Mean iron study, pregnancy and inflammatory indicators changes in of the group that abstained from soil consumption at different visits

VARIABLE		Baseline	STC (W4)	ITC (W7)	End oral	*p*-value
S Fe (µg/dL)	**Med**	22.5*$	24!	29*!	28$	<0,001
	IQR	17-28.3	18-31	21-41	20-42	
Trans. (g/L)	**X**	4.02	3.72	3.84	3.80	0,323
	SD	0.92	0.83	1.13	0.72	
Ferritin (µg/L)	**Med**	7	8	8	7	0,038
	IQR	4-12.5	5-16	5-15	5-14	
TIBC (µg/L)	**Med**	467$*!	441!	430$	441*	0,001
	IQR	432-516	411-484	404-481	389-477	
TSAT (%)	**Med**	4.8*!	5.1	6.9!	6.9*	<0,001
	IQR	3.5-6.3	3.5-8.1	4.9-10	4.3-10.7	
hCG (IU/L)	**Med**	0$^!	1&!	1$*	0*&^	<0,001
	IQR	0-1	0-1	0-1	0-0	
cRP (mg/L)	**Med**	0.5	0.5	0.5	0.5	0,336
	IQR	0.4-0.6	0.4-0.8	0.4-0.8	0.3-0.6	

(X = mean; SD = standard deviation; Med = median; IQR = interquartile range; S Fe= serum iron; Trans. = transferrin; TIBC = total iron binding capacity; TSAT = transferrin saturation; hCG = human chorionic gonadotropin; cRP = c-reactive protein; STC = short-term change, ITC = intermediate-term change; *$&!^ = means / medians with same special symbol signify statistical significant change within that variable; W4 = week 4; W7 = week 7)

4.5.1.4 Mean changes of the group that continued with soil consumption

Table 4.18 Mean anthropometric, body composition and general health indicators changes of the group that continued with consumption of soil

VARIABLE		Baseline	STC (W4)	ITC (W7)	End oral	*p*-value
Weight (kg)	**X**	64.8	64.1	64.4	64.8	0,808
	SD	16.5	19.3	17.1	17.1	
BMI (kg/m^2)	**X**	25.1	24.9	25.1	24.8	0,744
	SD	6.3	7.5	6.4	6.6	
Fat %	**X**	28.9	29.6	29.3	28.8	0,498
	SD	7.7	7.0	7.7	7.9	
Water %	**X**	47.7	47.9	47.6	48.2	0,468
	SD	6.6	6.8	6.8	7.1	
Muscle %	**X**	29.8	29.5	29.7	29.9	0,489
	SD	4.0	3.8	4.1	4.1	
Cal. req./day	**X**	2171	2155	2182	2179	0,721
	SD	247	207	266	249	
Waist (cm)	**X**	85.6	85.5	83.1	83.7	0,710
	SD	15.0	13.5	14.6	13.8	
Hip (cm)	**X**	103.5	104.4	103.0	102.3	0,381
	SD	12.8	11.6	13.0	13.8	
WHR	**X**	0.82	0.82	0.81	0.82	0.593
	SD	0.07	0.07	0.08	0.07	
Bust (cm)	**X**	92.5*	92.3	90.4	89.9*	0,599
	SD	14.2	12.7	14.2	14.3	
SBP (mmHg)	**X**	130.4	127.7	129.8	123.8	0,527
	SD	28.0	23.1	20.0	21.3	
DBP (mmHg)	**X**	85.0	82.6	83.8	80.2	0,498
	SD	20.1	17.4	13.3	11.9	
Pulse (beats/min)	**X**	81.5	82.2	82.0	83.9	0,063
	SD	11.7	12.6	12.6	12.1	

(X = mean; SD = standard deviation; SBP = systolic blood pressure; Cal. Reg/day = calorie requirement per day DBP = diastolic blood pressure; BMI = body mass index; WHR = waist:hip ratio; STC = short-term change, ITC = intermediate-term change; * = means / medians with same special symbol signify statistical significant change within that variable; W4 = week 4; W7 = week 7)

The mean changes of anthropometric, body composition and general health indicators were not statistically or clinically different for all the indicators as depicted in Table 4.18. Signifying that there were no differences observed during the different study periods in all the measured variables. The exception was bust, where the Post Hoc test showed a statistically significant decrease from baseline to end of oral (p 0.002), even though the repeated measures ANOVA was not significant (p 0.599).

A repeated measures ANOVA with a Greenhouse-Geisser correction determined that there was a statistically significant change for RDW between week 4 versus baseline and week 10 $F(1.84, 60.64) = 10.59$, $p < 0.001$, this was as a result of a decrease in the mean of week 4. Haemoglobin concentration revealed a statistically significant change with the overall ANOVA p 0.042. Conversely, the post hoc test did not reveal any significant change among the time periods. The red blood cell parameters' means were below the reference range, except for red blood cell count and MCHC which were normal and RDW which was increased, as captured in Table 4.19

Platelet count increased from baseline to week 4 and week 7 but decreased by week 10, the change from baseline to the end of oral iron therapy was 2% (338 – 326 = 8/338). All the other blood cell counts did not show a pattern and were not significantly different during the different oral iron therapy study visits, as shown in Table 4.19. The means and medians of WBC parameters and platelets were within reference ranges.

Table 4.19 Mean blood cell count changes of the group that continued with soil consumption at different visits

VARIABLE		Baseline	STC (W4)	ITC (W7)	End oral	p-value
RBC ($x10^3/\mu L$)	X	4.01	4.12	4.06	4.05	0.645
	SD	0.42	0.47	0.40	0.43	
Hb (g/dL)	X	9.01	9.31	9.28	9.31	0,042
	SD	1.42	1.52	1.58	1.77	
HCT (%)	X	28.8	29.3	29.1	29.3	0,064
	SD	3.5	3.8	3.8	4.3	
MCV (fl)	X	72.0	71.4	72.1	72.6	0,069
	SD	8.7	8.7	9.4	9.8	
MCH (pg)	X	22.7	22.8	23.0	23.1	0.073
	SD	3.6	3.8	4.0	4.2	
MCHC (g/dl)	X	31.4	31.7	31.7	31.5	0,145
	SD	2.0	1.7	1.9	2.0	
RDW (%)	X	17.8$	16.9$#	17.3	17.6#	<0,001
	SD	2.3	1.9	2.0	2.3	
PLT ($x10^9/L$)	X	338	352	355	326	0,460
	SD	78	82	107	106	
WBC ($x10^9/L$)	X	4.99	5.46	4.94	5.25	0,252
	SD	1.63	1.59	1.40	1.43	
Neutrophil #	X	2.31	2.46	2.28	2.32	0,163
	SD	0.89	1.0	0.86	0.78	
Lymphocyte #	X	2.20	2.35	2.25	2.37	0,494
	SD	0.87	0.70	0.77	0.89	
Monocyte #	X	0.31	0.37	0.32	0.36	0,088
	SD	0.17	0.16	0.15	0.18	
Eosinophil #	Med	0.07	0.08	0.07	0.08	0,912
	IQR	0.05-0.13	0.06-0.14	0.05-0.13	0.06-0.18	
Basophil #	X	0.03	0.04	0.03	0.03	0,130
	SD	0.02	0.03	0.01	0.02	

(X = mean; SD = standard deviation; Med = median; IQR = interquartile range; RBC = red blood cell; Hb = haemoglobin; HCT = haematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; RDW = red cell distribution width; PLT = platelet; WBC =white blood cell; # = $x10^9/L$; STC = short-term change, ITC = intermediate-term change; *$&! = means / medians with same special symbol signify statistical significant change within that variable; W4 = week 4; W7 = week 7)

The mean of BUN showed a statistically significant increase from baseline to end of oral iron study, as indicated by (34, -2.992) *p* 0.005. Serum creatinine did not show a statistically significant change. The means for both variables were still within the reference range for both study periods as summarised in Table 4.20.

The change in means of albumin and ALP indicated statistically significant decreases from baseline to end of oral iron study, as indicated by (34, 2.185) *p* 0.036 and (30, 3.631), *p* 0.001, respectively. ALT and AST did not show a statistically significant change as illustrated in Table 4.20. The changes did not alter the results in such a way that they were out of reference ranges

The change in mean and medians of sodium, phosphate and zinc showed statistically significant differences from baseline to end of oral therapy as seen with (35, 4.201) *p* <0.001; *Z* = -2.967, *p* 0.003 and (34, 3.692) *p* 0.001, respectively. Sodium and zinc showed a decrease from baseline to end of oral therapy, while phosphate increased. Potassium, magnesium and calcium that did not show a statistically significant change, as depicted in Table 4.20. The medians and means results of all variables for both study periods were still within their respective reference ranges. The exception was potassium where the end of oral therapy was below the range, plus sodium had the study period's means below the range.

Table 4.20 Mean general clinical chemistry changes of the group that continued with soil consumption at different visits

VARIABLE		Baseline	End oral	*p*-value
BUN (mg/dL)	**X**	9.67	11.74	0,005
	SD	2.22	3.53	
S Creat. (mg/dl)	**X**	0.76	0.79	0,233
	SD	0.17	0.20	
Albumin (g/L)	**X**	42.6	39.6	0,036
	SD	5.6	7.9	
ALP (U/L)	**X**	71.0	59.1	0,001
	SD	21.8	21.5	
AST (U/L)	**Med**	22.5	23	0,644
	IQR	19.8-28.3	20-28	
ALT (U/L)	**X**	16.2	17.3	0,179
	SD	4.9	5.2	
K (mmol/L)	**X**	3.52	3.42	0,088
	SD	0.62	0.49	
Na (mmol/L)	**X**	129.7	126.5	<0,001
	SD	3.0	5.4	
Phos (mmol/L)	**X**	1.11	1.27	0,003
	SD	0.96-1.27	1.12-1.4	
Mg (mg/dL)	**X**	2.24	2.29	0,568
	SD	0.29	0.31	
Ca (mg/dL)	**X**	9.38	9.20	0,277
	SD	0.97	0.86	
Zinc (umol/L)	**X**	12.92	10.87	0,001
	SD	4.93	2.94	

(X = mean; Med = median; SD = standard deviation; IQR = interquartile range; BUN = blood urea nitrogen; S Creat. = serum creatinine; ALP = alkaline phosphatase; AST = aspartate aminotransferase; ALT = alanine aminotransferase; K = potassium; Na = sodium; Phos = phosphate; Mg = magnesium; Ca = Calcium)

As depicted in Table 4.21, there were statistically significant differences for serum iron, TIBC, TSAT and HCG between the different follow-up periods, χ^2 = 8.436, p 0.038; χ^2 = 14.767, p 0.002; χ^2 = 17.233, p <0.001 and χ^2 = 41.082, p < 0.001, respectively. The medians of serum iron, ferritin and TSAT lingered below the reference range for all oral iron therapy visits.

Table 4.21 Mean iron study, pregnancy and inflammatory changes of the group that continued with consumption of soil during at different visits

VARIABLE		Baseline	STC (W4)	ITC (W7)	End oral	p-value
S Fe (µg/dL)	Med	21*!	21.5$	24*$	28!	0,038
	IQR	14-27	16.8-29.8	17-41	19-39	
Trans. (g/L)	Med	4.1	4.02	3.85	3.71	0,477
	IQR	3.56-4.98	3.42-4.73	3.48-4.31	3.45-4.33	
Ferritin (µg/L)	Med	6	6	6	8	0,261
	IQR	5-8	4-8.5	5-9	4-10	
TIBC (µg/L)	Med	496*!	483$	434*$	421!	0,002
	IQR	439-554	436-546	399-486	394-496	
TSAT (%)	Med	4.3$&	4.8*	5.9&	6.9*$	0,011
	IQR	3.1-6.1	3.3-6.6	3.9-9.0	3.9-9.5	
hCG (IU/L)	Med	0*$	1.0*&	1$~	0&~	<0,001
	IQR	0-1	1.0	0.5-1	0-0	
cRP (mg/L)	Med	0.5	0.55	0.4	0.5	0,516
	IQR	0.3-0.6	0.38-0.8	0.3-0.7	0.3-0.6	

(Med = median; IQR = interquartile range; S Fe= serum iron; Trans. = transferrin; TIBC = total iron binding capacity; TSAT = transferrin saturation; hCG = human chorionic gonadotropin; cRP = c-reactive protein; STC = short-term change, ITC = intermediate-term change; *$&! = means / medians with same special symbol signify statistical significant change within that variable; W4 = week 4; W7 = week 7)

Contrary to transferrin and TIBC which were above the reference range, while end of oral therapy for transferrin was within range. Transferrin, ferritin and CRP did not indicate a statistically significant difference for all study visits. HCG medians were all within the reference range even though week 4 and week 7 were higher than baseline and end of oral iron therapy.

4.5.2 IV iron therapy result

At the end of oral iron therapy (week 10) blood was analysed to ascertain the response of each participant. Participants that responded to oral iron continued with oral iron for the entire duration of the study. The rest were given an opportunity to consent for IV iron therapy. The dose was calculated utilising the Ganzoni formula. The required dosage was administered within a week if multiple doses were required. Follow-up was scheduled 2 weeks after receiving the last IV dose (week 12), and then again at the end of the study (week 14). The entire follow-up after IV therapy was four weeks.

This section of the findings was summarised in this particular fashion. Firstly, a presentation of compliance which is measured by the number of doses that were administered versus the amount that was required, based on the formula calculation. Secondly, the mean changes were discussed for the entire group as an entity, then the findings were summarised for the two groups; the one that abstained from geophagia during the study followed by the one that continued with geophagia during the study period.

4.5.2.1 Compliance for IV iron administration

The calculated dose requirement to achieve a Hb level of 12.5 g/dl for the entire study group was 394 mg iron, that signified 2 doses of 200 mg. The administered median dose was similar to the calculated one as depicted by $p>0.05$ in Table 4.22.

Table 4.22 Mean calculated compliance for IV iron administration for the entire population and both groups

		Median (IQR)	Min - Max	*p*-value
Entire group	Calculated dose	394 (262 – 626)	22 - 1147	0,0863
	Administered dose	400 (400 – 700)	200 - 1200	
	Calculated no. of doses	2 (2 – 3.5)	1 - 6	0,73477
	Administered no. of doses	2 (2 – 3.5)	1 - 6	
Abstain group	Calculated dose	361 (241 – 543)	22 - 1075	0,23089
	Administered dose	400 (350 – 600)	200 - 1000	
	Calculated no. of doses	2 (2 – 3)	1 – 5	0,84172
	Administered no. of doses	2 (1.8 – 3)	1 – 5	
Continue group	Calculated dose	462 (290 – 645)	162 - 1147	0,20971
	Administered dose	600 (400 – 800)	200 - 1200	
	Calculated no. of doses	3 (2 – 4)	1 – 6	0,77536
	Administered no. of doses	3 (2 – 4)	1 – 6	

A similar trend was observed for group A while group B had a median of 3 doses. However, the difference was not statistically significant for both

the calculated dose (*p* 0.1201) and the number of doses administered (*p* 0.0521). Calculated dose and number of doses for group A vs group B did not show a statistically significant difference *p*-value of 0.1201 and 0.0521, respectively.

4.5.2.2 Mean changes of IV iron of the entire group at different visits

A repeated measures ANOVA with a Greenhouse-Geisser correction determined that there was a statistically significant decrease for pulse $F(1.59, 90.83) = 6.13$, p 0.006. However, when the Bonferroni adjustment for multiple comparison calculation was undertaken, the comparisons were above the revised significant value of 0.017 (which were 0.019 & 0.040). The mean changes of all other anthropometric, body composition and general health indicators were not statistically or clinically different for all the indicators as depicted in Table 4.23, signifying that there were no differences observed during the different study periods in all the measured variables.

Table 4.23 Mean anthropometric, body composition and general health indicators changes of the entire group at different visits

VARIABLE		Baseline	STC (W12)	End IV (W14)	*p*-value
Weight (kg)	**X**	67.1	65.8	65.7	0.931
	SD	19.5	15.7	16.4	
BMI (kg/m^2)	**Med**	24.2	23.7	25.1	0.283
	IQR	20.6-30.5	21.1-28.9	20.5-29.4	
Fat %	**Med**	27.9	26.9	27.0	0.188
	IQR	23.4-34.4	23.5-33.8	24.1-34.5	
Water %	**X**	47.7	47.3	47.9	0.651
	SD	6.6	6.8	6.3	
Muscle %	**X**	30.0	29.9	29.7	0.616
	SD	4.6	4.5	3.8	
Cal. req./day	**X**	2181	2189	2189	0.943
	SD	237	246	230	
Waist (cm)	**X**	84.8	84.1	85.5	0.558
	SD	17.9	14.7	14.7	
Hip (cm)	**X**	103.6	104.0	103.2	0.792
	SD	15.6	11.9	12.5	
WHR	**Med**	0.80	0.80	0.81	0.080
	IQR	0.75-0.87	0.74-0.85	0.76-0.87	
Bust (cm)	**Med**	88	88	89	0.070
	IQR	82-102	82-101	81-101	
SBP (mmHg)	**X**	125.0	124.7	121.3	0.628
	SD	19.8	19.5	15.5	
DBP (mmHg)	**X**	80.1	80.7	77.8	0.450
	SD	13.1	14.1	12.2	
Pulse (beats/min)	**X**	81.8	78.1	78.6	0.006
	SD	12.3	10.2	9.3	

(X = mean; SD = standard deviation; Med = median; IQR = interquartile range; SBP = systolic blood pressure; Cal. Reg/day = calorie requirement per day DBP = diastolic blood pressure; BMI = body mass index; WHR = waist:hip ratio; STC = short-term changes; W12 = week 12; W14 = week 14)

The repeated measures ANOVA with Greenhouse-Geisser correction and Friedman test determined that there were statistically significant increases for red blood cell parameters; RBC, Hb, HCT, MCV, MCH and RDW between IV baseline, week 12 and week 14 $F(1.31, 82.68) = 16.33$, $p < 0.001$; $F(1.19, 75.16) = 53.44$, $p < 0.001$; $F(1.26, 79.47) = 73.36$, $p < 0.001$; $\chi^2 = 48.925$, $p < 0.001$; $F(1.19, 74.47) = 19.27$, $p < 0.001$ and $\chi^2 = 22.574$, $p < 0.001$, respectively. The means and medians were now within the reference ranges for the red blood cell parameters, except for RDW where all visits were above the range, as depicted in Table 4.24.

The repeated measures ANOVA with a Greenhouse-Geisser correction and Friedman test determined that there were statistically significant changes for PLT, lymphocyte and basophil between IV baseline, week 12 and end of IV therapy $\chi^2 = 20.614$, $p < 0.001$; $F(1.23, 77.33) = 6.37$, p 0.009 and $\chi^2 = 10.157$, p 0.006, respectively. Post hoc analysis for lymphocyte with Wilcoxon signed-rank tests was conducted with a Bonferroni correction applied, resulting in a revised significance level set at p 0.017 which was not met by week 10 versus week 14 (p 0.020). The median platelet count dropped by 16% (321 – 269 = 52/321) from IV baseline to the end of the study. All the other blood cell counts did not reveal a particular pattern, were not statistically different during the different oral iron therapy study visits, plus their means and medians were with the reference ranges as shown in Table 4.24.

Table 4.24 Mean blood cell count changes of the entire study group at different study visits

VARIABLE		Baseline	STC (W12)	End IV (W14)	*p*-value
RBC ($x10^3/\mu L$)	**X**	4.08*	4.43*	4.34*	<0.001
	SD	0.47	0.52	0.44	
Hb (g/dL)	**X**	9.7*!	11.6*	11.6!	<0.001
	SD	1.8	1.1	0.9	
HCT (%)	**X**	30.2*!	36.1*	36.2!	<0.001
	SD	4.4	3.3	2.6	
MCV (fl)	**Med**	76.0*	82.0*	84*	<0.001
	IQR	67.0-81	77.5-87.1	78-88.6	
MCH (pg)	**X**	23.9*	26.4*	27.1*	<0.001
	SD	4.0	2.7	2.6	
MCHC (g/dl)	**X**	32.0	32.2	32.3	0.435
	SD	1.75	1.76	1.76	
RDW (%)	**Med**	17.1*!	19.0*	20.5!	<0.001
	IQR	15.9-19.4	17.1-22.4	16.7-22.4	
PLT ($x10^9/L$)	**X**	321*	289!	269*!	<0.001
	SD	254-363	258-341	228-291	
WBC ($x10^9/L$)	**Med**	5.09	5.10	4.95	0.315
	IQR	4.20-6.00	4.05-6.30	4.03-6.00	
Neutrophil #	**Med**	2.3	2.63	2.51	0.718
	IQR	1.86-2.87	1.89-3.48	1.86-3.08	
Lymphocyte #	**X**	2.38	2.06	2.06	0.009
	SD	0.76	0.71	0.71	
Monocyte #	**Med**	0.30	0.29	0.28	0.171
	IQR	0.21-0.43	0.19-0.40	0.20-0.36	
Eosinophil #	**Med**	0.08	0.08	0.09	0.699
	IQR	0.04-0.17	0.05-0.13	0.05-0.14	
Basophil #	**Med**	0.03	0.04*	0.03*	0.006
	IQR	0.03-0.05	0.03-0.05	0.02-0.04	

(X = mean; SD = standard deviation; Med = median; IQR = interquartile range; RBC = red blood cell; Hb = haemoglobin; HCT = haematocrit, MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; RDW = red cell distribution width; PLT = platelet; WBC =white blood cell; # = $x10^9/L$; STC = short-term changes, W12 = week 12; W14 = week 14; *$&! = means / medians with same special symbol signify statistical significant change within that variable)

The mean of serum creatinine showed a statistically significant increase from IV baseline to end of IV iron study, as indicated by (72, -3.181) *p* 0.024. BUN did not show a statistically significant change. The means for both variables were still within the reference range for both study periods as presented in Table 4.25.

The medians of albumin and AST indicated statistically significant changes from IV baseline to end of IV iron study, as indicated by $Z = -4.583$, $p < 0.001$ and $Z = -2.108$, p 0.035, respectively. Albumin increased from IV baseline whereas AST decreased, as demonstrated in Table 4.25. The changes did not alter the results so that they were out of reference ranges. The other two liver enzymes did not change statistically and clinically, owing to $p > 0.05$ plus the means being within the reference ranges.

The medians of potassium, sodium, phosphate, magnesium and calcium's mean showed statistically significant increases from IV baseline to end of IV iron therapy as seen with $Z = -2.440$, p 0.015; $Z = -3.945$, $p < 0.001$; $Z = -4.662$, $p < 0.001$; $Z = -2.999$, p 0.003 and (73, 5.395) p 0.001, respectively. Zinc did not show a statistically significant change for both study periods as presented in Table 4.25. The medians and means results of both study periods were still within their respective reference ranges for all variables. The only exception was sodium which was below the range for both IV baseline and end of oral therapy.

Table 4.25 Mean general health clinical chemistry changes in the entire study group during different study visits

VARIABLE		Baseline	End IV (W14)	*p*-value
BUN (mg/dL)	**X**	11.20	11.28	0.857
	SD	3.46	3.34	
S Creat. (mg/dl)	**X**	0.80	0.86	0.002
	SD	0.16	0.18	
Albumin (g/L)	**Med**	40	44	<0.001
	IQR	38-43	41-46.5	
ALP (U/L)	**Med**	52.5	62	0.587
	IQR	42.8-69.3	42.8-73	
AST (U/L)	**Med**	23.0	21.0	0.035
	IQR	19.0-29.0	17-27	
ALT (U/L)	**Med**	16	16	0.711
	IQR	13-20	13-19.5	
K (mmol/L)	**Med**	3.5	3.6	0.015
	IQR	3.3-3.7	3.3-4.0	
Na (mmol/L)	**Med**	127.0	131.8	<0.001
	IQR	123-129	129-136	
Phos (mmol/L)	**Med**	1.27	1.39	<0.001
	IQR	1.12-1.38	1.25-1.66	
Mg (mg/dL)	**Med**	2.2	2.30	0.003
	IQR	2.0-2.3	2.1-2.5	
Ca (mg/dL)	**X**	9.3	9.9	<0.001
	SD	0.7	0.7	
Zinc (umol/L)	**X**	11.20	11.64	0.216
	SD	2.61	2.20	

(X = mean; Med = median; SD = standard deviation; IQR = interquartile range; BUN = blood urea nitrogen; S Creat. = serum creatinine; ALP = alkaline phosphatase; AST = aspartate aminotransferase; ALT = alanine aminotransferase; K = potassium; Na = sodium; Phos = phosphate; Mg = magnesium; Ca = Calcium; STC = short-term changes; W14 = week 14)

The repeated measures ANOVA with Greenhouse-Geisser correction determined that there were statistically significant decreases for transferrin & TIBC between the different IV follow-up periods $F(1.67, 105.33) = 71.02$, $p < 0.001$ and $F(1.83, 128.35) = 90.99$, $p < 0.001$, respectively.

Table 4.26 Mean iron study, pregnancy and inflammatory indicators changes of the entire study groups at different study visits

VARIABLE		Baseline	STC (W12)	End IV (W14)	*p*-value
S Fe (µg/dL)	**Med**	28*!	61.0*	59.0!	<0.001
	IQR	19.3-39.8	46.0-84.0	43.0-73.5	
Trans. (g/L)		3.75*!	2.92*	3.00!	<0.001
	X	3.43-4.32	2.6-3.3	2.79-3.56	
	SD				
Ferritin (µg/L)	**Med**	8*	44*	30.0*	< 0.001
	IQR	5-12	26-84	16-43	
TIBC (µg/L)	**X**	436.5*!	355.6*	369.8!	<0.001
	SD	65.4	71.4	68.3	
TSAT (%)	**Med**	6.9*!	17.4*	17.4!	<0.001
	IQR	4.3-9.6	12.8-23.1	11.4-23.0	
hCG (IU/L)	**Med**	0*	0*!	0!	<0.001
	IQR	0-0	0-1	0-0	
CRP (mg/L)	**Med**	0.5	0.50	0.50	0.162
	IQR	0.3-0.6	0.4-0.8	0.4-0.7	

(X = mean; SD = standard deviation; Med = median; IQR = interquartile range; S Fe= serum iron; Trans. = transferrin; TIBC = total iron binding capacity; TSAT = transferrin saturation; hCG = human chorionic gonadotropin; cRP = c-reactive protein; STC = short-term changes, W12 = week 12; W14 = week 14; *$&! = means / medians with same special symbol signify statistical significant change within that variable)

Additionally, there were statistically significant differences for serum iron, ferritin, TSAT and HCG between the different follow-up periods, $\chi^2 = 47.348$, $p < 0.001$; $\chi^2 = 108.863$, $p < 0.001$; $\chi^2 = 59.521$, $p < 0.001$ and $\chi^2 = 59.660$,

p < 0.001, respectively. However, CRP did not show any pattern or significant changes among the different follow-up periods, as depicted in Table 4.26. All the variables' means and medians were within their respective reference ranges, except for IV baseline.

4.5.2.3 Mean changes in the group that abstained from soil consumption

The mean changes of anthropometric, body composition and general health indicators were not statistically or clinically different for all the indicators as seen in Table 4.27. This was supported by similar means and repeated measures ANOVA Greenhouse-Geisser correction with p values of above 0.05 for all variables. Signifying that there were no differences observed during the different study periods in all the measured variables.

Table 4.27 Mean anthropometric, body composition and general health indicators changes of the group that abstained from soil consumption

VARIABLE		Baseline	STC (W12)	End IV (W14)	*p*-value
Weight (kg)	**X**	66.6	66.0	65.8	0.963
	SD	14.6	14.8	15.4	
BMI (kg/m^2)	**X**	25.7	25.2	25.4	0.811
	SD	6.0	5.6	6.1	
Fat %	**X**	29.6	28.9	29.1	0.913
	SD	7.2	7.1	7.2	
Water %	**X**	47.3	47.4	48.0	0.703
	SD	6.3	6.8	6.1	
Muscle %	**X**	30.1	30.4	29.9	0.525
	SD	5.0	4.5	3.7	
Cal. req./day	**X**	2181	2203	2198	0.941
	SD	229	233	211	
Waist (cm)	**X**	85.7	84.3	85.7	0.716
	SD	13.8	13.2	13.7	
Hip (cm)	**X**	103.2	104.5	104.2	0.479
	SD	12.0	11.6	12.0	
WHR	**Med**	0.82	0.80	0.81	0.345
	IQR	0.77-0.88	0.75-0.85	0.76-0.89	
Bust (cm)	**Med**	92	87	89	0.812
	IQR	83-105	81-102	81-102	
SBP (mmHg)	**Med**	127	121	121	0.692
	IQR	114-135	111-132	111-129	
DBP (mmHg)	**X**	80.1	79.8	78.2	0.635
	SD	14.1	12.5	12.8	
Pulse (beats/min)	**X**	80.0	77.5	78.0	0.319
	SD	12.4	9.6	9.5	

(X = mean; SD = standard deviation; Med = median; IQR = interquartile range; SBP = systolic blood pressure; Cal. Reg/day = calorie requirement per day DBP = diastolic blood pressure; BMI = body mass index; WHR = waist:hip ratio; STC = short-term changes, W12 = week 12; W14 = week 14)

The repeated measures ANOVA with Greenhouse-Geisser correction determined that there were statistically significant changes for RBC, Hb, HCT, MCV, MCH, MCHC, RDW and PLT between the different IV study periods' follow-up $F(1.33, 49.24) = 8.39$, p 0.003; $F(1.29, 47.78) = 33.27$, p <0.001; $F(1.30\ 48.19) = 36.68$, p <0.001; $F(1.29, 47.89) = 7.69$, p 0.004; $F(1.34, 49.72) = 7.14$, p 0.006; $F(1.44, 53.30) = 5.11$, p 0.017; $F(1.57, 57.94) = 15.99$, p <0.001 and $F(1.42, 52.52) = 5.35$, p 0.015, respectively. The red blood cell parameters' means that increased from baseline and the follow-up periods' means were now within their respective reference ranges, except for RDW, that had all IV study periods' means above the reference range. However, MCHC difference between time periods did not meet the Bonferroni adjustment level of 0.017, plus it was the only one that showed a decrease.

Dissimilar to the red blood cell parameters' pattern, the platelet count showed a decrease from IV baseline, yet the means of all IV study periods were within the reference range. The mean platelet count dropped by 16% (314 – 263 = 51/314) from IV baseline to the end of the study. All the white blood cell counts did not reveal a particular pattern, were not significantly different during the different oral iron therapy study visits and were within the reference ranges, as shown in Table 4.28.

Table 4.28 Mean blood cell count changes of the group that abstained from soil consumption at different visits

VARIABLE		Baseline	STC (W12)	End IV (W14)	*p*-value
RBC ($x10^3/\mu L$)	**X**	4.01*	4.40*	4.34	0.003
	SD	0.50	0.48	0.46	
Hb (g/dL)	**X**	10.0*!	11.7*	11.4!	<0.001
	SD	1.7	1.01	1.01	
HCT (%)	**X**	30.9*!	35.8*	36.4!	<0.001
	SD	4.4	3.4	3.0	
MCV (fl)	**X**	75.7*	81.8	84.4*	0.004
	SD	8.5	6.5	7.7	
MCH (pg)	**X**	24.6*	26.7	26.7*	0.006
	SD	3.7	2.7	2.5	
MCHC (g/dl)	**X**	32.4	32.6	31.7	0.017
	SD	1.5	1.4	2.0	
RDW (%)	**X**	17.3*!	19.4*	21.6!	<0.001
	SD	2.4	3.8	4.2	
PLT ($x10^9/L$)	**X**	314	289*	263*	0.015
	SD	79	94	57	
WBC ($x10^9/L$)	**X**	5.34	5.34	5.08	0.346
	SD	1.64	1.69	1.50	
Neutrophil #	**Med**	2.30	2.47	2.5	0.707
	IQR	1.87-3.07	1.96-3.41	1.85-3.02	
Lymphocyte #	**X**	2.39	2.12	2.02	0.128
	SD	0.65	0.69	0.72	
Monocyte #	**X**	0.33	0.34	0.30	0.170
	SD	0.19	0.19	0.15	
Eosinophil #	**Med**	0.08	0.08	0.09	0.101
	IQR	0.04-0.14	0.04-0.15	0.05-0.14	
Basophil #	**Med**	0.03	0.03	0.03	0.592
	IQR	0.03-0.05	0.03-0.04	0.02-0.04	

(X = mean; SD = standard deviation; Med = median; IQR = interquartile range; RBC = red blood cell; Hb = haemoglobin; HCT = haematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; RDW = red cell distribution width; PLT = platelet; WBC =white blood cell; # = $x10^9$/L; STC = short-term changes, W12 = week 12; W14 = week 14; *$&! = means / medians with same special symbol signify statistical significant change within that variable)

Serum creatinine and BUN did not show a statistically significant change. The means for both variables were within the reference range for both study periods as presented in Table 4.29.

The mean of albumin showed statistically significant increases from IV baseline to end of IV iron study, as indicated by (39, -5.008) p <0.001, respectively. The liver enzymes and protein assessment values were within range for all variables, as shown in Table 4.29.

The median of potassium plus means of sodium, phosphate, magnesium and calcium showed statistically significant increases from IV baseline to end of IV iron therapy as seen with Z = -2.263, p 0.024; (39, -3.646) p 0.001; (39, -3.199) p 0.003; (39, -3.096) p <0.001 and (39, -3.999) p <0.001, respectively. There was no statistically significant change in zinc for both study periods, as presented in Table 4.29. The medians and means results of both study periods were still within their respective reference ranges for all variables. The only exception was sodium which was below the range for both baseline and end of oral therapy.

Table 4.29 Mean general health clinical chemistry indicators changes of the group that abstained from soil consumption at different visits

VARIABLE		Baseline	End IV (W14)	*p*-value
BUN (mg/dL)	**X**	10.91	11.67	0.200
	SD	3.51	3.40	
S Creat. (mg/dl)	**X**	0.81	0.84	0.083
	SD	0.13	0.13	
Albumin (g/L)	**X**	40.8	44.1	<0.001
	SD	3.6	4.0	
ALP (U/L)	**Med**	62.5	65	0.125
	IQR	42-80	47-73	
AST (U/L)	**Med**	24	22	0.282
	IQR	18-29	17-30	
ALT (U/L)	**Med**	15	15	0.876
	IQR	13-20	13-20	
K (mmol/L)	**Med**	3.5	3.6	0.024
	IQR	3.3-3.7	3.4-4	
Na (mmol/L)	**X**	127.6	133.1	0.001
	SD	6.3	7.9	
Phos (mmol/L)	**X**	1.25	1.43	0.003
	SD	0.24	0.26	
Mg (mg/dL)	**X**	2.14	2.31	0.004
	SD	0.25	0.34	
Ca (mg/dL)	**X**	9.35	9.94	<0.001
	SD	0.64	0.74	
Zinc (umol/L)	**X**	11.47	11.96	0.322
	SD	2.52	2.29	

(X = mean; Med = median; SD = standard deviation; IQR = interquartile range; BUN = blood urea nitrogen; S Creat. = serum creatinine; ALP = alkaline phosphatase; AST = aspartate aminotransferase; ALT = alanine aminotransferase; K = potassium; Na = sodium; Phos = phosphate; Mg = magnesium; Ca = Calcium; STC = short-term changes; W14 = week 14)

The repeated measures ANOVA with Greenhouse-Geisser correction determined that there were statistically significant decreases for transferrin & TIBC between the different IV follow-up periods $F(1.60, 52.88) = 37.03$, $p < 0.001$ and $F(2, 74) = 90.99$, $p < 0.001$, respectively. For TIBC Greenhouse-Geisser correction was not applied because Mauchly's Test of Sphericity was non-significant.

Table 4.30 Mean iron study, pregnancy and inflammatory indicators changes of the groups that abstained from soil consumption at different visits

VARIABLE		Baseline	STC (W12)	End IV (W14)	*p*-value
S Fe (µg/dL)	**Med**	28*!	56*	67.5!	<0.001
	IQR	20-42	45.5-86.5	29.8	
Trans. (g/L)	**X**	3.80*!	2.98*	3.10!	<0.001
	SD	0.72	0.49	0.62	
Ferritin (µg/L)	**Med**	7*	41*	22*	<0.001
	IQR	5-14	25.5-74.5	27.6	
TIBC (µg/L)		431.2*	349.3*	370.5*	<0.001
	X	62.6	65.7	70.3	
	SD				
TSAT (%)	**Med**	6.9*!	16.6*	16.8!	<0.001
	IQR	4.3-10.7	12.6-26.6	10.7-26.2	
hCG (IU/L)	**Med**	0*	0*!	0!	<0.001
	IQR	0-0	0-1	0-0	
cRP (mg/L)	**Med**	0.5*	0.6*	0.5	0,022
	IQR	0.3-0.6	0.4-0.8	0.4-0.7	

(X = mean; SD = standard deviation; Med = median; IQR = interquartile range; S Fe= serum iron; Trans. = transferrin; TIBC = total iron binding capacity; TSAT = transferrin saturation; hCG = human chorionic gonadotropin; cRP = c-reactive protein; STC = short-term changes, W12 = week 12; W14 = week 14; *$&! = means / medians with same special symbol signify statistical significant change within that variable)

There were also statistically significant differences for serum iron, ferritin, TSAT, CRP and HCG between the different follow-up periods, $\chi^2 = 31.801$, $p < 0.001$; $\chi^2 = 55.192$, $p < 0.001$; $\chi^2 = 33.211$, $p < 0.001$; $\chi^2 = 7.600$, p 0.022 and $\chi 2 = 36.000$, $p < 0.001$, respectively. Furthermore, the different variables' means and medians were within the reference range for week 12 and week 14, as depicted in Table 4.30.

4.5.2.4 Mean changes in the group that continues with soil consumption

A repeated measures ANOVA with a Greenhouse-Geisser correction determined that there was a statistically significant decrease between IV baseline and end of week 14 for pulse $F(1.33, 27.94) = 10.38$, p 0.002. The mean changes of all other anthropometric, body composition and general health indicators were not statistically or clinically different for all the indicators as revealed in Table 4.31. Signifying that there were no differences observed during the different study periods in all the measured variables

Table 4.31 Mean anthropometric, body composition and general health indicators changes of the group that continued with consumption of soil

VARIABLE		Baseline	STC (W12)	End IV (W14)	*p*-value
Weight (kg)	**X**	64.8	65.6	65.6	0,894
	SD	17.1	16.9	17.7	
BMI (kg/m^2)	**X**	24.8	25.8	25.5	0,614
	SD	6.6	6.5	6.5	
Fat %	**X**	28.8	29.6	29.3	0,669
	SD	7.9	8.0	7.7	
Water %	**X**	48.2	47.1	47.8	0,419
	SD	7.1	6.9	6.7	
Muscle %	**X**	29.9	29.2	29.6	0,699
	SD	4.1	4.5	4.0	
Cal. req./day	**X**	2179	2170	2177	0,946
	SD	249	263	254	
Waist (cm)	**X**	83.7	83.2	85.3	0,648
	SD	13.8	17.0	16.1	
Hip (cm)	**X**	102.3	103.2	102.0	0,524
	SD	13.8	12.8	13.2	
WHR	**X**	0.82	0.81	0.84	0.416
	SD	0.07	0.09	0.13	
Bust (cm)	**X**	89.9	91.9	93.0	0.358
	SD	14.3	14.0	14.3	
SBP (mmHg)	**X**	123.8	124.9	121.1	0,863
	SD	21.3	24.1	13.2	
DBP (mmHg)	**X**	80.2	81.8	77.3	0.625
	SD	11.9	16.1	11.5	
Pulse (beats/min)	**X**	83.9*	78.9*	79.3	0,002
	SD	12.1	11.0	9.05	

(X = mean; SD = standard deviation; SBP = systolic blood pressure; Cal. Reg/day = calorie requirement per day DBP = diastolic blood pressure; BMI = body mass index; WHR = waist:hip ratio; STC = short-term changes, W12 = week 12; W14 = week 14; *$&! = means / medians with same special symbol signify statistical significant change within that variable)

The repeated measures ANOVA with Greenhouse-Geisser correction determined that there were statistically significant changes for RBC, Hb, HCT, MCV, MCH, RDW, PLT, lymphocyte and basophil (no Greenhouse-Geisser correction) between the different IV therapy follow-up visits $F(1.27, 31.61) = 8.06$, p 0.005; $F(1.11, 27.68) = 23.18$, $p < 0.001$; $F(1.25, 31.18) = 47.69$, $p < 0.001$; $F(1.13, 28.33) = 38.73$, $p < 0.001$; $F(1.04, 25.86) = 13.49$, p 0.001; $F(1.55, 38.86) = 5.99$, p 0.009; $F(1.50, 37.40) = 6.18$, p 0.009; $F(1.13, 28.18) = 4.40$, p 0.041 and $F(2, 50) = 9.24$, $p < 0.001$, respectively. The red blood cell parameters' means that increased from baseline and the follow-up periods' means were now within their respective reference ranges, except for RDW where all IV visits were above the reference range and MCH which remained below the reference range, as seen in Table 4.32. Furthermore, the Post Hoc test for RDW showed significant changes which did not meet the Bonferroni adjustment level.

Dissimilar to the red blood cell parameters' increasing pattern, the platelet count showed a downward trend from IV baseline to end of IV therapy, yet the means of both follow-up periods' means were within the reference range. As captured in Table 4.32, the mean platelet count dropped by 19% (326 – 263 = 63/338) from IV baseline to the end of the study. The Post Hoc test of lymphocyte did not show a statistically significant change among the time periods. The basophil count increased during the short-term period and dropped again.

Table 4.32 Mean blood cell count changes of the group that continued with soil consumption at different visits

VARIABLE		Baseline	STC (W12)	End IV (W14)	*p*-value
RBC ($x10^3/\mu L$)	**X**	4.05!	4.47*!	4.34*	0,005
	SD	0.43	0.58	0.46	
Hb (g/dL)	**X**	9.31*!	11.5*	11.4!	<0,001
	SD	1.77	1.13	1.01	
HCT (%)	**X**	29.3*!	36.5*	36.3!	<0,001
	SD	4.3	3.1	3.0	
MCV (fl)	**X**	72.6*	82.2*	84.4*	<0,001
	SD	9.8	7.5	7.7	
MCH (pg)	**X**	23.1*	26.0*	26.7*	0,001
	SD	4.2	2.7	2.5	
MCHC (g/dl)	**X**	31.5	31.7	31.7	0,228
	SD	2.0	2.1	2.0	
RDW (%)	**X**	17.6	20.7	21.6	0,009
	SD	2.3	4.9	4.2	
PLT ($x10^9/L$)	**X**	326*	311	263*	0,009
	SD	106	96	57	
WBC ($x10^9/L$)	**X**	5.25	5.12	5.08	0.423
	SD	1.43	1.51	1.50	
Neutrophil #	**Med**	2.32	2.69	2.74	0.076
	IQR	1.83-2.74	1.81-3.47	1.87-3.11	
Lymphocyte #	**X**	2.37	1.98	2.02	0.041
	SD	0.89	0.76	0.72	
Monocyte #	**X**	0.36	0.31	0.30	0.304
	SD	0.18	0.17	0.15	
Eosinophil #	**Med**	0.08	0.08	0.09	0.188
	IQR	0.06-0.18	0.06-0.11	0.06-0.13	
Basophil #	**X**	0.034	0.045*	0.025*	<0.001
	SD	0.02	0.02	0.02	

(X = mean; SD = standard deviation; Med = median; IQR = interquartile range; RBC = red blood cell; Hb = haemoglobin; HCT = haematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; RDW = red cell distribution width; PLT = platelet; WBC =white blood cell; # = $x10^9/L$; STC = short-term changes, W12 = week 12; W14 = week 14; *$&! = means / medians with same special symbol signify statistical significant change within that variable)

All the other full blood count parameters did not reveal a particular pattern, were not significantly different during the different IV iron therapy study visits, plus the means and medians were within reference ranges.

The mean serum creatinine indicated a statistically significant increase from IV baseline to end of IV iron study, as indicated by (33, -2.661) p 0.012. BUN did not show a statistically significant change. The means for both variables were within the reference range for both study periods as shown in Table 4.33.

The median albumin indicated a statistically significant increase from IV baseline to end of IV iron study, as indicated by Z = -2.510, p 0.012. The liver enzymes did not show statistically significant changes in all variables. All the liver variables' means and medians were within range, as illustrated in Table 4.33.

The mean of phosphate, medians of sodium and calcium showed statistically significant increases from IV baseline to end of IV iron therapy as seen with (33, -4.281) p <0.001; Z = -2.075, *p* 0.038 and Z = -3.036 p 0.002, respectively. There were no statistically significant changes for potassium, magnesium and zinc for both study periods, as depicted in Table 4.33. The medians and means results of both study periods were still within their respective reference ranges for all variables. The only exception was sodium which was below the range for both baseline and end of oral therapy, plus IV baseline for potassium that was below the reference range.

Table 4.33 Mean general health clinical chemistry indicators changes of the group that continued with soil consumption at different

VARIABLE		Baseline	End IV (W14)	*p*-value
BUN (mg/dL)	**X**	11.55	10.83	0,318
	SD	3.56	3.27	
S Creat. (mg/dl)	**X**	0.79	0.87	0,012
	SD	0.19	0.23	
TP (g/L)	**Med**	83	90	0,214
	IQR	78-93	84-96	
Albumin (g/L)	**Med**	39	44	0,012
	IQR	36-44	41-46	
ALP (U/L)	**X**	58.7	57.0	0,467
	SD	21.4	20.4	
AST (U/L)	**Med**	23	21.0	0,050
	IQR	20-28	17-24.8	
ALT (U/L)	**X**	17.1	16.5	0,622
	SD	5.2	3.8	
K (mmol/L)	**X**	3.42	3.60	0,151
	SD	0.49	0.44	
Na (mmol/L)	**Med**	127.0	131	0,038
	IQR	123-129	129-133	
Phos (mmol/L)	**X**	1.31	1.54	<0,001
	SD	0.30	0.42	
Mg (mg/dL)	**Med**	2.20	2.30	0,223
	IQR	2.00-2.40	2.10-2.55	
Ca (mg/dL)	**Med**	9.1	9.8	0.002
	IQR	8.7-9.6	9.3-10.4	
Zinc (umol/L)	**X**	10.86	11.11	0.469
	SD	2.90	2.05	

(X = mean; Med = median; SD = standard deviation; IQR = interquartile range; BUN = blood urea nitrogen; Creat. = serum creatinine; TP = total protein; ALP = alkaline phosphatase; AST = aspartate aminotransferase; ALT = alanine aminotransferase; K = potassium; Na = sodium; Phos = phosphate; Mg = magnesium; Ca = Calcium; STC = short-term changes, W14 = week 14)

The repeated measures ANOVA determined that there were statistically significant differences for transferrin, total iron binding capacity & TSAT between the different IV follow-up periods $F(2, 58) = 33.22$, $p < 0.001$; $F(2, 64) = 29.06$, $p < 0.001$ and $F(2, 64) = 39.54$, $p < 0.001$, respectively. The changes resulted in the means and medians of the variables being within the reference range for all variables pertaining to the IV follow-up visits, as captured in Table 4.34.

Table 4.34 Mean iron study, pregnancy and inflammatory indicators changes of the groups that continued with soil consumption at different visits

VARIABLE		Baseline	STC (W12)	End IV (W14)	*p*-value
S Fe (µg/dL)	**Med**	28*!	61.5*	59.0!	<0.001
	IQR	19-39	48.5-78.8	43.0-73.5	
Trans. (g/L)	**X**	3.81*!	3.01*	3.14!	<0.001
	SD	0.64	0.76	0.58	
Ferritin (µg/L)	**Med**	8*	55.5*	30.0*	<0.001
	IQR	4-10	28.3-87.5	16-43	
TIBC (µg/L)	**X**	442.8*!	362.8*	369.0!	<0.001
	SD	69.1	77.7	67.1	
TSAT (%)	**X**	7.8*!	19.1*	17.5!	<0.001
	SD	5.1	8.2	7.9	
hCG (IU/L)	**Med**	0*!	0*	0!	<0.001
	IQR	0-0	0-1	0-0	
cRP (mg/L)	**Med**	0.45	0.50	0.50	0.607
	IQR	0.3-0.6	0.33-0.88	0.40-0.70	

(X = mean; SD = standard deviation; Med = median; IQR = interquartile range; S Fe= serum iron; Trans. = transferrin; TIBC = total iron binding capacity; TSAT = transferrin saturation; hCG = human chorionic gonadotropin; cRP = c-reactive protein; STC = short-term changes, W12 = week 12; W14 = week 14; *$&! = means / medians with same special symbol signify statistical significant change within that variable)

Plus, there were statistically significant differences for serum iron, ferritin and HCG between the different follow-up periods, $\chi^2 = 16.534$, $p < 0.001$; $\chi^2 = 54.189$, $p < 0.001$ and $\chi^2 = 24.043$, $p < 0.001$, respectively. However, CRP did not show any pattern or significant changes among the different follow-up periods.

4.5.3 Comparison of oral and IV

Comparison of changes in RBC and iron study indicators that were primarily utilised to assess the response to IDA treatment was split into two components. One entailed the comparison of parameters from baseline to the end of each therapy form, i.e. oral baseline versus the end of oral therapy and IV baseline versus the end of IV therapy. The second aspect involved mean difference comparison, implying subtracting the end of therapy from baseline and calculating the average change utilising the differences. The parameters that were selected were: Hb, MCV, MCH, ferritin, TIBC and TSAT. Factorial ANOVA was also performed to determine which group achieved superior correction of IDA.

4.5.3.1 Summary of oral and IV therapy changes for the entire study group

The repeated measures ANOVA with Greenhouse-Geisser correction determined that there were statistically significant differences for Hb, MCH and TIBC (no Greenhouse-Geisser correction) pertaining to both baselines and end of both iron therapy forms $F(1.34, 87.39) = 98.64$, p <0.001;

$F(1.26, 82.88) = 398.63$, $p < 0.001$ and $F(2, 142) = 114.47$, $p < 0.001$, respectively. The Friedman test indicated statistically significant differences for MCV, ferritin and TSAT between the two baselines and end of each therapy form, $\chi^2 = 52.625$, $p < 0.001$; $\chi^2 = 97.432$, $p < 0.001$ and $\chi^2 = 80.083$, $p < 0.001$, respectively.

When scrutinising Table 4.35, it can be observed that the changes brought about by oral therapy were minimal compared to IV therapy. At baseline the approximate percentage of participants who had values that were within the reference range for Hb, MCV and MCH were ±7% (mean plus 2SD = 12.2 g/dl = maximum); <25% (75th percentile = 79 fl) and <16% (mean plus SD = 26.7 pg), respectively. At the end of oral therapy, the situation was as follows; 16% (mean plus SD = 11.5 g/dl); >25% (75th percentile = 81 fl) and 25% (75th percentile = 27 pg), for Hb, MCV and MCH, respectively. Increased percentages were seen between IV baseline and end of IV therapy with Hb having >50% (mean = 11.6 g/dl); MCV had ±70% (25th percentile = 78 fl) and MCH had >50% (mean = 27.1 pg). The net change for correction into the reference ranges normal values based on red blood cell parameters for oral vs IV therapies in Hb, MCH and MCV were approximately 9% vs 34%, 5% vs 40% and 7% vs 25%, respectively.

Table 4.35 Mean baseline and end of therapy changes in the IDA treatment markers of the entire study population

VARIABLE		Baseline	End Oral / IV baseline	IV end	*p*-value
Hb	**X ± SD [25th -75th] (Min-Max)**	9.2 ±1.5* [8.3 – 10.3] (4.8 – 12.2)	9.7 ±1.8* [8.5 – 11.0] (5.2 – 12.3)	11.6 ± 0.9* [11.1 – 12.3] (9.2 – 14.1)	<0.001
MCV	**Med [25th -75th] (Min-Max)**	74.0* [67.0 – 79.0] (52.0 – 93.3)	76* [67.0 – 81.0] (53.0 – 93.0)	84* [78.0 – 88.6] (68.0 – 101.5)	<0.001
MCH	**X ± SD [25th -75th] (Min-Max)**	23.2 ± 3.5* [20.7 – 26.2] (14.4 – 30.0)	23.9 ± 4.0* [20.7 – 27.0] (13.8 – 31.9)	27.1 ± 2.6* [25.4 – 28.8] (21.0 – 34.3)	<0.001
Ferritin	**Med [25th -75th] Min-Max)**	6.5# [4.0 – 10.0] (2.0 – 42.0)	8* [5.0 – 12.0] (1.0 – 32.0)	26*# [15.0 – 44.0] (4.0 – 143.0)	<0.001
TSAT	**Med [25th -75th] Min-Max)**	4.5* [3.4 – 6.1] (1.6 – 31.4)	6.9* [4.3 – 9.6] (2.2 – 39.6)	17.4* [10.8 – 25.2] (3.2 – 36.6)	<0.001
TIBC	**X ± SD [25th -75th] (Min – Max)**	483.0 ± 77.6* [432-531] (261-652)	436.5 ± 65.4* [391.8-482.5] (298-619)	369.8 ± 68.3* [318.5-417.5] (252-561)	<0.001

(25th = 25th percentile; 75th = 75th percentile; Hb = haemoglobin; HCT = haematocrit; IV = intravenous; MCV = mean corpuscular volume; Med = median; SD = standard deviation; TIBC = total iron binding capacity; TSAT = transferrin saturation; X = mean)

Table 4.35 further demonstrate that for oral iron study results the percentage participants who were within reference range for ferritin, TSAT and TIBC was <10% (75th percentile = 10µg/dl), <2% (75th percentile = 6.1%) and <16% (mean plus SD = 405 µg/l), respectively. For end of oral ferritin had <25% (75th percentile = 12 µg/dl), <25% for TSAT (75th

percentile = 9.6%) while TIBC had >25% (25th percentile = 392 µg/l). End of IV revealed the following percentages: 75% for ferritin (25th percentile = 15 µg/dl); TSAT showed >50% (median = 17,4%) but <75% (25th percentile = 10.8%) while <75% (75th percentile = 418 µg/l) was obtained for TIBC. The mean percentage increase into the reference range for oral vs IV therapies for ferritin, TSAT and TIBC was estimated at 5% vs 50%; 5% vs 40% and 9% vs 45%, respectively.

Table 4.36 Mean calculated differences of oral versus IV therapies in the IDA treatment markers of the entire study group

VARIABLE		Oral	IV
Haemoglobin	**X / Median** **[25th -75th]** **(Min-Max)**	0.53 / 0.50 [0 – 1] (-2.4 – 4.1)	2.09 / 2.00 [0.8 – 3.2] (-0.4 – 5.7)
MCV	**X / Median** **[25th -75th]** **(Min-Max)**	1.51 / 2.00 [0.0 – 3.0] (-6.8 – 10.7)	10.46 / 10.00 [6.0 – 15] (-1.0 – 29.2)
MCH	**X / Median** **[25th -75th]** **(Min-Max)**	0.69 / 0.70 [0.0 – 1.3] (-2.7 – 4.0)	3.54 / 3.40 [1.7 – 5.0] (0.0 – 9.7)
Ferritin	**X / Median** **[25th -75th]** **(Min-Max)**	1.18 / 0.0 [-1.0 – 2.0] (-22.0 – 20.0)	24.07 / 17.00 [7.0 – 33.5] (-16.0 – 134.0)
TSAT	**X / Median** **[25th -75th]** **(Min-Max)**	2.64 / 1.37 [-0.1 – 3.9] (-23.5 – 34.8)	7.87 / 5.92 [1.0 – 13.1] (-12.2 – 33.4)
TIBC	**X / Median** **[25th -75th]** **(Min – Max)**	-44.33 / -40.5 [-88.5 – 9.3] (-217 – 97)	-63.25 / -72 [-101 – -33] (-207 – 105)

(25th = 25th percentile; 75th = 75th percentile; Hb = haemoglobin; HCT = haematocrit; IV = intravenous; MCV = mean corpuscular volume; Med = median; SD = standard deviation; TIBC = total iron binding capacity; TSAT = transferrin saturation; X = mean)

The mean / median difference achieved by 50% of participants for oral vs IV therapy for haemoglobin, MCV and MCH increased by 0.5 g/dl vs 2 g/dl; 2 fl vs 10 fl and 0.7 pg vs 3.4 pg, while the top 25% of participants achieved a similar rate at 1 g/dl vs 3.2 g/dl, 3 fl vs 15 fl and 1.3 pg vs 5.0 pg, respectively. The differences between oral and IV therapy were 3x, 5x and 3x more in favour of IV therapy for Hb, MCV and MCH, respectively.

Furthermore, as captured in Table 4.36, it's observed that an improvement in the top 25% of participants for iron study parameters of oral versus IV therapy indicated a similar trend as the red blood cell indices. Where improvements of 2 µg/dl vs 33.5 µg/dl were observed for ferritin, TSAT showed 3.9% vs 13.1% and -9.3 µg/l vs -33 µg/l for TIBC. In addition, the mean / median improvements were similar with 0 µg/dl vs 17 µg/dl, 2.64% vs 7.87% and -40.5 µg/l vs -72 µg/l for ferritin, TSAT and TIBC, respectively. The difference between oral and IV therapy was 16x, 3x and 2x more in favour of IV therapy for ferritin, TSAT and TIBC, respectively.

4.5.3.2 Summary of oral and IV therapy changes for the group that abstained from soil consumption throughout the oral therapy phase

The repeated measures ANOVA with Greenhouse-Geisser correction determined that there were statistically significant differences for Hb, MCV, MCH and total iron binding capacity (no Greenhouse-Geisser correction) pertaining to both baselines and end of both iron therapy forms $F(1.45, 56.38) = 62.95$, $p < 0.001$; $F(1.12, 43.59) = 20.03$, $p < 0.001$; $F(1.12, 43.68)$

= 20.89, p <0.001 and $F(2, 76) = 67.78$, p <0.001, respectively. The Friedman test indicated statistically significant differences for ferritin and TSAT between the two baselines and end of each therapy form, χ^2 = 44.243, $p < 0.001$ and χ^2 = 47.744, $p < 0.001$, respectively. However, ferritin's significant change was only observed for IV and not oral therapy. In Table 4.37, it can be observed that the changes brought about by oral therapy were minimal compared to IV therapy. At baseline the approximate percentage of participants who had values that were within the reference range for Hb, MCV and MCH were 0% (maximum= 10.4 g/dl); 25% (75^{th} percentile = 79.8 fl) and <16% (mean plus SD = 27.1 pg), respectively. At the end of oral therapy, the situation was as follows; >16% (mean plus SD = 11.7 g/dl) but <25% (75^{th} percentile =11.3 g/dl); >25% (75^{th} percentile = 82 fl) and >25% (75^{th} percentile = 27.5 pg), for Hb, MCV and MCH, respectively. Increased percentages were seen between IV baseline and end of IV therapy with Hb having <75% (25^{th} percentile = 11.4 g/dl); MCV had ±70% (25^{th} percentile = 78 fl) and MCH had >50% (mean = 27.4 pg). The net change for correction into the reference ranges of red blood cell parameters for oral vs IV therapies in Hb, MCH and MCV were approximately 20% vs 50%, 5% vs 40% and 10% vs 25%, respectively.

Table 4.37 Mean baseline and end of therapy changes in the IDA treatment markers of the abstained from soil consumption study group

VARIABLE		Baseline	End Oral IV baseline	IV end	*p*-value
Hb	**X ± SD** **[25th -75th]** **(Min – Max)**	9.4 ±1.5* [8.7 – 10.4] (4.8 – 10.4)	10.0 ±1.7* [9.2 – 11.3] (5.2 – 12.3)	11.8 ± 0.7* [11.4 – 12.3] (9.4 – 12.9)	<0.001
MCV	**X ± SD** **[25th -75th]** **(Min – Max)**	73.6 ± 8.6* [67.0 – 79.8] (54.0 – 93.3)	75.7 ± 8.9* [69.0 – 82.0] (53.0 – 93.0)	83.4 ± 7.4* [78.0 – 88.8] (70.0 – 101.5)	<0.001
MCH	**X ± SD** **[25th -75th]** **(Min – Max)**	23.7 ± 3.4* [20.7 – 26.2] (15.4 – 29.7)	24.6 ± 3.7* [21.9 – 27.5] (14.9 – 31.5)	27.4 ± 2.7* [25.9 – 29.0] (22.8 – 34.3)	<0.001
Ferritin	**Med** **[25th -75th]** **(Min – Max)**	7* [4.0 –12.5] (2.0 – 42.0)	7# [5.0 – 14.0] (3.0 – 32.0)	22*# [15.0 – 44.5] (8.0 – 128.0)	<0.001
TSAT	**Med** **[25th -75th]** **(Min – Max)**	4.8* [3.5 – 6.3] (2.3 – 31.4)	6.8* [4.3 – 10.7] (2.8 – 39.6)	16.8* [10.6 – 26.2] (4.1 – 36.6)	<0.001
TIBC	**X ± SD** **[25th -75th]** **(Min – Max)**	474.4± 80.0* [431.8-515.5] (261-652)	431.2± 62.5* [389-477] (298-539)	370.5 ± 70.3* [316.5-401.5] (261-561)	<0.001

(25th = 25th percentile; 75th = 75th percentile; Hb = haemoglobin; HCT = haematocrit; IV = intravenous; MCV = mean corpuscular volume; Med = median; SD = standard deviation; TIBC = total iron binding capacity; TSAT = transferrin saturation; X = mean)

Furthermore, as depicted in Table 4.37, for oral iron baseline study results the percentage participants who were within reference range for ferritin, TSAT and TIBC were <25% (75th percentile = 12.5 µg/dl), <5% (75th percentile = 6.3%) and >16% (mean plus SD = 396 µg/l), respectively. For end of oral therapy ferritin had <25% (75th percentile = 14 µg/dl), <25% for TSAT (75th percentile = 10.7%) while TIBC had >25% (25th percentile =

389 µg/l). End of IV revealed the following percentages: 75% for ferritin (25th percentile = 15 µg/dl); TSAT showed >50% (median = 16.8%) but <75% (25th percentile = 10.8%) while <75% (75th percentile = 402 µg/l) was obtained for TIBC. The approximate net change for correction into the reference ranges for oral vs IV therapies for ferritin, TSAT and TIBC were estimated at 5% vs 50%; 10% vs 35% and 9% vs 50%, respectively.

The mean / median increase achieved by 50% of participants for oral vs IV therapy for haemoglobin, MCV and MCH were 0.66 g/dl vs 1.85 g/dl; 2 fl vs 8 fl and 1.1 pg vs 3.2 pg, while the top 25% of participants achieved a similar rate at 1.1 g/dl vs 2.7 g/dl, 3 fl vs 11 fl and 1.4 pg vs 4.2 pg, respectively. The difference between oral and IV therapy was 3x, 4x and 3x more in favour of IV therapy for Hb, MCV and MCH, respectively.

Furthermore, as captured in Table 4.38, it's observed that an improvement in the top 25% of participants for iron study parameters of oral versus IV therapy indicated a similar trend as observed for red blood cell parameters. Where improvements of 2 µg/dl vs 29.8 µg/dl were observed for ferritin, transferrin saturation showed 6.0% vs 15.9% and -2.8 µg/l vs -31.3 µg/l for TIBC. In addition, the mean / median improvements were similar with 0 µg/dl vs 17 µg/dl, 2.7% vs 8.4% and -42.5 µg/l vs -61.6 µg/l for ferritin, TSAT and TIBC, respectively. The difference between oral and IV therapy was 16x, 3x and 2x more in favour of IV therapy for ferritin, TSAT and TIBC, respectively.

Table 4.38 Mean calculated differences of oral versus IV therapies in the IDA treatment markers of the group that abstained from soil consumption

VARIABLE		Oral	IV
Haemoglobin	**X / Median**	0.66 / 0.80	1.85 / 1.80
	[25th -75th]	[0.2 – 1.1]	[0.8 – 2.7]
	(Min – Max)	(-2.4 – 2.8)	(-0.4 – 5.7)
MCV	**X / Median**	2.11 / 2.00	8.48 / 8.00
	[25th -75th]	[1.0 – 3.0]	[6.0 – 11.0]
	(Min – Max)	(-4 – 8)	(-1.0 – 24.0)
MCH	**X / Median**	0.89 / 1.1	3.11 / 3.20
	[25th -75th]	[0.3 – 1.4]	[1.4 – 4.2]
	(Min – Max)	(-2.7 – 4)	(0.0 – 9.7)
Ferritin	**X / Median**	0.8 / 0.0	23.4 / 17
	[25th -75th]	[-1.0 – 2.0]	[7.0 – 29.8]
	(Min – Max)	(-22.0 – 20.0)	(-16 – 109)
TSAT	**X / Median**	2.7 / 1.1	8.4 / 6.4
	[25th -75th]	[-0.2 – 6.0]	[2.5 – 15.9]
	(Min – Max)	(-23.5 – 34.8)	(-12.2 – 33.4)
TIBC	**X / Median**	-42.5 / -42.5	-61.6 / -57.5
	[25th -75th]	[-80.5 – -2.8]	[-99.5 – -31.3]
	(Min – Max)	(-170 – 62)	(-207 – 105)

(25th = 25th percentile; 75th = 75th percentile; Hb = haemoglobin; HCT = haematocrit; IV = intravenous; MCV = mean corpuscular volume; Med = median; SD = standard deviation; TIBC = total iron binding capacity; TSAT = transferrin saturation; X = mean)

4.5.3.3 Summary of oral and IV therapy changes for the group that continued with soil consumption throughout oral therapy

The repeated measures ANOVA with Greenhouse-Geisser correction determined that there were statistically significant differences for Hb, MCV, MCH and total iron binding capacity (no Greenhouse-Geisser correction) pertaining to IV baselines and end of IV iron therapy; $F(1.22, 30.46) = 36.28$, $p < 0.001$; $F(1.22, 31.81) = 52.75$, $p < 0.001$; $F(1.12, 29.21) = 21.94$,

p <0.001 and $F(2, 64) = 47.20$, p <0.001, respectively. The Friedman test indicated statistically significant differences for ferritin and TSAT between the IV baselines and end of IV therapy, $\chi^2 = 54.500$, $p < 0.001$ and $\chi^2 = 32.788$, $p < 0.001$, respectively. However, for TIBC and TSAT there was a statistical significant change for oral therapy as well. Similar to entire study population and Group A, the changes brought about by oral therapy were minimal compared to IV therapy in Group B, as illustrated in Table 4.39. At baseline the approximate percentage of participants who had values that were within the reference range for Hb, MCV and MCH were 0% (maximum= 11.1 g/dl); >16% (mean plus SD = 80.7 fl) and <16% (mean plus SD = 26.3 pg), respectively. At the end of oral therapy, the situation was as follows: <16% (mean plus SD = 11.2 g/dl); <25% (75^{th} percentile = 79.3 fl) and >16% (mean plus SD = 27.3 pg), for Hb, MCV and MCH, respectively. Increased percentages were seen between IV baseline and end of IV therapy with Hb having 50% (mean = 11.5 g/dl); MCV had 75% (25^{th} percentile = 80 fl) and MCH had <50% (mean = 26.9 pg). The net change of correction into reference ranges for red blood cell parameters of oral vs IV therapies in Hb, MCH and MCV were approximately 10% vs 40%, 8% vs 50% and 5% vs 30%, respectively.

Table 4.39 Mean baseline and end of therapy changes in the IDA treatment markers of the group that continued with soil consumption

VARIABLE		Baseline	End Oral IV baseline	IV end	*p*-value
Hb	**X ± SD [25th - 75th] (Min – Max)**	9.0 ±1.4* [7.8 – 10.2] (6.3 – 11.1)	9.3 ±1.8# [7.9 – 10.6] (5.4 – 12.2)	11.5 ± 1.0*# [10.9 – 12.2] (9.2 – 14.1)	<0.001
MCV	**X ± SD [25th - 75th] (Min – Max)**	72.0 ± 8.7* [67.0 – 78.1] (52.0 – 89.0)	72.6 ± 9.8# [65.0 – 79.3] (53.0 – 93.0)	84.6 ± 7.7*# [80.0 – 88.6] (68.0 – 101.0)	<0.001
MCH	**X ± SD [25th - 75th] (Min – Max)**	22.7 ± 3.6* [20.0 – 25.3] (14.4 – 30.0)	23.1 ± 4.2# [19.6 – 26.5] (13.8 – 31.9)	26.9 ± 2.6*# [25.4 – 28.8] (21.0 – 32.2)	<0.001
Ferritin	**Med [25th - 75th] (Min – Max)**	6* [5.0 –8.0] (2.0 – 14.0)	8# [4.0 – 10.0] (1.0 – 22.0)	30*# [16.0 – 43.0] (4.0 – 143.0)	<0.001
TSAT	**Med [25th - 75th] (Min – Max)**	4.3* [3.1 – 6.1] (1.6 – 14.4)	6.9* [3.9 – 9.5] (2.2 – 24.4)	17.4* [11.4 – 23.0] (3.2 – 35.0)	<0.001
TIBC	**X ± SD [25th - 75th] (Min – Max)**	492.3± 74.8* [439-554] (316-613)	442.8 ± 69.1* [394-496] (334-619)	369.0 ± 67.1* [325-427] (252-512)	<0.001

(25th = 25th percentile; 75th = 75th percentile; Hb = haemoglobin; HCT = haematocrit; IV = intravenous; MCV = mean corpuscular volume; Med = median; SD = standard deviation; TIBC = total iron binding capacity; TSAT = transferrin saturation; X = mean)

In addition, as shown in Table 4.39, for oral iron baseline study results the percentage participants who were within reference range for ferritin, TSAT

and TIBC were 0% (maximum = 14 µg/dl), 0% (maximum = 14.4%) and <16% (mean minus SD = 418 µg/l), respectively. For end of oral iron therapy ferritin had <25% (75th percentile = 10 µg/dl), <25% for TSAT (75th percentile = 9.5%) while TIBC had >25% (25th percentile = 394 µg/l). End of IV revealed the following percentages: >75% for ferritin (25th percentile = 16 µg/dl); TSAT showed >50% (median = 17.4%) but <75% (25th percentile = 10.8%) while >50% (mean = 369 µg/l) but <75% (75th percentile = 427 µg/l) was obtained for TIBC. The approximate net change to within the reference range for oral vs IV therapies for ferritin, TSAT and TIBC was estimated at 10% vs 60%; 10% vs 40% and 9% vs 50%, respectively.

The mean / median difference achieved by 50% of participants for oral vs IV therapy for haemoglobin, MCV and MCH increased by 0.5 g/dl vs 2 g/dl; 2 fl vs 10 fl and 0.7 pg vs 3.4 pg, while the top 25% of participants achieved a similar rate at 1 g/dl vs 3.2 g/dl, 3 fl vs 15 fl and 1.3 pg vs 5.0 pg, respectively. The difference between oral and IV therapy was 3x, 5x and 3x more in favour of IV therapy for Hb, MCV and MCH, respectively.

Furthermore, as captured in Table 4.40, it's observed that an improvement in the top 25% of participants for iron study parameters of oral versus IV therapy indicated a similar trend as the red blood cell indices. Where improvements of 2 µg/dl vs 33.5 µg/dl were observed for ferritin, TSAT indicated 3.9% vs 13.1% and -9.3 µg/l vs -33 µg/l for TIBC. In addition, the mean / median improvements were similar with 0 µg/dl vs 17 µg/dl, 2.64% vs 7.87% and -40.5 µg/l vs -72 µg/l for ferritin, TSAT and TIBC, respectively.

The difference between oral and IV therapy was 16x, 3x and 2x more in favour of IV therapy for ferritin, TSAT and TIBC, respectively.

Table 4.40 Mean calculated differences of oral versus IV therapies in the IDA treatment markers of the group that continued with soil consumption

VARIABLE		Oral	IV
Haemoglobin	**X / Median**	0.37 / 0. 35	2.20 / 2.00
	[25th -75th]	[-0.2 – 0.8]	[0.7 – 3.9]
	(Min – Max)	(-1.5 – 4.1)	(-0.4 – 5.1)
MCV	**X / Median**	0.77 / 1.0	11.93 / 11.70
	[25th -75th]	[-2.1 – 3.3]	[4.0 – 16.7]
	(Min – Max)	(-6.8 – 10.7)	(2.0 – 29.2)
MCH	**X / Median**	0.44 / 0. 35	3.75 / 3.3
	[25th -75th]	[0.3 – 1.13]	[1.8 – 5.5]
	(Min – Max)	(-2.5 – 4.0)	(0.2 – 9.2)
Ferritin	**X / Median**	1.64 / 4.32	24.67 / 17.00
	[25th -75th]	[-0.25 – 3.25]	[7.25 – 31.75]
	(Min – Max)	(-6 – 17)	(-10 – 134.0)
TSAT	**X / Median**	2.52 / 2.13	8.78 / 9.41
	[25th -75th]	[-0.13 – 3.54]	[0.65 – 30.82]
	(Min – Max)	(-7.14 – 18.7)	(-7.14 – 30.82)
TIBC	**X / Median**	-46.6 / -40.0	-84.3 / -82
	[25th -75th]	[-96 – 15]	[-108 – -40.5]
	(Min – Max)	(-217 – 97)	(-378 – 64)

(25th = 25th percentile; 75th = 75th percentile; Hb = haemoglobin; HCT = haematocrit; IV = intravenous; MCV = mean corpuscular volume; Med = median; SD = standard deviation; TIBC = total iron binding capacity; TSAT = transferrin saturation; X = mean)

4.5.3.4 Factorial ANOVA analysis to check the effect of groups

In the previous section it was observed that there were slight changes noted between Group A and B. In addition, one of the objectives of the study was to evaluate if the abstaining group would lead to a better response to oral iron therapy. Thus factorial ANOVA analysis was undertaken to ascertain if belonging to a particular group made a difference based on oral and IV therapy, baseline versus the end of therapy for each type.

The variables that were subject to factorial ANOVA were red blood cell parameters: RBC, haemoglobin, MCV, MCH & RDW; iron study parameters: serum iron, ferritin, transferrin, total iron binding capacity & TSAT; platelet count; c-reactive protein and eosinophil count. The only variable that indicated a statistically significant change between the groups was MCV.

A factorial ANOVA with Greenhouse-Geisser correction determined that there was a statistically significant difference for MCV pertaining to total oral versus IV iron therapy $F(1.17, 76.10) = 13.9.6$ $p < 0.001$. The means ± SDs for Group A versus B were 73.0 ± 8.5 vs 72.8 ± 9.1, 75.1 ± 8.8 vs 73.8 ± 9.4 and 80.5 ± 6.2 vs 88.9 ± 6.8, for oral baseline, end of oral/IV baseline and end of IV therapy, respectively. The significant difference was observed at the end of IV iron therapy phase. The rest of the above-mentioned variables' statistical data are captured in Appendix K, for ease of reading.

CHAPTER 5

DISCUSSION

5.1 INTRODUCTION

Data collection and implementation of the study protocol as described in the methodology section spanned approximately 18 months. This intense and laborious undertaking's purpose was to compare oral and intravenous iron therapy by performing an intervention (*in vivo*) study that entailed the following objectives: firstly, to determine the possible impact of certain geophagic practices on the development of iron deficiency anaemia. Secondly, to determine if bleeding, gastrointestinal and menstrual factors, could have played a role in the development of IDA. Thirdly and fourthly, to ascertain the effectiveness of oral and IV iron therapy in geophagia cases of IDA and finally, to compare iron therapy modes. The significance of the findings that were presented in the previous chapter is elaborated upon by utilising the same sequence and logic as captured in the objectives above.

5.2 GEOPHAGIC PRACTICES

The participants have started with soil consumption at an early age as the median years of consumption was eight years and the mean participant's age was 32 years. From this it can also be insinuated that once people start they do not seem to stop, especially looking at 30 years being the maximum

consumption of soil. This is especially important since geophagic individuals find it hard to quit the habit (Ekosse & Jumbam 2000). There was no significant difference between Group A and Group B in terms of the number of years of consumption, thus randomization was successful pertaining to years of soil consumption.

The amount of soil consumed in a day by participants was high as the median was a quarter of a kilogram with a maximum of 1.5 kg. There was no statistically significant difference based on the amount of soil consumed by groups, thus differences observed in the different group was not influenced by the different amount of soil consumed.

The soil colour most commonly consumed was black, approximately 60%, which is the prevalent type in Botshabelo. This is key because black soil has been associated with the removal of potassium. The second most common was khaki, followed by red and yellow. Khakhi may be the colour of Calabar chalk. Red is thought to be rich in iron but this has not been proven correct. Majority of participants consumed one soil colour while two soil colours were half that of one colour. This signifies that geophagic individuals are particular as to what they prefer and where they obtain their geophagic material (Young *et al.* 2010; Perridge *et al.* 2011).

However; there was no direct correlation between the years of consumption and the amount of soil consumed in relation to the severity of red blood cell and iron study parameters that are associated with IDA. The lack of direct correlation may be due to many confounding factors that are involved in the

development of IDA. However, this does not eliminate the possible contribution of these aspects in the development of IDA in geophagia. Furthermore, other associations or correlation calculations were not undertaken which could reveal a different picture. This type of correlation study has not been performed before. This might be due to IDA being worsened by soil further removing dietary iron or soil coating or changing the GIT surface, thus interfering with iron absorption (Pebsworth *et al.* 2013; Seim *et al.* 2013).

5.3 BLEEDING ASSESSMENT

Bleeding is one of the major cause of IDA, thus platelet function assessment, menstrual and occult blood assessments were undertaken. Platelets play a pivotal role in haemostasis thus if there is a quantitative or qualitative abnormality bleeding is encountered. The platelet numbers were summarised with the FBC results, and thus will not be discussed in this section. PFA results were only obtained for 25% (20/80) of the study population. Only 5% (1/20) had an abnormal PFA result. At face value, it can be concluded that platelet function is not associated with the development of IDA in geophagia. However, it must be noted that a representative sample was not obtained, thus the results may be misleading.

The female reproductive system is one of the most predominant sites of blood loss, which means loss of iron resulting in IDA. And that is why

females are prone to IDA. The median blood loss per menstrual cycle was 51ml with (75^{th} percentile of 82ml) a maximum of 337ml. Heavy menstrual bleeding, >80ml loss per menstrual cycle (Shivani & Ruchika 2017), was seen in 25% of the participants. The lady with monthly losses of 337ml was advised to visit the clinic where contraceptives were prescribed while she was waiting for gynaecology appointment. Her cycle improved somewhat while taking contraceptives. An average coefficient variation (CV) of 20% was observed from month to month for those ladies that submitted more than one pictogram. This high CV could have been due to irregular menstruation, influenced by participants who received contraceptives that decreased the amount of blood loss and the normal internal variation that is expected (Sharkar *et al.* 2008; Elbartarny *et al.* 2014). Heavy menstrual blood loss is associated with development of IDA, thus this could partly explain the role played by menstrual blood loss. However, since heavy menstrual blood loss is not found in most cases, it may signify a developmental contribution while soil consumption may represent the contributory aspects to IDA in geophagia. Furthermore, there was no correlation between menstrual blood loss and the severity of IDA based on red blood cell and iron study parameters. This could be due to heavy menstrual bleeding being encountered in only 25% of cases and these findings confirm the complexity of IDA development in geophagia. If a larger population could be obtained, those findings may present a different picture.

An accurate assessment of the contribution of GIT bleeding could not be determined, due to the low number of samples collected (32/84 = 38%) and only 4% (3/84) having samples for both start and end of study samples. The reason for the low numbers was participants being uncomfortable handling faeces. It was essential to determine if abstaining from soil consumption could result in positive samples being negative at the end of the study. The low number of participants with positive occult blood indicates that GIT may not be a major factor in Geophagia. However, further investigations are needed to ascertain the role of GIT bleeding.

Bleeding is major contributor to development of IDA. However, its role in geophagia is still to be determined. A look at platelet function and GIT bleeding seems to indicate that these avenues might not be significant, as observed from the results. However, it must be borne in mind that the sample size was not representative of study population, thus such conclusion cannot be accepted. With menstrual blood loss, 25% of participants had heavy menstrual blood flow. That is a significant finding that warrants further investigation.

5.4 DISCUSSION OF BASELINE PROFILE OF THE ENTIRE STUDY POPULATION

The selection criteria were designed for choosing individuals with mild hypochromic microcytic anaemia and ferritin levels that are associated with IDA. From the 320 screened participants only 84 met the inclusion criteria

at a rate of 26% which was comparable to the odds ratio of 1.4 -2.5 (95% CI) that a geophagic person would have IDA as reported by Raphuthing *et al.* (2014). The drop-out rate was 10% (8/84) which was lower than what is expected for this kind of study, e.g. 18% (Macdougall *et al.* 2014a) and 22% (Froessler *et al.* 2013). The reasons were due to the process followed to obtain consent. Knowing where participants lived enabled easy follow-up and this approached made participants more comfortable. Three of the 45 participants in the abstain group achieved the desired response to oral iron hence they continued with oral therapy. The reason for the response to oral therapy could be because the participants have not consumed soil for a long period and they don't consume a significant amount of soil. As observed by the results. However, since there are only three participants it was decided not to analyse their results separately as the small size might lead to misrepresentation of data analysis as the population is not representative.

5.4.1 General health profile of the entire study group

The age criteria for the study group was within inclusion criteria except for a 15-year-old. This participant was included because her haemoglobin level was low, her geophagic practice was intense plus she had difficulty concentrating at school. The parents together with the participants vehemently request that she be admitted to the study as she was consuming copious amounts of soil. Thus the participant consented and the mother assented to her participation.

A quarter of participants showed a tendency towards hypertension, with 16% having stage 2 hypertension (>160 systolic or >100 diastolic). This is comparable to the 24.4% found by Copper *et al.* (1997) and Steyn *et al.* (2001). Bradsaw *et al.* (2011) found a rising trend in the prevalence of hypertension for females in all age groups over a period of 10 years (1998 – 2008). The average prevalence for the study's age group in 1998 was 21% while in 2008 it was 30%. The prevalence in a recent study for Soweto women - which unfortunately was of 40 – 60-year-olds - was between 50% and 56% (Gómez-Olive *et al.* 2017). This was comparable to 24% – 44 % for 1998 and 34% – 63 % for 2008 studies of 40-60-year-olds. It should also be noted that the prevalence increases with age (Bradsaw *et al.* 2011; Gómez-Olive *et al.* 2017). The reasons for the increase is related to the development of secondary diseases, sedentary lifestyle and hypertension having latent symptoms. Secondary diseases that result in the development of hypertension include artery, kidney, heart or endocrine diseases. Another reason is the lack of testing during the younger ages as people remain oblivious to the presence of the "silent killer" called hypertension. The final reasons that may contribute is a lifestyle consisting of stress, poor diet or lack of exercise leading to obesity (Gómez-Olive *et al.* 2017).

Based on BMI, WHR and body fat %, more than 50% of participants presented with a common but serious health condition in South Africa, obesity. The problem was even worse in 25% of participants as they were classified in the high-risk group. This scenario is strongly supported by just

above 16% of participants having ideal muscle mass, as a fat percentage is inversely proportional to muscle mass. One of the theories of geophagia is its theoretical association with hunger. However, most of the participants were not malnourished but obese. The obesity in their case could be related to incorrect dietary habits and lack of exercise.

5.4.2 Full blood count profile of the entire study group

The red blood cell parameters revealed a hypochromic microcytic anaemia with anisocytosis (variation in cell size). The red blood cell count's mean was within range with only a quarter of participants with decreased red blood cell count. The bone marrow compensation for IDA is to increase the number of cell division, thus producing an increased number of small red blood cell in an attempt to wait for the iron to be available (Worwood *et al*. 2017). Once that approach fails, then less haemoglobin is added resulting in the production of hypochromic red blood cells. The 75th percentile of MCH, MCV and Hb were below the reference range signifying hypochromic microcytic anaemia was observed in more than 75% of participants. MCHC was decreased in between 50% and 25% of participants, contrary to MCH with more than 75% of participants. The reason for this discrepancy in measures that both determine the amount of haemoglobin within red blood cells is as a result of MCHC being insensitive to small changes in Hb content that affect MCH much more (Worwood *et al.* 2017). The discrepancy is also caused by MCH measuring the mean weight of Hb in the cell while MCHC

measured the mean concentration (Hoffbrand & Moss 2016:35). Another feature was the increased RDW as cells of different sizes were produced, RDW was increased in >84% of participants signifying anisocytosis. This picture was expected as the inclusion criteria were aimed at participants with IDA.

Furthermore, it was highlighted that less than 5 % of participants had higher haemoglobin values than suggested by inclusion criteria. This phenomenon can be explained by the factors that follow. As far as ferritin levels were concerned, if the concentration was above 15 µg/dl but below 30 µg/dl, coupled with a hypochromic microcytic anaemia picture, the participants were accepted in the study pending further evaluation during baseline where CRP concentrations were taken into account. A haemoglobin level allowance of about 0.5 g/dl was granted because of instrument measurement error and timing of blood collection. It must be noted that it was extremely difficult for participants to qualify, and this factor necessitated the flexibility of criteria.

The platelets and white blood cell parameters provided interesting discussion pointers. Increased platelet is associated with bleeding (Hoffbrand & Moss 2016: 173), which is a major cause of IDA. Only 25% of participants had increased platelet count. Another theory that has come to the fore is that increased platelet count is encountered in the absence of iron (Kulnigg-Dabsch *et al.* 2012). All the white blood cell parameters were within the reference ranges signifying that the immune system was intact.

In addition, there was no parasitic infestation to mention as eosinophil counts were normal. The lack of parasitic infestation was similar to other findings in the literature (Kutalek *et al.* 2010; Young *et al.* 2010; Perridge *et al.* 2011; Raphuthing *et al.* 2014) and dissimilar to others (Geissler *et al.* 1998). The difference may be embedded in the location of the geophagic soils, with others being in the tropics (Geissler et al. 1998) while the others are in dryer parts of the world (Perridge *et al.* 2011).

5.4.3 Clinical chemistry general health and iron study profile of the entire study group

For mean kidney function test results only the maximums were increased, as means plus 2SD were within range, signifying that only one participant had increased kidney function test results. Creatinine further revealed that <25% had levels below the range. This is of no clinical consequence as only increased values are important (Yilma *et al.* 2019). These findings signified that the kidney function of the participants was not compromised.

More than 84% of participants had normal albumin and liver enzyme levels as depicted in Table 4.5 by their mean plus SD results. In addition, for all liver enzymes, it was just the maximum that was above the range, signifying that the liver function of the participants was normal. However, a few decreased levels were seen but these do not pose a serious concern for the participants' health. The reason for monitoring liver enzymes was to be on

the lookout for hepatocellular damage, which can influence the ferritin level because the liver is a storage organ for iron (Hoffbrand *et al.* 2016: 28).

Decreased level of sodium and potassium was encountered in 75% and 50% of participants, respectively. While between 16% and 25% had increased magnesium plasma levels. This effect might have been brought about by soil removing these minerals and probably the soil contains an increased level of magnesium. Black coloured soil from South Africa has previously been associated with hypokalaemia (Chaushev *et al.* 2003) which might explain the decreased potassium level. Contrary to the above-mentioned minerals; calcium, phosphate and zinc were within range with the exception of minimum and maximum. This signified that soil did not affect the levels of these minerals.

During IDA serum iron, ferritin and TSAT decreases while the TIBC and transferrin increases. Serum iron, ferritin and TSAT decrease because there is less iron within the circulation. This was evidenced by decreased levels in >84% of participants of the former iron study parameters. In addition, the means plus SD of TSAT was 10%. Levels <10% are strongly associated with IDA (Worwood *et al.* 2017). Serum iron, ferritin and TSAT are decreased because iron is utilised for synthesis of all cells that require iron. Therefore, there is insufficient iron to circulate in the serum, to be stored as ferritin and transferrin is not saturated as less iron is transported in circulation (Hoffbrand & Moss 2016: 31). TIBC and transferrin levels are increased in IDA as the body attempts to obtain as many iron molecules as

possible. Thus, TIBC showed 85% of participants with increased levels while transferrin had between 50% and 75%.

Normal levels of HCG and CRP indicated that the participants were not pregnant and inflammation was not detected in the participants. No pregnant women were allowed in the study due to the uncertainty of complications for the foetus concerning IV iron. CRP was performed to eliminate inflammation which is used as a differential diagnostic tool for anaemia of chronic disease (ACD). In ACD, which is caused by chronic inflammation and infection, CRP and hepcidin are increased. Hepcidin prevents iron release from macrophages and absorption from GIT through ferroportin degradation. The diagnostic laboratory findings of ACD have decreased serum ferritin, normal TIBC and increased ferritin (Hoffbrand & Moss 2016: 37). The increase in ferritin is not only affected by iron storage but by the fact that ferritin is an acute phase protein, which is increased during inflammatory disorders.

5.4.4 General health profile of both study groups

The lack of significant differences in the anthropometric, body composition and general health indicators, signifies that randomization produced two similar groups. Thus randomization worked effectively and changes observed after treatment were due to the intervention and not because of dissimilar starting points.

5.4.5 Full blood count profile of both study groups

There were no statistically significant changes in the full blood count parameters, thus the hypochromic microcytic anaemia was akin in both groups at baseline. In addition, this finding signifies that randomization produced two similar groups. Thus changes observed after treatment were not due to differences between the groups themselves. Similarly, there was no significant difference in bleeding, parasitic infestation and immune system variables of participants based on platelet, eosinophil and white blood cell parameters, respectively.

5.4.6 Clinical chemistry general health and iron study profile of both study groups

The kidney and liver function variables did not show statistically significant changes in both groups. Similarly; minerals, iron studies, pregnancy determining variable (HCG) and inflammatory (CRP) indicators were similar for both groups. All the above data indicated that randomisation was successful. In spite of that, it was observed that the means of serum iron, ferritin, TSAT and HGC were slightly higher for Group A The phenomenon was caused by the outlier which was depicted by the maximum, while Group B's mean transferrin was increased for a similar reason.

5.5 INTERVENTION STUDY DISCUSSION

This section formed the basis of comparing oral versus IV iron therapies, plus the effect of soil on GIT and iron absorption. The comparison was undertaken by first evaluating the effect of oral followed by IV therapy, and then ending with a comparison of the two therapies. By dividing the participants into two groups, the direct and indirect effect of soil on GIT lining and iron absorption could be determined.

5.5.1 Oral iron therapy discussion

Oral iron therapy is the mainstay for therapy of IDA but its challenges include non-response to therapy due to compliance issues caused by side effects and health status of the individual. Compliance of participants determines whether therapy will succeed or not, thus two methods were implemented to evaluate it. Side effects were also recorded to ascertain if the medication has untoward health effects that could have decreased the level of compliance. Oral iron therapy was monitored at different time intervals while clinical chemistry general health indicators, which was composed of kidney, liver function test and minerals' level, were performed at the beginning and the end of the study periods.

5.5.1.1 Compliance for oral medication

Compliance was much higher in this study (97%) than other studies, e.g. 67% (Anker & von Haehling 2012). This might be explained by the least

number of side effects encountered due to the type of oral preparation utilised (Geisser 2007), motivation of participants to quit the habit, following up participants at their own homes, participants feeling that they would let the research team down and/or the individualised follow-up of participants by the research team. There was no statistically significant difference between the calculated and declared compliance calculation. This signified that the participants could recall the times that they missed doses and they were honest about it.

The calculated compliance percentage was slightly higher than the declared in all cases and the calculated exceeded 100%, as evidenced by the maximum. This resulted from the inclusion of excess medication that was consumed by participants above the recommended dose. Overdose was not captured on the questionnaire where the primary objective was to determine the declared missed doses instead of overdose. The reasons why participants took more medication than required were the palatable taste and some thinking that it will remove the desire for soil. Nevertheless, some participants' compliance left much to be desired with minimum values from 23 to 43% for Group B and 47 to 74% for Group A. The reason for the continuous group having lower minimums were because they were less motivated. However, the abstain group participants thought that oral treatment will work if they keep to the regime.

There was no preference for either pills or capsule since there was no statistically significant difference between the two oral therapy types, even

based on groups. However, an exception was observed for the calculated combination of both oral therapies. Where the median of Group A was 6% higher than Group B's median. Most importantly it should be noted that this was not of clinical significance since both compliances were above the acceptable threshold of 67% (Anker & von Haehling 2012).

5.5.1.2 Discussion of mean changes of oral iron therapy of the entire group at different visits

There were no statistically significant differences in any of the anthropometric, body composition and general health indicators of the entire study population for different visits during the different oral therapy follow-up periods. This was expected since oral iron therapy should not have an effect on these variables. Their reason for the inclusion of these variables was to assess the general health of the participants and to observe if their health suddenly deteriorates. Sudden health deterioration would affect other measured variables.

According to the repeated measures ANOVA there were no statistically significant differences for all full blood count parameters. However, the post-hoc analysis showed a statistically significant change from baseline to end of oral therapy in Hb, HCT, MCV and MCH. These differences were not of clinical significance as the expected response in Hb is an increase of 2g/dl in 3-4 weeks (Jimenez *et al.*, 2015, Bonovas *et al.*, 2016, Martin-Malo *et al.* 2019), the mean Hb change was only 0.5g/dl over a ten-week

period. The reason why ANOVA did not pick up the change was that it was a small difference while the other six comparisons did not show any difference, thus concealing the small difference. These results signify that time and increased doses of oral iron did not have a significant impact on therapy outcome. The oral iron haematological findings are contrary to those observed in IBD, CHF, CKD and pregnancy where anaemia corrected (Becuzzi *et al.* 2014, Toblli & Genaro 2015, Bonovas *et al.* 2016, Zhang *et al.* 2016). This correction was observed with a similar dosage and follow-up periods.

Bleeding, a leading cause of IDA can be indicated by an increase in PLT count, the platelet count did not show statistically significant change because the end of oral therapy was 6% lower than baseline. There is a theory that iron can lead to an increased propensity to infection (Carrier *et al.* 1989; Tompkins *et al.* 2001; Seril *et al.* 2002). However, the white blood cells including eosinophil count did not show any effect These findings indirectly eliminated infections and parasitic infestation (Hoffbrand & Moss 2016: 89, 96).

Clinical chemistry general health variables, kidney function tests showed a statistically significant increase from baseline to end of oral therapy. However, this increase was not clinically significant as the mean and median did not change so that the increased ones were above the reference range.

Liver function tests followed a similar pattern with albumin and ALP showing a decrease while ALT increased. The changes were not clinically significant. The was no significant difference observed for AST. The slight increase in ALT was mitigated by finding normal AST and ALP concentration. Iron therapy was not supposed to affect liver and kidney functions; these organs' functions were evaluated to assess the general health of the participants. However, the liver is the major storage site for iron. Thus damage to it could have resulted in increased iron level, coupled with increased liver enzymes, plus iron overload affects the liver (Hoffbrand & Moss 2016:42)

Minerals are removed or supplemented depending on the soil's mineralogy. Sodium and zinc showed a decrease while phosphate increased. The mean and median changes were within the reference ranges thus not clinically significant. All other minerals did not change significantly. Due to consumption of different soil types the variation in mineral concentration could be due the varied effects of these soils as their contents differ.

The expected therapeutic response with iron therapy was an increase in serum iron, ferritin and TSAT. All these showed a statistically significant increase especially at ITC and end of oral therapy. However, the response was not of clinical significance. TIBC and transferrin were expected to decrease during iron therapy, and this was the case with both. However, only TIBC was statistically significant for the same time period, while for

transferrin the post-hoc test failed to show the significant change between different visit because the Bonferroni adjustment was not met although the ANOVA was significant. These iron study findings were contrary to those observed in IBD, CHF, CKD and pregnancy where oral iron corrected iron deficiency (Becuzzi *et al.* 2014; Toblli & Genaro 2015; Bonovas *et al.* 2016; Zhang *et al.* 2016). This correction was observed with a similar dosage and follow-up period. HCG showed a significant change which was due to a few outliers in STC and ITC. Since most values for HCG were zero, a few values of 3 or 5 affected the statistics negatively causing a significant change. It should be noted that the participants were not pregnant as the IQR was well within the reference range. This significant change was ignored as is was of no clinical significance. The CRP results of all visits did not show a significant change for all visits, signifying that no inflammation or infection was observed in the participants. The CRP finding strongly supports the above-mentioned normal WBC finding.

5.5.1.3 Discussion of mean changes of oral iron of the group that abstained from soil consumption, at different visits

There were no statistically significant changes in the anthropometric, body composition and general health indicators, except for pulse which showed a significant p-value for ANOVA. But the post-hoc test did not meet the Bonferroni adjustment, and therefore was not statistically significant. The reason why ANOVA was significant was that at ITC, the pulse increased

and then dropped at the end of oral. Most importantly this change was not clinically significant.

The red blood cell parameters: Hb, HCT, MCV and MCH showed a statistically significant increase from baseline to end of oral therapy. Although these changes were important statistically, they were not clinically significant because the changes were small. RDW showed a statistically significant change with ANOVA but post-hoc it could not reveal any significant visits' variation. Oral iron did not correct the red blood cell parameters of the abstain group sufficiently as the expected therapy outcomes were not encountered.

Platelet indicated a statistically significant decrease from baseline to end of oral therapy. There is a theory that thrombocytosis results when thrombopoiesis occur in the absence of iron (Kulnigg-Dabsch *et al.* 2012). Thus iron therapy, although not sufficient could be the reason why a 10% decrease was seen even though the effect was not pronounced similar to that encountered in RBC parameters. Platelet count decrease was not clinically significant. White blood cells parameters did not show any significant changes, thus infection and parasitic infestation were not an issue.

Kidney function was not affected, although BUN had a statistically significant increase and serum creatinine did not. The changes were not clinically significant. BUN can be affected by other factors such as diet,

while creatinine is not (Juraschek *et al*. 2013), further supporting the fact that kidney function was intact.

The liver did not indicate any clinically significant changes either, even though there were statistically significant changes. ALT increased, ALP and albumin decreased, while AST remained the same from a statistical point of view. Due to the non-significant clinical changes and two liver function tests changing in opposite directions, it can be concluded that liver function is on track. This implies that the increase in ferritin level is not associated with liver problems.

Sodium and zinc showed a statistically significant decrease, however, the changes were not clinically significant. All other minerals did not show any significant changes. This signifies that the soils might have different cation exchange capacity that results in absorption of certain minerals (Abrahams *et al.* 2013).

TIBC decreased, serum iron and TSAT increased, although ferritin was statistically significant, it did not reveal a statistically significant difference in post-hoc analysis. These changes were as expected because they imply that the treatment was minimally successful but the disappointing fact was that they were not clinically significant. Due to the less than expected effect on iron studies, this implies that iron absorption was not optimal, possibly caused by changes to the GIT lining. CRP did not change throughout the oral iron study period. Therefore, non-absorption of iron could not have been due to the presence of hepcidin. HCG showed statistically significant

changes because higher means of HCG were found during STC and ITC. However, no participants were pregnant and thus clinical significance was eliminated.

5.5.1.4 Discussion of mean changes of oral iron of the group that continued with soil consumption, at different visits

There were no statistically significant changes in the anthropometric, body composition and general health indicators except for bust which decreased from baseline until the end of oral iron therapy. The determining factors of breast size are hereditary and physiological. Physiologically size is determined by adipose tissue due to estrogen stimulation and gladular tissue which increase during pregnancy (Lim *et al.* 2018). However, none of these factors could be affected by iron therapy or consumption of soil. Therefore, the results were seen as an anomaly and the fact that the change was not clinically significant deemed the change not important.

Hb concentration increased from baseline to STC and then remained the same for the rest of the visits. RDW decreased at week 4 and returned to the previous level for week 7 and 10, and this change was the reason for the statistically significant change. Furthermore, at STC visit RBC, Hb and HCT increased while RDW and MCV decreased. This was perplexing as the MCV and RDW were also expected to increase. The MCV and RDW phenomenon was not understood, but having said that it must be noted that the changes were not statistically and clinically significant.

Platelet count did not change significantly, indicating that iron therapy is not working in this group. The lack of significant changes in red blood cell parameters and platelet signified that iron therapy was not effective in this group. This could imply that soil interfered with iron therapy absorption over and above the changes to the GIT lining. In comparison to Group A, Group B did not show statistically significant changes while Group A did. White blood cell count did not change thus no inflammation or parasitic infestation was observed.

BUN showed a statistically significant increase while serum creatinine was not significant. However, the changes in both were not clinically significant, thus kidney function was not affected as expected. BUN is reabsorbed while creatinine is not, consequently it may be a reflection on the reabsorption function of the kidney. However, since creatinine is intact and changes were not of clinical significance then kidney function was not affected from the beginning to the end of oral therapy.

There were statistically significant decreases found for albumin and ALP, while AST and ALT remained the same. The statistical significant decreases were not clinically significant, indicating that liver function was intact. The decreases were counteracted by the other enzyme concentrations being normal.

Statistical significant decreases were seen in zinc and sodium while phosphate increased. However, the changes were not clinically significant, plus the other minerals were within range. Soil has differing effects on

different minerals thus different effects are observed. Due to participants consuming different soil types, different effects are observed.

Serum iron and TSAT increased from baseline to STC, ITC and end of oral therapy, contrary to TIBC which decreased for the same time period. Implying that an increase in therapy iron concentration had an impact on iron study results. It must be noted that the changes were not clinically significant indicating that iron therapy was not very effective in this study group. Ferritin did not show significant change as expected because most iron that is absorbed is utilised for red blood cell synthesis, not diverted to storage. It should be noted that as iron becomes available then transferrin level decreases as part of the regulatory mechanism of iron absorption.

Iron studies showed small changes while red blood cells and platelets did not, in this particular group. This signifies that some iron was absorbed, although it was not sufficient to produce a significant change in the RBC and platelets There was no untoward inflammation involved since CRP level did not change significantly. Thus the lack of response to oral therapy could not have been due to inflammation that would have been coupled with increased hepcidin. HCG increased at STC and ITC, which resulted in a statistically significant difference with the baseline and end of iron therapy. However, there were no pregnant participants, thus clinical significance was not established.

5.5.2 Intravenous iron therapy discussion

Effective treatment was evaluated based on whether a haemoglobin level of 12.5 g/dl is achieved. Due to the formula calculation determining the amount of iron that should be administered, compliance had to be determined. Compliance was calculated and compared between two groups, so as to evaluate if the groups were associated with different responses.

5.5.2.1 Compliance for IV therapy

Compliance to IV iron was very high because there were not statistical differences between the required iron concentration and number of doses for all population groups. Group B required slightly higher doses of iron that was due to the Hb level being lower than that of group A. High compliance rate indicated that participants received the approximate amount of iron that their bodies required, thus the desired effect should be obtained.

5.5.2.2 Discussion of mean changes of the entire group of IV iron at different visits

The anthropometric, body composition and general health indicators did not change significantly for all the follow-up visits of IV iron therapy, except for pulse which showed a statistical significant ANOVA change that did not meet the Bonferroni adjustment for multiple analyses on post-hoc analysis. The decrease from IV baseline to the other two follow-up visits was noted

even though it was not of clinical significance. The decrease could be as a result of an increase in haemoglobin level, thus the heart need not compensate by increasing heart rate. One of the compensatory mechanism of anaemia is increased heart rate (Hoffbrand & Moss 2016: 20).

The statistically significant increase in all red blood cell parameters, except MCHC that remained the same, indicated that IV iron therapy was efficient. This was substantiated by changes being of clinical significance, as all parameters increased into the reference range. However, RDW increased even further because normal sized cells were produced, increasing anisocytosis further.

The platelet count indicated a statistically significant decrease, although it remained within the reference range and thus not clinically significant. In addition, platelets' reference range is wide thus a clinically significant change would require a drastic change. The drastic change that would have resulted in a decreased platelet count would have signified another problem altogether, bleeding resulting from lack of platelet. It was a significant change nonetheless because it was a change of 16%. This is important because thrombopoiesis in the presence of iron deficiency tends to increase the platelet count (Kulnigg-Dabsch *et al.* 2012).

The white blood cell parameters did not show statistically significant changes, except for basophil where STC and end of IV therapy were statistically different but not of clinical significance. Notwithstanding the

statistically significant change, all the white blood descriptive statistics were within the reference range for all white blood cell parameters. Indicating that infection, inflammation and parasitic infestation were not seen in the participants at all IV visits.

Kidney function was not affected during IV iron therapy period. Even though there was a statistically significant increase in serum creatinine, the increase was not of clinical significance, means were still within range and <2.5% of participants may have had increased values. The normal kidney function is supported by no change BUN. When kidney problems occur usually both BUN and creatinine levels increase (Yilma *et al.* 2019).

Liver function tests showed an increase in albumin and a decrease in AST, while ALP and ALT did not show statistically significant differences. The changes were not of clinical significance. Moreover, not all enzymes were affected, this was an indication that liver function was spot on at the beginning and end of IV iron therapy.

All minerals, except zinc, showed a statistically significant increase. All the changes were not of clinical significance as the means were within range for both study periods. The exception was sodium which was still below level in-spite of the increase. This might be as a result of participants not consuming soil as stated in the side effects questionnaire feedback.

The iron study results of both STC and end of IV therapy were within range. The statistical and clinically significant differences observed were according to the expected IV iron therapy effectiveness standard. By

circumventing the absorption in the gut, IV iron therapy showed the expected results. Where iron, ferritin and TSAT increased while transferrin and TIBC decreased. This was caused by the availability of iron in the system because circulation, storage and transferrin binding were increased. The level of transferrin decreased as the body does not need to obtain iron in the body, invariably decreasing the binding capacity.

5.5.2.3 Discussion of mean changes of IV iron of the group that abstained from soil consumption, at different visits

There were no clinical or statistically significant changes for all the anthropometric, body composition and general health indicators. Signifying that IV iron did not affect these variables. No effect was expected as these parameters were assessed to ascertain the health status of participants, not the effects of iron therapy.

All red blood cell parameters showed clinical and statistically significant increases at both follow-up visits, signifying effective therapy. The peculiar result was a statistical but not clinical significant decrease for MCHC observed during the IV study period. However, the post-hoc test did not meet the Bonferroni adjustment. MCHC was not expected to drop and the reason for this is an enigma. MCHC is not normally affected by small changes in Hb content, the change has to be severe to have an impact. Adding to the peculiarity is that the decrease in MCHC was coupled with an increase in Hb and MCH. However, it must be borne in mind that the

change wasn't of clinical significance. All red blood cell parameters were now within reference ranges, except RDW which increased further because red blood cell lifespan is 120 days (Hoffbrand & Moss 2016: 12). Thus the newly formed normal-sized and old small-sized cells further contributed to the anisocytosis.

Platelet count significantly decreased based on statistics and percentage change (16%). However, the change was not clinically significant as all results were within the reference range. This finding supports the theory of iron therapy leading to decreased platelet count. White blood cell parameters were within the reference and did not differ significantly for all variables at all visits. No inflammation or parasitic infestation was observed during the IV iron therapy phase.

The functioning of the kidney and liver were not affected at IV baseline and end of IV therapy. No significant differences were observed for both kidney function parameters. Albumin increased statistically but was not clinically significant. Divergent to albumin, liver enzymes' results did not change significantly, meaning liver function was sound at both the beginning and end of IV iron therapy.

All minerals showed a statistically significant increase, except zinc where the increase was not statistically significant. These results refute the theory of zinc being the suspected cause of geophagia or zinc being exchanged for iron absorption, but soil type and content should be taken into consideration when this decision is considered. All the mineral changes

were not of clinical significance. However, sodium means were still decreased for the end of IV therapy. These mineral changes could signify that during the absence of soil consumption more minerals are available for absorption. Especially since it was captured in the side effects questionnaire of IV STC that participants did not crave or consume soil post IV therapy.

The iron study results produced the expected response which was observed by both statistical and clinically significant changes in all variables, at both follow-up IV therapy visits. Ferritin decreased at the end of IV therapy because ferritin concentration is not accurate post IV therapy (Bonovas *et al.* 2016; Martin-Malo *et al.* 2019). There was no inflammation as observed by no changes in CRP and all descriptive statistics being within the reference range. The absence of inflammation or infection-finding was supported by the absence of changes in WBC parameters. HCG showed a statistically significant change between baseline and STC. Most importantly, this change was not of clinical significance as none of the participants was pregnant.

5.5.2.4 Discussion of mean changes of IV iron of the group that continued with soil consumption, at different visits

No statistical or clinically significant changes were observed for all anthropometric, body composition and general health parameters except pulse. Pulse declined from IV baseline to IV STC. Even though pulse might

be affected by factors like temperature and emotional state, the most probable reason for a drop in pulse might be an increase in Hb which decreased the compensation by the heart (Hoffbrand & Moss 2016:20). The drop in pulse rate was seen in this group because the haemoglobin level improved from a lower level contrary to group A.

All the red blood cell parameters increased significantly on a clinical and statistical basis, except MCHC. The response of red blood cell parameters to IV iron therapy was as expected for successful therapy. This signified that IV therapy successfully corrected hypochromic microcytic anaemia in this group.

Platelet count decreased statistically and percentage-wise (19%), but was not clinically significant. The white blood cell parameters were not significantly different and were within the reference ranges for all the visits. A statistically significant change was observed in basophil between STC and end of IV therapy. Basophil change was not of clinical significance. The changes that would be considered of clinical significance are nowhere near those that have been observed in this study. For those diseases, the changes to basophil count would have to be much higher. Diseases associated with increased basophil count are an inflammatory response, myeloproliferative neoplasms and cancer (Hoffbrand & Moss 2016: 90). Furthermore, inflammation theory is refuted by the normal CRP and WBC findings, while the degree of increase refuted the myeloproliferative neoplasms and cancer. In addition, these diseases are associated with

other unrelated peripheral blood changes that were not observed in this case.

A significant increase in serum creatinine was not of clinical importance, while BUN decreased for the same period, although not significant. Generally, in cases of kidney failure, both levels increase. Thus, the current finding does not support kidney problems. However, BUN can be affected by other factors thus leading to a decreased level. Albumin was the only one that showed a statistical but not clinically significant increase. While the livers enzymes did not differ significantly, indicating that the liver is functioning well or was not damaged.

Sodium, phosphate and calcium indicated statistically significant increases to the end of the study. The increase in potassium, phosphate and zinc was not statistically significant. Although all the minerals increased, it must be noted that the changes were not of clinical significance except for potassium that increased into the reference range. This could be due to the absence of soil consumption, thus no interference of soil with the absorption of minerals. The black coloured soil, which is mostly consumed in Botshabelo, has been associated with hypokalemia (Chaushev *et al.* 2003).

Statistical and clinically significant changes were observed in all the iron study variables. This indicated that IV iron therapy was efficient in correcting ID. The end of study ferritin decreased from STC because ferritin assessment after IV therapy is not accurate for eight weeks

(Bonovas *et al.* 2016; Martin-Malo *et al.* 2019) because ferritin is utilised for the formation of new red blood cells to correct the anaemia. There were no infections or inflammation as there were no significant changes in CRP, plus the mean results were all within the reference range. HCG had a statistically significant difference between IV baseline and STC, but it was not of clinical significance as no participants were pregnant.

5.5.3 Comparison of oral and intravenous iron therapies

Oral therapy was below par compared to effects seen in other disciplines or diseases like in pregnancy (Khalafallah & Dennis 2012, Becuzzi *et al.* 2014; Holm *et al.* 2017, 2017a), gastroenterology (Abitbol *et al.* 2015; Dignass *et al.* 2015, Bonovas *et al.* 2016) and heart failure (Swedberg *et al.* 2013, Zhang *et al.* 2016). This fact further supports the interference of soil with the bioavailability of iron and changes that may have resulted from damage of GIT by soil's granularity.

5.5.3.1 Oral and IV therapy changes for the entire study group

Oral therapy had a minimal effect on IDA red blood cell indicators while IV therapy had a pronounced impact. Although there was a statistically significant increase with oral therapy, the increase was not of clinical significance. More participants increased into the reference range for IV therapy, the mean change for IV was 3x to 5x that of oral. In addition, IV therapy achieved a mean haemoglobin concentration change of 2g/dl, as

expected, when appropriate correction takes place (Jimenez *et al*. 2015, Bonovas *et al.* 2016, Hoffbrand & Moss 2016: 36). MCV had the most pronounced change while the effect of Hb and MCH were similar. MCV is the first red blood cell parameter to plummet due to the body's compensatory mechanism of increasing cell division, thus the drop facilitated the sharp increase. Contrary to Hb and MCH that were not severely decreased.

A similar trend was observed in the iron study indicators where oral had minimal effect as compared to IV therapy. More participants were within reference range after IV therapy, IV therapy achieved correction as per recommended changes and ferritin at the end of IV therapy improved 16X more than with oral therapy. Mean TSAT was well below the margin for IDA (<10%) post oral therapy, while post IV therapy >16% was achieved signifying a normal value. Ferritin achieved the greatest change while TSAT and TIBC changes were similar. This can be explained by iron overload in circulation due to IV therapy where a copious amount of iron was delivered at once. This iron was diverted to storage and then synthesis of red blood cells occurred from storage (Hoffbrand & Moss 2016: 29). In addition, oral iron has been shown to achieve ferritin correction much slower than IV iron therapy (Beck-da-Silva *et al.* 2013, Bonovas *et al.* 2016, Martin-Malo *et al.* 2019). It is for that reason that oral iron therapy was undertaken for three to six months so as to replenish the iron stores.

Most studies that have compared oral versus IV therapy found no difference between the two therapies when it comes to correction of IDA (Beck-da-Silva *et al.* 2013, Bonovas *et al.* 2016, Martin-Malo *et al.* 2019). However, in the current study, it was found that oral iron did not correct IDA. This scenario inferred that soil interfered with iron absorption and soil might have damaged or coated the mucosal surface thus interfered with soil absorption. There was a small difference between the two groups for oral iron, with group A achieving statistically significant changes within the parameters used to monitor red blood cell changes response, unlike group B. Although participants abstained from soil consumption, oral iron therapy was not effective therefore implying malabsorption. The participants that continued with soil absorption did not produce a statistically significant change implying that soil could also have interfered with iron absorption or continued to affect the GIT lining.

5.5.3.2 Oral and IV therapy changes for group A

The red blood cell parameters and iron study indicators indicated a minor but statistically significant increase for oral therapy, except ferritin. Signifying that oral therapy for ten weeks did not achieve the expected response for iron therapy. The non-clinical significant change might have been brought about by soil which modified the GIT absorptive surface. There might have been a recovery in the absorptive surface as the increase was only significant at the end of therapy but not at STC or ITC.

Furthermore, the increase in iron concentration did not seem to have played a significant role since STC and ITC did not change significantly.

All red blood cell and iron study variables showed a clinical and statistically significant increase for IV therapy. Indicating that IV iron therapy corrected the IDA in most participants. Circumventing the GIT absorption route and providing iron directly in the vein achieved the desired results, as most participants corrected into the normal range and the mean change achieved the expected therapy outcome. The red blood cell parameters mean changes of IV therapy were 3x higher than that of oral therapy. The mean change in ferritin for IV was 16X higher than oral therapy, while TSAT and TIBC were close at 3X and 2X, respectively. Oral iron cannot be stored if it is need for synthesis, thus when absorbed iron is utilised to correct the deficit instead of diverting the iron to storage, contrary to IV therapy, where surplus iron is available in circulation causing it to be diverted to storage, although temporarily.

5.5.3.3 Oral and IV therapy changes for group B

Dissimilar to the abstain group, oral therapy did not produce a significant change for all red blood cell parameters and iron study variables, except TSAT and TIBC which produced statistical, but non-clinical significant changes. Red blood cell parameters were not changed because the amount of iron absorbed was not substantial, for two plausible reasons. One, absorptive surface changes caused by soil consumption resulting in

malabsorption. Two, soil reducing the bioavailability of oral iron therapy. TSAT and TIBC reflect the current situation, unlike ferritin which reflects storage. Thus when a small amount of iron becomes available, a change can be observed. However, it must be noted that TSAT and TIBC changes were not of clinical significance.

Nevertheless, what was similar to group A was the clinical and statistically significant increase encountered for both red blood cell and iron study indicators for IV therapy. In addition, the mean changes were also similar for red blood cell parameters (with Hb >2g/dl) and iron study variables as for the abstain group. The only exception was MCV which was 5X higher in IV than oral compared to 4X in the abstain group. IV therapy produced clinically significant response unlike oral therapy, pointing to its efficacy. The reasons why MCV produced a higher multiple could be attributed to the lower starting point for group B in relation to group A. Owing to iron ineffective absorption because of soil interference with absorption, in group B. Contrary to the abstain group where oral iron increased MCV from baseline to end of oral therapy. Thus MCV remained low in group B at IV baseline while oral therapy increased MCV in group A. The effect of MCV was based on IV iron therapy and not oral iron therapy. Being in the abstain group or control group did not play a significant role during the IV therapy. Thus, the change is not significant overall, plus clinically as well.

5.5.3.4 Factorial ANOVA results discussion

There were statistically significant differences noticed for oral therapy in the abstain group which were not observed in group B. Did this imply that abstain group responded better than group B to oral iron? Factorial ANOVA was undertaken to address this question. The lack of statistically significant difference between group A and B for red blood cell, iron study, platelet, inflammatory and parasitic indicators with the factorial ANOVA analysis, signified that neither abstaining or continuing with soil consumption made a difference. Therefore, it is not just soil that may directly interfere with iron absorption but soil could have altered the GIT lining resulting in malabsorption.

CHAPTER 6

CONCLUSIONS AND IMPLICATIONS OF THE STUDY

6.1 INTRODUCTION

The primary aim of the study was to compare oral versus IV iron therapy in relation to the treatment of IDA in geophagia. The aim was achieved by investigating the objectives: evaluation of geophagic practices, bleeding assessment, oral iron, IV iron therapy and the comparison of both types of therapy. The methodology that was followed enabled the achievement of goals. The research questions that were addressed were centred on whether and how soil could cause iron malabsorption. Firstly, could soil through its constituents interfere with iron absorption? Secondly, could soil coat the GIT absorptive surface interfering with iron absorption? Thirdly, could soil due to its granular nature change the absorptive surface of the GIT? Fourthly, could soil change the GIT environment, e.g. increases pH, thus interfering with iron absorption? The study design of having two groups could infer if soil would contribute to malabsorption.

6.2 GEOPHAGIC PRACTICES

Participants consumed a significant amount of soil on a daily basis, the period of consumption was lengthy and black was the predominant colour of the soil. However, geophagic practices of participants did not contribute directly to the

severity of changes in the parameters that were used to diagnose IDA. Thus the major contribution of soil may be related to the following two factors: soil due to its granular nature could alter the GIT absorbance surface thus interfering with iron absorption; geophagic soils decreased the bioavailability of therapy iron thus interfering with absorption.

6.3 BLEEDING ASSESSMENTS

There was no correlation between menstrual blood loss and severity of IDA based on red blood cell and iron study parameters. Platelet function abnormality may not be a major contributor to the blood loss in geophagia that hypothetically could be related to the loss of iron. Gastrointestinal bleeding also did not seem to play a significant role in the development of IDA. Nonetheless, an unequivocal conclusion could not be reached for the contribution of platelet function and GIT bleeding because of the sample numbers were not representative.

6.4 INTERVENTION STUDY

The study was composed of a mix of the general population, who are affected by known lifestyle diseases like hypertension and obesity. In general, the participants were fairly healthy and did not have diseases that could be perceived as confounding factors. The liver, kidney and inflammatory evaluation parameters were normal at different follow-up periods. The

selection criteria were met and randomization was effective, thus the two groups were similar.

6.4.1 Oral iron therapy

Oral iron therapy was below par for the treatment of IDA in geophagia and abstinence from soil did not play a significant role. These effects were assessed utilising both red blood cell and iron study parameters. These effects were observed in spite of acceptable compliance by participants. This signifies that even in the absence of soil in the GIT, iron was still not effectively absorbed. This signifies that soil has a lasting effect of at least ten weeks on the GIT absorption surface resulting in iron malabsorption. Therefore, soil directly interfered with therapy iron absorption and caused malabsorption on the GIT absorptive surface.

6.4.2 IV iron therapy

IV iron therapy achieved clinical correction of IDA, according to expected criteria. Bypassing the GIT proves that malabsorption due to soil interference and or modification of absorptive surface was the problem because the red blood cell and the iron study parameters responded adequately to IV therapy.

6.4.3 Oral versus IV therapies

IV therapy was superior to oral therapy for IDA treatment in geophagia. This was indicated by a clinically significant change that occurred during IV therapy contrary to oral. There was no difference between the abstain and continue groups, signifying that it was not just soil by itself that decreased the bioavailability of iron. Thus a change to GIT absorptive surface also played a significant role. However, the group that abstained from soil consumption showed statistically significant changes but clinically non-significant changes pertaining to the red blood cell parameters. Contrary to the group that continued with soil absorption where this change was not observed. This further supports the concept that besides the GIT changes soil also interfered with oral iron absorption.

6.5 THE OVERALL GENERAL CONCLUSION

Geophagic soils interfere with iron absorption in GIT leading to iron malabsorption. IV iron therapy was superior to oral iron therapy for the treatment of IDA in geophagia. Therefore, the use of IV iron therapy is advocated for the treatment of IDA in geophagia. The finding that oral iron therapy was not effective for the correction of IDA is unique to geophagia cases because in other disciplines oral therapy corrected the IDA. Although IV iron is expensive financially, it is the cost-effective course of therapy because of disease symptom burden. Considering that the patient will not respond to oral iron therapy for a period of more than ten weeks, a poor

quality of life will be experienced while the patient is treated with oral iron. Depending on the duration it takes for the GIT to correctly absorb iron, the financial burden may increase to the same level of IV therapy.

6.6 THE STUDY IMPLICATIONS

The comparison of two therapy modes, oral and IV iron, has not been undertaken in geophagia. The reasons for this phenomenon is due to the lack of knowledge about the effect of soil on dietary iron absorption, the underestimation of geophagia prevalence and geophagia's contribution to the resistance to oral iron therapy. These findings emphasised that the protocol of treating IDA in geophagia with oral iron first and then IV iron when oral iron is neither effective nor beneficial to the patient, society and the health care system. The patient will carry an unwarranted burden of symptoms for a long time and this may even affect future pregnancy, especially because geophagia affects mostly females of child bearing age. The patient will not be productive as expected thus not contributing as effectively to society through their career. The health care system will carry the financial burden when an ineffective treatment is administered by following the current protocol. The findings of this study imply that IV therapy should be considered as first choice in therapy in cases of IDA in geophagia. However, more studies need to be undertaken to increase critical mass in this area of research as results are scanty.

6.6.1 Future research

The findings of this study have set the stage and increased the appetite for investigating the underlying principles of iron absorption in the presence of geophagia within the human GIT. *In vitro* soil studies are needed to investigate which theory is responsible for the malabsorption and to investigate the time it will take for GIT absorption surface to recover so that oral iron therapy can be effective. Furthermore, a multi-centre study involving numerous participants and studies investigating the effect of different IV preparations are warranted to confirm the finding of this study and ascertain which IV preparation would be more effective. Moreover, to ascertain the contribution of bleeding, especially haemostatic abnormalities and menstrual blood loss, to the development of IDA in geophagia.

6.6.2 Recommendations for clinical use

The main finding of this study, that oral iron therapy is not effective for the treatment of IDA in geophagia, implies that patients treated with the current protocol will endure the symptom burden while therapy is not effective. This will lead to patients with IDA being less productive, having a decreased quality of life level and will negatively affect the future generation due to the effects of IDA on pregnancy. Thus, this will have a direct impact on the patients and an indirect effect via socio-economic effect. In patients who fail to respond to oral iron therapy it is recommended that an enquiry about geophagia should be made, since geophagia is more prevalent than it is

currently thought. If geophagia is found, then effective therapy should be administered. IV therapy should be advocated for the treatment of IDA in geophagia, instead of oral first and then IV. This signifies that an evidence based new treatment protocol has been developed via the findings of this study.

6.7 LIMITATIONS

Although the findings of this study are changing the landscape of iron therapy in geophagia, this study was not with-out limitations. The results' generalizability is limited to two arms, single location design and a relatively small number. A larger multi-centre study for strengthening the case presented by these results is warranted. The limited number of participants was due to financial implications. However, it was worthwhile since great compliance and effective follow-up of participants was achieved as evidenced by the low drop-out rate. If a single IV iron injection was available, then the cost of the study and burden on participants would have been mitigated. Furthermore, the absence of similar studies in literature meant that comparison had to be drawn from other disciplines where oral versus IV therapy comparison studies were undertaken.

The absence of placebo and washout period that was a product of unsuccessful design of placebo medication could be seen as a limitation. However, no change in oral therapy indicates that the placebo effect was

not evident, since iron levels were measured at each visit then the interference that the washout period would have caused was not apparent. The absence of a representative sample in relation to haemostatic abnormality screening tests and GIT bleeding signified that a conclusion on their contribution could not be reached. The low turn-out was due to cultural reasons because participants were not comfortable providing their faecal samples and the participants not informing the research team once they were on their period.

CHAPTER 7

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APPENDIX: A

ETHICAL APPROVAL

UNIVERSITY OF THE FREE STATE
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2014-02-06

REC Reference nr 230408-011
IRB nr 00006240

MR LF MOGONGOA
DEPT OF HEALTH SCIENCES
FACULTY OF HEALTH AND
ENVIRONMENTAL SCIENCES
CENTRAL UNIVERSITY OF TECHNOLOGY
BLOEMFONTEIN

Dear Mr Mogongoa

ECUFS NR 17/2014
MR LF MOGONGOA **DEPT OF HEALTH SCIENCES, FACULTY OF HEALTH AND ENVIRONMENTAL SCIENCES, CUT**

PROJECT TITLE: ORAL AND INTRAVENOUS IRON THERAPY IN GEOPHAGIC WOMEN WITH IRON DEFICIENCY ANAEMIA.

1. You are hereby kindly informed that at the meeting held on 4 February 2014 the Ethics Committee granted conditional approval without ethical issues as the Evaluation Committee report has to be submitted to the Ethics Committee before a final approval letter can be issued.

2. Committee guidance documents: Declaration of Helsinki, ICH, GCP and MRC Guidelines on Bio Medical Research. Clinical Trial Guidelines 2000 Department of Health RSA; Ethics in Health Research: Principles Structure and Processes Department of Health RSA 2004; Guidelines for Good Practice in the Conduct of Clinical Trials with Human Participants in South Africa, Second Edition (2006); the Constitution of the Ethics Committee of the Faculty of Health Sciences and the Guidelines of the SA Medicines Control Council as well as Laws and Regulations with regard to the Control of Medicines.

3. Any amendment, extension or other modifications to the protocol must be submitted to the Ethics Committee for approval.

4. The Committee must be informed of any serious adverse event and/or termination of the study.

5. All relevant documents e.g. signed permission letters from the authorities, institutions, changes to the protocol, questionnaires etc. have to be submitted to the Ethics Committee before the study may be conducted (if applicable).

6. A progress report should be submitted within one year of approval of long term studies and a final report at completion of both short term and long term studies.

7. Kindly refer to the ETOVS/ECUFS reference number in correspondence to the Ethics Committee secretariat.

8. Thus, this letter only serves as conditional approval.

Yours faithfully

.....

PROF WH KRUGER
CHAIR: ETHICS COMMITTEE

Cc Dr AD Jaftha
Prof L de Jager

APPENDIX: B

SCREENING QUESTIONNAIRE

GEOPHAGIC PRACTICES SCREENING QUESTIONNAIRE

Participant number: _____

A. PERSONAL INFORMATION

1. Date of interview: _____
2. District: _____

B. DEMOGRAPHIC INFORMATION

1. Gender: _____
2. Age: _____
3. Are you pregnant? : _____
4. Are you presently in the habit of eating soil? : _____
5. If **YES**, how often do you eat soil? :

- ☐ Once a month
- ☐ Once a week
- ☐ Once a day
- ☐ More than once a day

6. What is /are your reason (s) for eating soil?

- ☐ Standard practice (cultural, traditional, spiritual)
- ☐ Craving
- ☐ Medicinal value

☐ Supplement diet

☐ Ritualistic

☐ When hungry

☐ When pregnant

☐ Don't know

☐ Others; please specify_____

7. Do you crave soil? : ☐ Yes ☐ No

If **YES**; how often:

☐ Regularly monthly

☐ Regularly weekly

☐ Regularly daily

☐ Only when pregnant

8. When do you crave soil?

☐ Pregnant ☐ nauseous, but not pregnant ☐

☐ Lactating ☐ constipated

☐ Both pregnant and lactating ☐ feeling weak

☐ Having trouble sleeping ☐ others; specify _____

9. When pregnant, how often do you eat soil?

- ☐ Once a month
- ☐ Once a week
- ☐ Once a day
- ☐ Others; specify: _____

10. Do you eat any other non-food substance: ☐ yes ☐ no

If **YES**; name the substance: _____

11. How often do you eat this substance:

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

12. How much soil do you eat?

Handful ☐ Daily

Bag of soil ☐ Daily

Bag of soil ☐ Weekly

In the box put in the number of times the soil is consumed, based on the example

13. Do other people know you eat clay? ☐ Yes ☐ No ☐ Don't know

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

☐ Family members
☐ Extended family members
☐ Friends
☐ Others; specify: _____

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

☐ Positive
☐ Negative
☐ Indifferent
☐ Don't know

15. Is this practice of eating soil more common among certain members of the community?

☐ Yes ☐ No ☐ Don't know

16. If **YES,** specify:

_____

C. INDIGENOUS KNOWLEDGE

1. Which substances do you consume?

- ☐ Soil
- ☐ Clay
- ☐ Soil from termite mounds
- ☐ Other, Specify:

_____

2. How do you eat the substances:

- ☐ Wet
- ☐ Dry
- ☐ With other foods
- ☐ Other, specify:

_____

3. Where do you obtain your preferred substance?

- ☐ From nature
- ☐ Buy it
- ☐ Am given it
- ☐ Others, specify:

_____

4. What is the color of your preferred substance?

☐ Reddish	☐ yellowish
☐ Whitish	☐ khaki
☐ Blackish	☐ other, please

specify_____

APPENDIX: C

STUDY INFORMATION DOCUMENT

INFORMATION DOCUMENT

Study title: Oral and intravenous iron therapy in geophagic women with iron deficiency anaemia

Greetings: Participant of the research on iron deficiency in people who consume soil

Definition of terms:
-Geophagia: is the consumption of soil or clay for a minimum period of a month.
-Iron deficiency anaemia: Anaemia is defined as decreased level of haemoglobin. Haemoglobin is the major constituent of blood which carries oxygen to tissue and gives blood its red colour. Haemoglobin consists of iron, and iron is the component responsible for oxygen transport. Once you do not have enough iron in your body, you will not be able to form enough haemoglobin. Then you have iron deficiency anaemia because both iron and haemoglobin levels are decreased.
-Gastrointestinal tract: Is the digestive tract that is responsible for the digestion and absorption of food, thus the absorption of iron also takes place in the gastrointestinal tract. It start from the mouth and ends at the rectum; it includes the stomach, small and large intestines.
-Intravenous iron: Is a form of treatment where the iron is inserted directly inside the blood by using a drip.

Introduction:
We, LF Mogongoa from Central University of Technology, Dr AD Jafta from van Rensburg pathologist, Bloemfontein and the rest of the geophagia team, are doing research on comparing treatment methods of iron deficiency anaemia in people who consume soil. Research is just the process to learn the answer to a question. Since this is a research study and not routine care, certain procedures will be implemented that are not necessarily part of routine care. In this study we want to learn whether oral iron or intravenous iron therapy is the best mode for treatment of iron deficiency anaemia. It has been discovered that people who consume soil have iron deficiency anaemia and that soil may interfere with the absorption of iron from the gastrointestinal tract. If person has iron deficiency anaemia the normal mode of treatment is oral iron (in tablet form) for six months. If the oral iron therapy is not effective then intravenous iron therapy is administered. Therefore if soil interferes with oral iron absorption then this the oral route might not correct the iron deficiency, thus you will experience the symptoms of anaemia for a long time. The aim of the study is to find out in cases of people who consume soil, if the patient should receive intravenous iron therapy instead of oral iron first. In so doing, the burden of symptom and financial implications can be avoided by implementing a new protocol.

Invitation to participate: We are asking/inviting you to participate in the above mentioned research study.

What is involved in the study – this is an intervention study where 80 people from Botshabelo who consume soil and have iron deficiency anaemia will be given two different forms of approved iron treatment to investigate which one works best. The iron that is used for treatment is not experimental, thus it is iron that is used for treatment of iron deficiency anaemia if you were to consult your physician (doctor). 40 participants will be expected to refrain from consumption of soil for the duration of the study while the other 40 will continue with soil consumption.
The study will take 14 weeks to complete where you will be required to take an oral iron tablet for 10 weeks. There after you will be given a single intravenous iron with follow up for four weeks (please note that only one intravenous dose of iron will be administered and the four weeks will be used for follow up). Blood will be drawn at the following intervals: (1) recruitment to ensure you meet the criteria, (2) baseline – when the study commences, (3) three weeks later for short term changes, (4) at week 6 to assess the intermediate changes, (5) at week 10 to assess the end of oral iron therapy, (6) week 12 to assess short term changes, and (7) week 14 for end of study measurements. The amount of blood that will be drawn at each visit will be 20 millilitres which is approximately four teaspoons of blood. The blood will be drawn by LF Mogongoa and M Raphuthing predominantly, but occasionally blood will be collected by Dr(s) Brand and D Jafta. Another specimen that will be required will be faecal sample to assess if there is bleeding in the gastrointestinal tract. The specimen will be collected at week 1, 6, 10 and 14.
You will also be required to complete different questionnaires about your nutritional information, to eliminate diet as the cause of iron deficiency anaemia. Another questionnaire will be on sources of bleeding including menstrual cycle. Information about the geophagic practices (type of soil and how much you consume) will also be sought, through the use of a questionnaire. The final questionnaire will assess your wellbeing and the problems that you might have encountered due to the treatment. These questionnaires will be administered in different combination and at different intervals throughout the study, namely: recruitment, baseline, weeks 1, 3, 6, 10, 12, and 14.
The blood will be tested for full blood count to assess the level of haemoglobin, iron studies to determine if the anaemia is caused by iron; kidney and liver function screening tests to ascertain health. The faecal sample will be used for occult blood analysis, i.e. to screen for bleeding in the gastrointestinal tract.

Risks of being involved in the study: oral iron therapy can cause side effects like constipation and stomach cramps but this will be handled by changing the iron preparation. Intravenous iron can cause an allergic reaction by this will be prevented by administering a low molecular weight preparation, a small dose, observing, if necessary antihistamines will be administered. The medical practitioner (doctor) will administer the intravenous dose and will be available to deal with any allergic reaction. The other risk of intravenous iron therapy is iron overload but the administered iron dose will be based on the laboratory results and the participant's weight thus this risk is eliminated. If an adverse event occurs, you will be required to contact the researcher so that arrangements can be made for you to obtain medical care as soon as possible, the bill of study related events will be paid by the researcher.

Benefits of being in the study: Iron deficiency anaemia will be corrected and therefore symptoms of tiredness will become bearable. Due to menstrual cycle assessment and occult blood testing any source of bleeding that leads to iron deficiency anaemia will be identified and thus the research doctor can give advice on how to deal with them. The results of the screening tests for renal and liver function can assist in identifying other

problems in these organ systems. The hope is that if the anaemia is corrected then you will no longer have a craving for soil. In addition, you will be helping other people who consume soil to receive the correct treatment from the beginning.

Alternative procedures or courses of treatment that might benefit the subject: you can visit you nearest health facility and your doctor will examine you for signs of anaemia. If the signs are present, the doctor might prescribe oral iron therapy for six months. If oral iron therapy fails, your doctor might administer intravenous iron therapy.

The subject will be given pertinent information on the study while involved in the project and after the results are available. In addition, should any information or significant new findings develop during the course of the research which may relate to the subject's willingness to continue participation statement, it will also be provided.

Participation is voluntary, and refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled; the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled.

Reimbursements for "out of pocket" expenses: this study is not funded by a pharmaceutical company so there will not be reimbursement except for transportation and a meal. There will be compensation of R150 (one hundred and fifty rands only) if the participants are required to travel from Botshabelo to Bloemfontein (with their own means) for scheduled visits. R90 will be for traveling costs and R60 is for meal. The meal will be provided by the cafeteria on CUT campus, thus the participant will get a meal voucher. Alternatively, CUT mini busses will be used to transport the participants and only meal vouchers will be provided. It should be noted that 'no costs will be payable by the participant'.

Confidentiality: Efforts will be made to keep personal information confidential. Absolute confidentiality cannot be guaranteed. Personal information may be disclosed if required by law. Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as the Ethics Committee for Medical Research.

If results are published, this may lead to cohort identification.

Contact details of researcher(s) – for further information/reporting of study-related adverse events. Lebogang Mogongoa at 073 815 6767 or 071 1510 647 or 051 5073118 (office hours).

Contact details of Secretariat and Chair: Ethics Committee of the Faculty of Health Sciences, University of the Free State – for reporting of complaints/problems: Telephone number (051) 4052812

STUDY INFORMATION DOCUMENT

Sehlooho: Boithuto ka mokgoa o lokileng bakeng sa ho phekola khaello ya madi ka lebaka la 'iron' mmeleng ya basadi ba jang mobu.

Mantsoe a sebedisoang boithutong

Geophagia: ke hoja mobu nakong e etsang khoedi le ho feta

Iron deficiency anaemia: ke kgaelo ea haemoglobin. Haemoglobin ke karolo e kgolo ya mad . Ke yona e tsamaisang moya o hloekileng dikarolong tsohle tsa mmele, sena e se etsa ka thuso ea iron, hape e fa madi mobala o mokhubedu oa oona. Mme ha mmele o na le khaello ya iron, ho bolela hore madi ha a no tsamaisa moya o hloekileng mmeleng oa motho. Motho ya joalo ho thoe o na le kgaello ya madi.

Gastrointestinal tract: ke tsela eo dijo di e tsamaeang; ho tloha ka lehanong ho fihlela mmele o dintsa. Mme ke tseleng eena moo dimenerale/ dihaha mmele tse fumanoang dijong, di monyellang mading, hore mmele o tsebe ho di sebedisa.

Intravenous iron: ke tsela ya phekolo eo ka yona moriana o fuoa mokudi ka nale hore o kene mading eseng o tsamae tsela e telele.

Selelekela

LF Mogongoa ho tsoa Central University of technology, Free State; Dr AD Jafta ho tsoa van Rensburg pathologists, Bloemfontein, le bohle bao eleng karolo ya sehlopha, re etsa boithuto ka bomme ba jang mobu. Mooko tabe oa boithuto ba rona ke ho batlisisa mokhoa o lokoleng bakeng sa ho phekola khaello ya madi ho bomme ba jang mobu. Boithuto (research) ke mokgoa oa ho leka ho fumana tsela e bobebe ya ho thusa sechaba ka ho rarolla diqaka tse teng saense le ho batla tsela tsa ho thusa sechaba. Hona ke boithuto e seng pheko ea boloetse, tsela eo re tlo enka e kentse mehato e mengata hore re be le bopaki ba seo re se fumanang. Boithuto bona taba kgolo ke ho batlisisa hore na mokgoa o loketseng ho sebedisoa bakeng sa ho phekola kgaello ya madi bathong ba bomme ba jang mobu ke ofeng pakeng tsa dipidisi tsa iron le iron e kenyoang mading ka nale. Taba kgolo ele ho batlisisa hore pakeng tsa mekhoa ena e mmedi ya ho phekola kgaello ea madi ke ofeng o sebetsa ka matla ho feta o mong ke ofe. Boithutong bo entsoeng, ho fomanehile hore bomme ba jang mobu boholo, ba bonahala bana le kgaello ya mali mmeleng le hore mobu oo ba ojang o thibela mmele ho sebedisa iron e fumanehang dijong tseo ba dijang. Mme mokoa phekolo ho batho ba nang le kgaello ya madi ba saje mobu ke ho noa dipidisi tsa iron nako ya likgoedi tse tseletseng, ha ho se phetoho mokhudi a be a fuoa iron ka nale (drip). Ho batho ba jang mobu pidisi e kanna ya se sebetse hoba pelaelo ke hore mobu o sitisa mmele ho sebedisa kapa hona ho nka iron e dijong. Mme ho ka etsahala hore le e dipilising mobu o tla etsa ka ho tsoana kaha dijo le dipidisi di tsamaea tsela e tsooanang mmele oa motho. Ke ka lebaka lena boithuto bona bo hlokahalang ho sheba hore na ke mokoa ofe o lokeloang ho sebedisoa bakeng sa phekolo ya kgaello ya madi bommeng ba jang mobu. Taba kgolo ke ho fuputsa hore na

bathong ba jang mobu mohlomong mokgoa oa ho ba phekola ke ho qala ka moriana oa nale (drip). Hona ho tla thusa hore ba fumane pheko ka pele, le ditsenyehelo ha di no ba kalo kalo, hona le ha ba qala ka dipidisi ebe ha disa thusa ka mora dikgoedi tse tseletseng ke hona a tla fuoa nale. Ka hona ho bohlokoa hore boithuto bona bo etsuoe, mme bomme ba jang mobu ba tle ba kgole tholoana tse molemo.

Memo ya ho nka karolo: Re o mema re bile re kopa hore o nke karolo boithutong bo seng bo hlalositsoe.

Ntho tse akareditsoeng boithutong bona: Bona ke boithuto bo shebang se etsehalang sechabeng mme bo leke ho fumana mokoa oa ho thusa sechaba. Boithuto bo hloka bo'm'e ba mashome a robedi (80), ba phelang Botshabelo, ba jang mobu, ba nang le kgaelo ea madi mmeleng honka kararolo. Bo'm'e bana ba tla thusoa ka mekgoa e mmedi, ba tla qaloa ka dipidisi mme ho shejoe ebang ho na le phetoho, ha ele siko ba tla fuoa phekolo ka nale. Ho bohlokoa ho tseba hore dipidisi kapa yona pheko ka nale, ke dihlare tseo motho a neng a tla di fuoa ke ngaka bakeng sa kgaello ya madi. Ba nka karolo ba mashome a robedi ba tla aroloa ka dihlopha tse pedi (sehlopha ka seng se tla etsoa ka bomme ba mashome a mane {40}). Dihlopha tsena tse pedi di tla qaloa ka dipidisi, empa se seng se tla kopuoa ho noa dipidisi mme di tlohele hoja mobu, ha se seng se tla noa dipidisi ntse se ja mobu.

Boithuto bona bo lebeletsoe hore bo nke dibeke tse leshome le metso e mene {14}, mme dibeke tse leshome {10} di tla sebedisoa ho noa dipidisi, ebe ho ntse ho lekoloa hore na dipidisi di ya thusa kapa che. Haeba kamora dibeke tsena ha ho pheko, motho ka mong o tla fuoa moriana ka ente/nale (morina ona o fanoa ha 'ngoe) ebe bakeng sa dibeke tse nne {4}, monka karolo enoa o dula a shejoa hore na o thusehile kapa che. Dibekeng tsena tse leshome le metso e mene {14} monka karolo e mong le e mong o tla nkoa madi (a tla sebedisoa feela ho sheba hora na o na le kgaello, le hore thuso ea e fuoang e ya thusa na). Madi a tla nkuoa ka nako tsena: (1) qalong ha ho shejoa hore na motho o na le kgaello ya madi, hore a kene boithutong. (2) Ha boithuto bo qala hore ho sejoe hore ha ho qaloa ka dipisisi monka karolo madi a hae a ne a shebahala joang. (3) Kamora dibeke tse tharo a ntse a noa dipidisi, ho shejoa hore na phetoho ya nako e kgutsoanyane eteng. (4) Kamora dibeke tse t'seletseng ho shebooadithetoho bohareng ba phekolo. (5) Kamora dibeke tse leshome {10} e leng qetello ya ho noa dipidisi ho shejoa hore na dipidisi di sebeditse kapa ha diya fana ka thuso. (6) Bekeng ya leshome le metso e mmedi {12} ho shejoa diphetoho tsa nako e kgutsoanyane ka mora ho fuoa moriana ka ente (drip), ho batho bao dipidisi di sa thusang. (7) Ka mora beke tse leshome le metso e mene {14}, qetellong ya boithuto, ho tla etsuoa lihlahlobo tsa ho qetela.

Madi a tla nkuoa nako yohle eo ho nkuoang madi ke a etsang kgaba e yane makgetlo a mane {20millilitre} Madi a tla nkuoa ke LF Mogongoa le M Raphuthing boholo ba nako, empa ka nako tse ding Dr(s) Brand le Jafta ba tla

thusa. Bekeng ya pele {1}, ya bots'elela {6}, ya leshome {10} le leshome le metso e mene {14} ho tla hlokahala mantle (makaka) ele ho sheba hore na motho ha lahleheloe ke madi dibakeng (gastrointestinal tract) tse ding tse ka hare mmeleng.

Monka karolo e momg le e mong otla kopuoa ho araba dipotso ka dijo tseo a dijang, sena se etsoa ho bontsha hore dijo ha se sesosa sa kgaelo ya madi. Dipotso ka ho ya matsatsing, hape ele ho leka ho sheba hore na motho ka mong o lahleheloa ke madi a makae ka kgoedi, hape ele ho tlosa tahlehelo ea madi matsatsing ele sesosa sa kgaello ya madi. Dipotso tse ding e tla ba tse mabapi le ho ja mobu, hore na monka karolo ka mong o ja mobu o mokae, ha hae, o ja joang kapa ka mokoa ofeng, mobu oa o jang o fumaneha kae; tse ele ho ithuta ka mobu o jeoang. Le ho tseba hore na mobu o ka etsang mmeleng oa motho. Dipotso tsena di tla botsoa qalong, mahareng le qetellog ya boithuto (libekeng 1,3,6,10,12 le 14). Madi a tla hlahlojoa ho sheba boemo ba madi, le ho sheba kgaelo ya madi e bakoang ke kgaello ya iron. Tsebetso ya dipheo le sebete e tla shejoa ho sheba maemo a bophelo ba monka karolo. Mme mantle a tla hlahojoa bakeng sa madi ele ho sheba hore monka karolo ha lahleheloe ke madi bakeng tse ding tsa mmele tse sa tlamehang ho lahleheloa ke madi.

Ditlamorao tse seng molemo tsa ho nka karolo boithutong bona: Dipisisi tse tlo sebedisoa li kanna tsa fana ka ho sibeha le mala bathong ba bang, mme sena ha se etsahala ho tla fetoloa mokhoa kalafo (dipidisi). Moriana o tla fanoa ka nale (drip) o tla fanowa tlasa leihlo le ncho la ngaka, mme ngaka e tla thusa ha ho ka ba le ditlamorao. Ditlamorao tsa moriana oa nale ke ho fuoa ho feta ka moo motho a hlokang. Empa hona ho tla ho qojoa ka hore pele monka karolo a fuoa moriana ka nale ho tla shejoa boima ba motho ka mong, le sephetho sa diteko tsa madi hore mong le mong a fuoe ka ho mo lekana. Ho batho bang ho ka etsahala moriana wa nale(drip) o se ba tsoare hantle ka ho tlisa ho ruruha, letlalo le lekhubedu le makgopho a masesane (allergy). Mme ha sensa se etsahala, monka karolo o tla fuoa pheko (antihistamines). Ha ho ka etsahala hore monka karolo a kule ka lebaka la moriana kapa dipidisi tseo a di fuoang, o kgothaletsoa ho joetsa ya ekarabellang boithutong bona. Mme o tla fumantsoa pheko ka ho isoa ngakeng, mme chelete ya ngaka le meriana e tla pataloa ke boithuto eseng monka karolo.

Dintle tsa ho nka karolo boithutong bona: Kgaelo ya madi e tla phekoloa, mme ditla morao tsa ho ba le kgaello ya madi di tla nyenyefala. Ka ho hlahloba ho ya matsatsing ha motho ka mong le mantle (makaka) tahlehelo ya madi dibakeng tse ding ho tla fumanoa, mme ngaka e fane ka keletso. Ho hlahloba dipheo le sebete ho thusa ho hlahoba tsebetso ya ditho tse ding tsa mmele. Tsepo ke hore ha kgaello ya madi e ka lokisoa, takatso ya mobu e tla fukotseha, kapa e fele ho monka karolo e mong le e mong. Hape ka ho nka karolo ho tla thusa hore batho ba bang ba jang mobu ba fumana thuso e nepahetseng.

Mekoa e meng ea ho fumana thuso ntle le ho nka karololo: O ka ya setsing sa kokelo se pela hao, mme ngaka ya o hlahloba bakeng sa kgaello ya madi. Ha ngaka e fumana matsoao a kgaello ya madi, ngaka e tla ofa dipidisi for nako ya khoedi tse tsheletseng. Ha a ntse a sa thusahala a kanna a ofa moriana ka nale (drip).

Ba nka karolo ba tla fuoa lintlha ka botlalo ka boithuto nakong eo ba ntseng ba nka karolo le ka mora boithuto ha lintlha kaofela difeletse. Hore ba tsebe sephetho sa boithuto. Ha ho ka etsahale hore ha boithuto bo ntse bo tsoela pele, ho na le lintlha kapa ntlha e hlahang e amang monka karolo, mme e ka etsang a fetole maikutlo a hae ka ho nka karolo, monka karolo o tla tsibisoa, mme a nke qeto ka boena hore o ntse a tsoella kapa che.

Ho nka karolo boithutong bona kantle ho pateletso: Mong le mong ya nkang karolo ha a patelesoa ho nka karolo. Mong le mong o na le tokelo ea ho tlohela nako efe kapa efe ha a utloa a se a sa batle ho tsoela pele. Mme ho etsa joalo ha ho mo tlame ho patala letho, kapa ho lahleheloa ke melomo efe kapa efe eo a nang le ona joalo ka moahi wa Afrika-Boroa.

Ho pataloa hoa banka karolo: Boithuto bona ha bo thusoe ka chelete ke khoebo e rekisang kapa le e etsa dipidisi. Ka hona monka karolo ha tlo fuoa chelete bakeng sao ho nka karolo. Ha ho ka etsahala hore mong a ba batle chelete a re ke karolo ya boithuto, ho bohlokoa hore a se patale empa a tsibise ba ikarabellang hang hang.

Empa motho ka mong o tla patalloa ho palama le dijo nako eo a tlamehang ho tla bloemfontein ka lebaka la boituto (chelete e R90 ke ya ho palama ho ya Bloemfontein, mme R60 ke ea lijo ho bolelang hore motho ka mong o tla palamisoa ho tla Bloemfontein, mme a fuoe dijo ha le teng). Dijo di tla fuoa ke kantini ya CUT, mme banka karolo ha ba fuoe chelete, empa tikete ya fo fumana dijo kantining. Dikoloi tsa sekolo ke tsona tse tla sebedisoa ho lata bonka karolo Botshabelo ho ba tlisa CUT Bloemfontein, eseng tsa sechaba. Ho bohlokoa ho re banka karolo ba tsebe hore ha ba tlo patala letho ho nka karolo boithutong bona.

Confidentiality: Boithutong bona ho tlo etsoa ka hohle hohle hore dintlha tsa monka karolo e mong le mong tse kang mabitso le maemo a bona a bophelo dibolokehile. Mme boithuto bo tla sebedisa feela dinomoro tseo monka karolo e mong le e mong a tla e fuoa ha nka karolo. Mme boithuto bo tla etsa ka hohle ho se phatlalatse dintlha tse sa amaneng le boithuto.

Contact details of reseacher(s)- ha hona le linthla tse sa hlakang kapa tseo monka karolo a hlokang tlhakisetso ho tsona, kapa ho bolela ka ditlamorao tsa dipidisi kapa moriana oa boithuto a ka ikopanya le Lebogang Mogongoa ho 073 815 6767 kapa 071 151 0647 kapa 051 507 3118 (nakong tsa mosebetsi).

Dinomoro tsa Secretariat and chair: Ethics Committee of the Faculty of Health Scieces, University of Free State- ho bolela ka mathata a mabapi le boithuto. Tel number: (051) 405 2812.

APPENDIX: D

INFORMED CONSENT DOCUMENT

CONSENT DOCUMENT

FORM EC 31

CONSENT TO PARTICIPATE IN RESEARCH

PROJECT TITLE: Oral and intravenous iron therapy in geophagic women with iron deficiency anaemia

You have been asked to participate in a research study.

You have been informed about the study by ……...

You have been informed about any available compensation or medical treatment if an injury occurs as a result of study-related procedures.

Your participation in the study may be terminated by the investigator without your consent when it is discovered that you are pregnant.

You may contact Lebogang Mogongoa at 073 815 6767 or 071 151 0647 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 Sciences, UFS at the telephone number (051) 4052812 if you have questions about your rights as a research participant.

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 Date
(Where applicable)

_____ _____

Signature of Translator Date
(Where applicable)

APPENDIX: E

EXIT QUESTIONNAIRE

Exit interview questionnaire

Interviewer			
Visit week		Visit date	

Section A to be completed at the end of the study

Section A & B should be completed if the research participant terminates the study prematurely

Section A: Questionnaire on overall experience of the study:

Indicate how you feel about the following aspects of the study using 5 point scale (strongly disagree – 1, disagree – 2, neutral – 3, agree – 4, strongly agree – 5): recruitment, phlebotomy, communication, transportation, oral iron, IV iron, faecal samples and time management. In addition, at the end of the section feel free to recommend other questions that could be asked and other comments not covered in the questions.

Recruitment

The study objectives were explained thoroughly.

Strongly disagree	Disagree	Neutral	Agree	Strongly agree

The information document was easy to follow.

Strongly disagree	Disagree	Neutral	Agree	Strongly agree

I understood what my role in the project was going to be.

Strongly disagree	Disagree	Neutral	Agree	Strongly agree

When I signed consent I was given an opportunity to ask questions.

Strongly disagree	Disagree	Neutral	Agree	Strongly agree

The investigators made sure that I understood my role in the project.

Strongly disagree	Disagree	Neutral	Agree	Strongly agree

Any other comments that can improve the recruitment experience:

_____

_____

_____

Phlebotomy

I was treated with respect.

Strongly disagree	Disagree	Neutral	Agree	Strongly agree

The process was explained to me.

Strongly disagree	Disagree	Neutral	Agree	Strongly agree

The phlebotomist was caring.

Strongly disagree	Disagree	Neutral	Agree	Strongly agree

Any other comments that can improve the phlebotomy experience:

_____

_____

_____

Communication

The communication was professional

Strongly disagree	Disagree	Neutral	Agree	Strongly agree

I was spoken to in a caring, courteous and respectful manner

Strongly disagree	Disagree	Neutral	Agree	Strongly agree

The language that was used was understandable to me

Strongly disagree	Disagree	Neutral	Agree	Strongly agree

The phone calls were made at appropriate times

Strongly disagree	Disagree	Neutral	Agree	Strongly agree

The appointments were convenient

Strongly disagree	Disagree	Neutral	Agree	Strongly agree

Was the translation of questionnaires easy to follow?

Strongly disagree	Disagree	Neutral	Agree	Strongly agree

Any other comments that can improve the communication:

_____

_____

_____

Transportation

Was the transportation comfortable?

Strongly disagree	Disagree	Neutral	Agree	Strongly agree

Was the manner of driving acceptable?

Strongly disagree	Disagree	Neutral	Agree	Strongly agree

Did the drivers keep to the speed limit?

Strongly disagree	Disagree	Neutral	Agree	Strongly agree

Did you feel safe during transportation?

Strongly disagree	Disagree	Neutral	Agree	Strongly agree

Any other comments that can improve the transportation experience:

_____

_____

_____

Time management

Do you feel your time was respected?

Strongly disagree	Disagree	Neutral	Agree	Strongly agree

Please explain:

_____

_____

_____

Did we keep to the appointment?

Strongly disagree	Disagree	Neutral	Agree	Strongly agree

Any other comments that can improve time management:

_____

_____

_____

Oral iron therapy

Did the pill have an unbearable / unacceptable taste?

Strongly disagree	Disagree	Neutral	Agree	Strongly agree

Any other comments that can improve oral iron therapy:

_____

_____

_____

IV iron

Was the IV iron administered with care?

Strongly disagree	Disagree	Neutral	Agree	Strongly agree

Did you feel like you were properly monitored while the drip was administered?

Strongly disagree	Disagree	Neutral	Agree	Strongly agree

Any other comments that can improve the IV iron therapy experience:

_____

_____

_____

Faecal samples

Was the collection of faecal sample properly explained to you?

Strongly disagree	Disagree	Neutral	Agree	Strongly agree

Was the process of faecal sample collection easy for you? YES / NO
Please explain:

_____

_____

_____

How can the faecal sample collection be improved?

_____

_____

_____

Any other questions you feel should be included in this questionnaire that were not asked:

_____

_____

_____

_____

_____

_____

_____

_____

Section B: Questionnaire on reason for termination of study:

At which stage of the study did you terminate your participation (which week)?

_____

Indicate whether you terminated the study due to any of the following reason, please answer each question.

	YES	NO	Don't know
Negative treatment from study organizers			
Negative effects of the oral treatment			
Negative impact on schedule on my schedule			
Due to pregnancy			
	YES	NO	Don't know
Fear of IV iron stage			
Fear of phlebotomy			
Negative effects of phlebotomy			
Stigma from community members			
Family members or partner objection to study			
Found employment, study interferes with work			
Bad or speedy driving during transportation			
Organizers not keeping time for appointments			
Organizers not professional			
Please explain if organizers are not professional:_____			

State any other reason for leaving the study that is not mentioned above:

1) _____

2) _____

3) _____

4) _____

5) _____

The geophagia study group wishes to thank for participating in the research study. We hope we have touched your life in a positive manner. You have touched ours.

APPENDIX: F

SAMPLE SIZE CALCULATION RESULTS

Sample Size:X-Sectional, Cohort, & Randomized Clinical Trials	
Two-sided significance level(1-alpha):	95
Power(1-beta, % chance of detecting):	80
Ratio of sample size, Unexposed/Exposed:	1
Percent of Unexposed with Outcome:	5
Percent of Exposed with Outcome:	30
Odds Ratio:	8.1
Risk/Prevalence Ratio:	6
Risk/Prevalence difference:	25

	Kelsey	Fleiss	Fleiss with CC
Sample Size - Exposed	37	36	43
Sample Size- Nonexposed	37	36	43
Total sample size:	74	72	86

References

Kelsey et al., Methods in Observational Epidemiology 2nd Edition, Table 12-15
Fleiss, Statistical Methods for Rates and Proportions, formulas 3.18 &3.19
CC = continuity correction
Results are rounded up to the nearest integer.

APPENDIX: G

GEOPHAGIC PRACTISE QUESTIONNAIRE

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
Region:	Free State		
	Limpopo		
	North West		
	Gauteng		

17

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

27

5. Ethnic Group:

- 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		
2		
3		
4		
5		
6		
7		
8		

32-33 34

35-36 37

38-39 40

41-42 43

44-45 46

47-48 49

50-51 52

53-54 55

1

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

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

9. Occupation: _____ 60-61

10. Monthly income: R/Pula_____ 62-67

11. Highest educational level attained: 68
- No schooling
- Primary
- 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 52 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

6

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
47
48
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52
53
54
55
56
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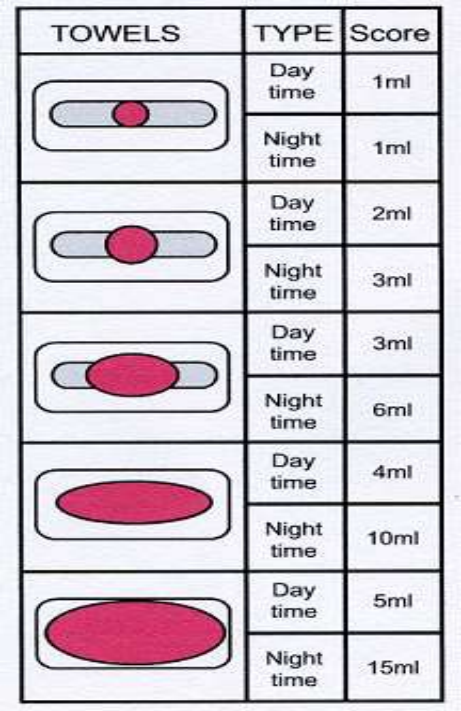
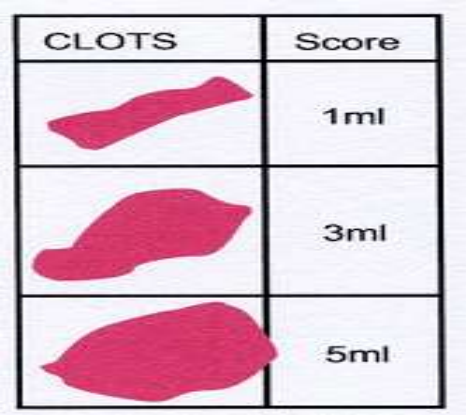
62
63
64
65
66
67
[ ][ ] 68-69
[ ][ ] 70-71
[ ][ ] 72-73

APPENDIX: H

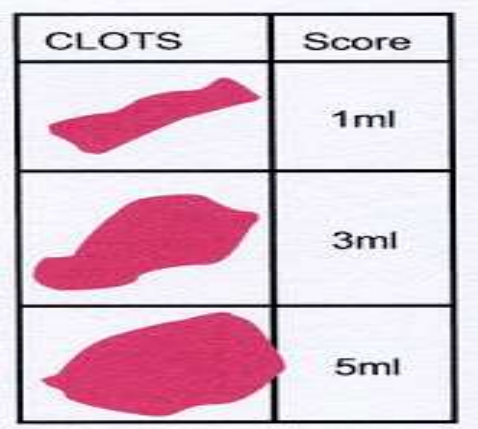
MENSTRUAL CHART INSTRUMENT



D2	D3	D4	D5	D6	D7	D8	D9	D10



D2	D3	D4	D5	D6	D7	D8	D9	D10



APPENDIX: I

SIDE EFFECTS QUESTIONNAIRE

Adverse effects questionnaire

Investigator		**Randomisation number**	
Subject initials		Subject number	
Visit number		Visit date	

When did you start consuming study medication? _____/_____/_____

Number of pills given _____ and left _____ (confirm by sight before giving the next set of pills)

Are there any missed doses? _____

If there are missed doses, which dates? _____

Number pills now given: _____

Name of other medication or supplement that you are currently taking:

_____

_____

_____

How long have you been consuming the said medication or supplement? _____

_____

When do you consume these medication or supplement? _____

_____

_____

Dosage of medication or supplement: _____

When do you prefer to take the study medication? _____

Do you take your study medication with Tea? YES NO

If YES: When (dates) and how many times from the last visit?_____

Have you ever consumed tea within an hour of taking your study medication? YES NO

If YES: When (dates) and how many times from the last visit?_____

Do you take your study medication with a meal? YES NO

If NO: Which days didn't you take the medication with a meal?_____

Have you had (or have) a challenge remembering to take your medication? _____

_____

What is your strategy for remembering to take the medication?_____

Have you had any illness or symptoms since your last visit? (NOTE: Record all in the table)

Which of those mentioned illnesses were diagnosed by a physician (date of diagnosis)?_____

_____

Which medication did you take for the illness' treatment? _____

For how long did you take the medication and what was the dose (i.e. three times a day)? _____

Is the illness resolved? YES NO

Since the last visit have you consumed soil? YES NO

If YES: Which days and approximately how much?_____

_____

Adverse effects	Duration	Intensity / severity	Causality	Treatment oral/intravenous	Outcome	Seriousness

Investigator signature		**Date**	

APPENDIX: J

MENSTRUAL ASSESSMENT QUESTIONNAIRE

Menstrual Blood Loss Assessment Questionnaire Date: _____

Interviewer: _____ Age of Participant: _____

Participant No: _____ Week number: _____

Geophagic group ☐ Non geophagic group ☐

Menstrual Patterns

1. At what age did you get your first menstrual period?
   - ☐ < 9 years
   - ☐ 9-10 years
   - ☐ 11-12 years
   - ☐ 13-14 years
   - ☐ 15-16 years
   - ☐ >17 years
2. Do you have your period every month?
   - ☐ Yes
   - ☐ No

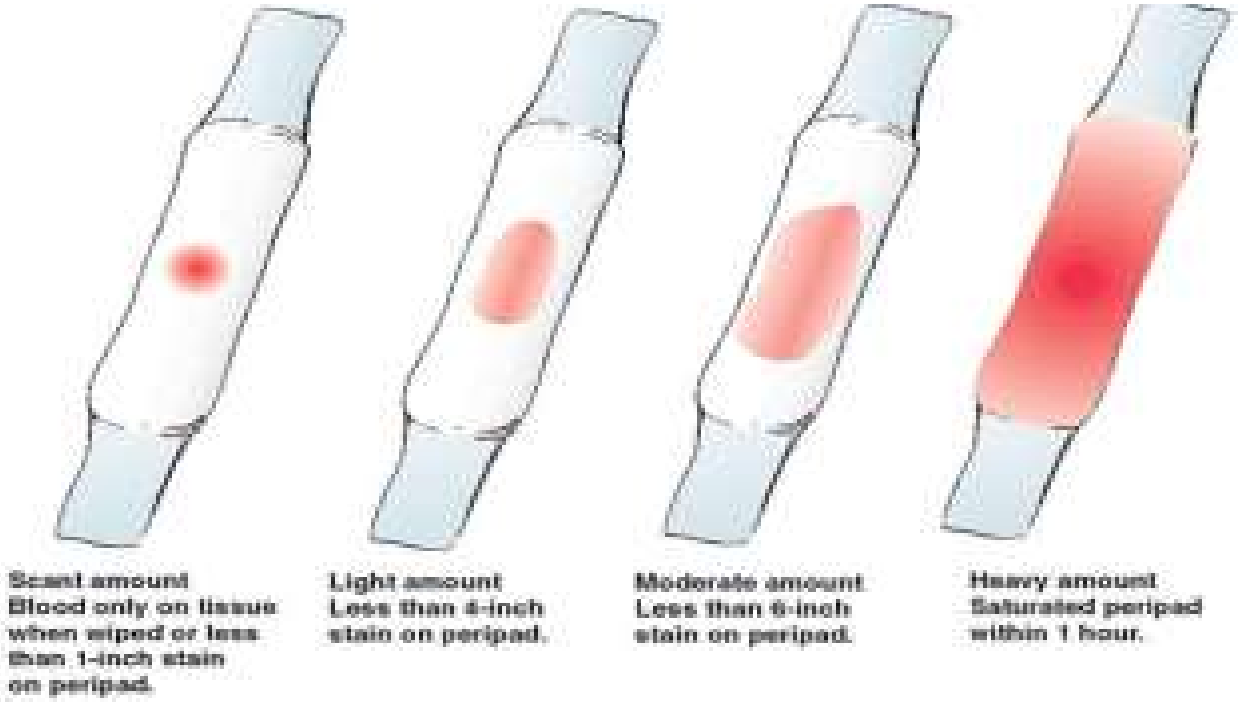
   2.1 If no, how often do you get your period?

   _____
3. How many days do your period last?

   _____
4. What kind of sanitation do you use during your periods?
   - ☐ Pads
   - ☐ Tampons
   - ☐ Cloth
   - ☐ Other

   If other, please state what you use _____
5. According to your choice of sanitation, how many do you use per day during your period?

   _____
6. Indicate how the majority of pads are filled (Use diagram below)



Type 1 Type 2 Type 3 Type 4

☐ ☐ ☐ ☐

6.1 Which one is the second most common (With reference to picture above)?

- ☐ Type1
- ☐ Type 2
- ☐ Type 3
- ☐ Type 4

7. How best would you describe your menstrual flow?

- ☐ Very light
- ☐ Light
- ☐ Moderate
- ☐ Heavy
- ☐ Very heavy

Common Menstrual Symptoms

8. Do you experience painful periods?

☐ Yes

☐ No

8.1 If yes, how would you rate the pain? Please refer to the pain scale attached

_____

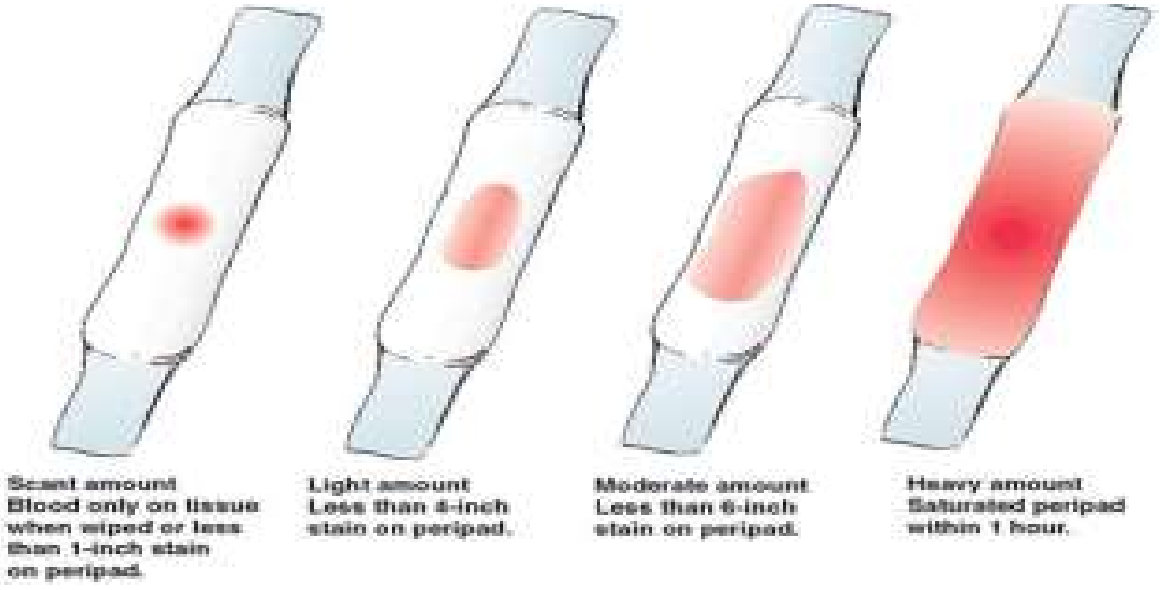
9. Do you experience blood spots or spotting (bleeding in between menstrual cycle)?

☐ Yes

☐ No

9.1 If yes, how long does it last?_____

9.2 How much blood do you lose? Use diagram below



Type 1 ☐ Type 2 ☐ Type 3 ☐ Type 4 ☐

9.3 What sanitation do you use during spotting?

9.3.1 If none, where do you observe the spotting?

_____

10. Do you experience staining of the bed at night during menstruation?

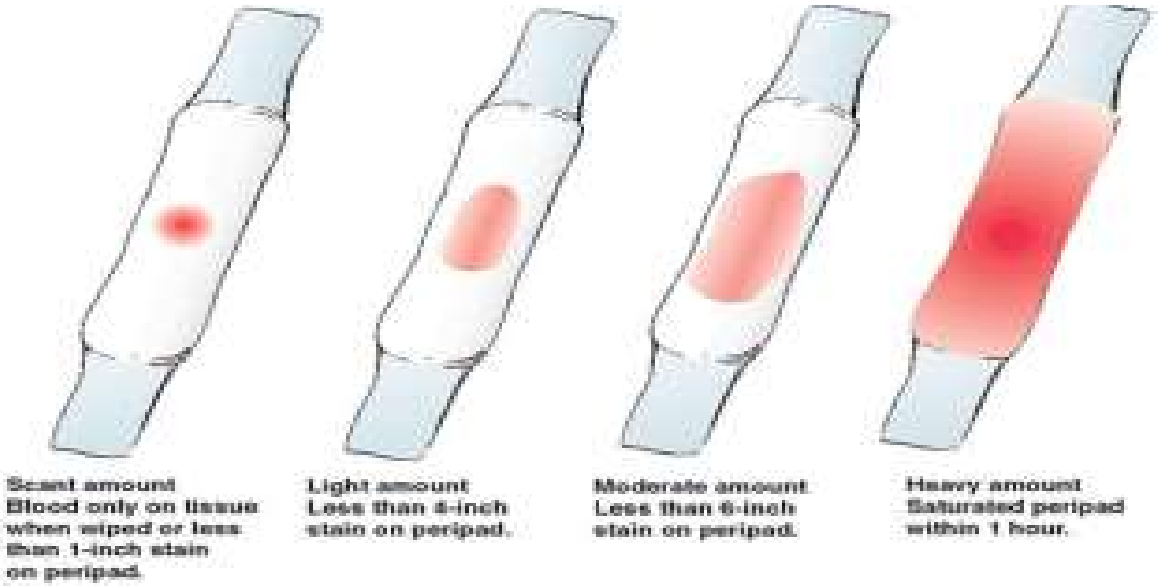
☐ Yes

☐ No

10.1 If yes, how often does it occur during your period?

_____

10.2 How big is the spot? Use the diagram below



Type 1 ☐ Type 2 ☐ Type 3 ☐ Type 4 ☐

Personal History

11. Please indicate with a **YES/NO/I DON'T KNOW** if you have ever been diagnosed with the following medical conditions;
    - Hypothyroidism

      Yes ☐ No ☐ I don't know ☐

    - Iron deficiency anaemia

      Yes ☐ No ☐ I don't know ☐

    - Lupus Erythematosus

      Yes ☐ No ☐ I don't know ☐

    - Cirrhosis of the liver

      Yes ☐ No ☐ I don't know ☐

12. Are you currently taking medication?
    ☐ Yes
    ☐ No

13. If yes please list the medication

    _____

14. Are you on birth control?
    ☐ Yes

    ☐ No

    14.1 If yes which type (Oral Contraceptive Pill, Intra-Uterine Device (IUD), other)?

    _____

    14.2 How long have you been on birth control?

    _____

    14.3 Have you changed your birth control method in the previous 3 months?

    ☐ Yes

☐ No

14.4 If yes, state the previous method and the method you are currently using.

Previous: _____

Current: _____

14.4.1 How long have you been on the current contraceptive method?

_____

15. Do you smoke cigarettes?

☐ Yes

☐ No

15.1 If yes, how many per day? _____

16. Do you consume alcohol?

☐ Yes

☐ No

16.1 If yes, which type of alcohol?

☐ Wine

☐ Beer

☐ Cider

☐ Spirits

☐ Homebrew

☐ Other, specify _____

16.2 If yes, how many glasses, bottles, cans: per day, week or month?

	per day	per week	per month
☐ Wine			
☐ Beer			
☐ Cider			
☐ Spirits			
☐ Homebrew			

☐ Other

Family History

17. Please indicate if anyone from your family has suffered from the following;

- Fibroids Yes ☐ No ☐ I don't know ☐
- Endometriosis Yes ☐ No ☐ I don't know ☐
- Thyroid Disorders Yes ☐ No ☐ I don't know ☐
- Bleeding disorders Yes ☐ No ☐ I don't know ☐

Sexual History

18. Are you sexually active?

☐ Yes

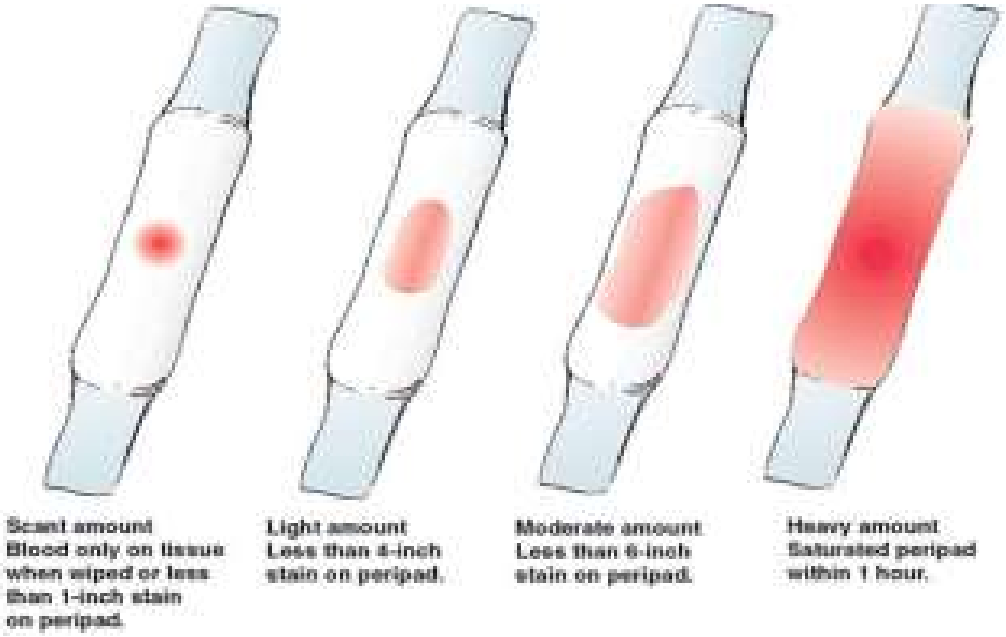
☐ No

19. Do you experience bleeding after sexual intercourse?

☐ Yes

☐ No

19.1 If yes, how much blood do you lose? Use diagram below



Type 1 ☐ Type 2 ☐ Type 3 ☐ Type 4 ☐

19.2 Have you seen a medical doctor about it?

☐ Yes

☐ No

20. If yes, what was the diagnosis?

_____

_____

21. Do you experience pain during sexual intercourse?

☐ Yes

☐ No

21.1 If yes where is the site of the pain (superficial within the vagina or deep within the pelvis)?

_____

21.2 Do you have vaginal discharge?

☐ Yes

☐ No

21.2.1 If yes, how does it present?

_____

21.2.2 Has it changed its appearance in the past 3 months?

☐ Yes

☐ No

21.3 If yes what is the nature of the discharge (smelly, colour, texture)

_____

Interference of Menstruation with Life Activities

22. Please indicate with a **YES/NO** where the following is relevant to you;

- Sexual activities Yes ☐ No ☐ I don't know ☐
- Attending classes/lectures Yes ☐ No ☐ I don't know ☐
- Completing assignment Yes ☐ No ☐ I don't know ☐
- Work Yes ☐ No ☐ I don't know ☐
- Relationship with family Yes ☐ No ☐ I don't know ☐
- Relationship with boyfriend Yes ☐ No ☐ I don't know ☐
- Sports and exercise Yes ☐ No ☐ I don't know ☐
- Relationship with friends Yes ☐ No ☐ I don't know ☐
- Social activities Yes ☐ No ☐ I don't know ☐

APPENDIX: K

ANOVA, FREIDMAN AND WILCOXON STATISTICAL VALUES NOT REPORTED IN THE THESIS

Table 4.9 Mean changes of oral iron therapy in anthropometric and general health indicators of the entire group at different

VARIABLE	df	df	F	p-value
Weight (kg)	1.632	102.277	0.319	0,682 gg
BMI	1.585	99.836	0.321	0.675 gg
Fat %	1.021	63.304	0.386	0,541 gg
Water %	1.126	68.675	0.237	0,656 gg
Muscle %	1.234	76.532	0.792	0,401 gg
Cal. r/d	1.160	71.919	0.219	0,678 gg
Bust (cm)	1.081	62.721	0.139	0,731 gg
Waist (cm)	1.450	85.540	0.425	0,590 gg
Hip (cm)	1.294	76.325	1.465	0,235 gg
WHR	1.891	111.572	0.108	0,887 gg
SBP	1.755	98.293	1.600	0,209 gg
DBP	1.734	95.372	1.007	0,360 gg
Pulse	1.964	100.89	1.563	0,215 gg

Table 4.12 Mean changes in iron study, pregnancy and inflammatory results of the entire study groups at different study visits

VARIABLE	df	df	F	p value
S Fe			(23.132)	<0,001 f
Trans. (g/L)	2.312	168.742	2.962	0,047 gg
Ferritin			(9.109)	0,028 f w
ICBT (µg/L)	2.480	195.958	9.634	<0,001 g
ISAT (%)			(15.580)	<0,001 f
hCG (IU/L)			(84.348)	<0,001 f
cRP (mg/L)			(3.381)	0,336 f

(Chi square)

Table 4.10 Mean changes blood cell counts of the entire study group at different study visits

VARIABLE	df	df	F	p value
RBC	1.424	111.048	0.931	0,369 gg
Hb (g/dL)	1.305	100.499	2.251	0,129 gg
HCT (%)	1.430	111.528	1.731	0,190 gg
MCV (fl)	1.124	87.670	1.093	0,307 gg
MCH (pg)	1.094	85.296	1.019	0,323 gg
MCHC	1.394	108.764	0.590	0,497 gg
RDW (%)	1.852	144.461	0.701	0,487 gg
PLT	2.379	185.526	2.721	0,059 gg
WBC	2.155	168.125	0.818	0,451 gg
Neutrophil #	2.217	161.831	0.527	0,809 gg
Lymphocyte	1.947	144.111	0.531	0,584 gg
Monocyte #	2.397	177.382	1.184	0,313 gg
Eosinophil #	2.187	159.660	0.590	0,570 gg
Basophil #	1.552	116.373	1.456	0.238 gg

Table 4.11 Mean changes in the general health clinical chemistry of the entire study group during different study visits

VARIABLE	df	Z t	p value
BUN (mg/dL)		-3.397	0,001 w
S Creat. (mg/dl)	68	-2.088	0,041 t
TP (g/L)		-4.406	<0,001 w
Albumin (g/L)		-2.871	0.004 w
ALP (U/L)		-5.143	<0,001 w
AST (U/L)		-0.615	0,538 W
ALT (U/L)		-3.177	0,001 W
K (mmol/L)		-1.569	0,117 w
Na (mmol/L)		-4.541	<0,001 w
Phos (mmol/L)		-2.354	0,019 w
Mg (mg/dL)		-0.226	0,821 w
Ca (mg/dL)	79	1.542	0,127 t
Zinc (umol/L)	77	5.116	<0,001 t

Table 4.13 Mean anthropometric and general health indicators of the group that abstained from consumption of soil at different visits

VARIABLE	df	df	F	p value
Weight (kg)	1.562	60.918	0.393	0,625 gg
BMI	1.474	57.485	0.304	0,672 gg
Fat %	1.019	39.746	0.091	0,769 gg
Water %	1.125	42.747	0.065	0,829 gg
Muscle %	1.287	50.192	0.437	0,561 gg
Cal. r/d	1.160	45.223	0.221	0,677 gg
Bust (cm)	1.084	39.035	0.029	0,882 gg
Waist (cm)	1.513	54.452	0.368	0,366 gg
Hip (cm)	1.332	47.969	0.933	0,928 gg
WHR	1.846	66.469	0.063	0,882 gg
SBP	1.688	57.387	1.098	0,332 gg
DBP	1.551	51.172	0.670	0,480 gg
Pulse	1.963	56.933	6.800	0,002 gg

Table 4.14 Mean blood cell count changes of the group that abstained from consumption of soil on different visits

VARIABLE	df	df	F	p value
RBC	1.398	61.532	0.889	0,383 gg
Hb (g/dL)	1.422	62.558	4.305	0,029 gg
HCT (%)	1.447	63.684	3.843	0,039 gg
MCV (fl)	1.119	49.233	8.185	0,005 gg
MCH (pg)	1.128	49.651	6.116	0,014 gg
MCHC	1.470	64.672	1.490	0,233 gg
RDW (%)	2.198	96.699	4.763	0,009 gg
PLT	2.156	94.886	2.382	0,094 gg
WBC	2.271	99.931	0.723	0,504 gg
Neutrophil #	2.191	85.450	0.661	0,532 gg
Lymphocyte	2.137	85.485	0.532	0,601 gg
Monocyte #	2.150	85.987	1.851	0,160 gg
Eosinophil #			(2.569)	0,463 f
Basophil #			(3.569)	0,312 f

(Chi square)

Table 4.16 Mean iron study, pregnancy and inflammatory indicators changes in of the groups that abstained from consumption of soil at different visit

VARIABLE	df	df	F	p value
S Fe			(81.707)	<0,001 f
Trans. (g/L)	2.316	92.624	1.158	0,323 gg
Ferritin			(8.402)	0,038 f
ICBT (µg/L)			(15.642)	0,001 f
ISAT (%)			(21.900)	<0.001 f
hCG (IU/L)			(43.467)	<0,001 f
cRP (mg/L)			(3.384)	0,336 f

(Chi square)

Table 4.15 Mean general health clinical chemistry indicators changes of the group that abstained from consumption of soil on different visit

VARIABLE	df	Z t	p value
BUN (mg/dL)	43	-2.343	0,024 t
S Creat. (mg/dl)	37	-1.722	0,094 t
TP (g/L)	43	4.907	<0,001 t
Albumin (g/L)	42	2.821	0,007 t
ALP (U/L)	37	4.974	<0,001 t
AST (U/L)		-0.408	0,683 w
ALT (U/L)		-2.495	0,013 w
K (mmol/L)		0.695	0,487 w
Na (mmol/L)		-3.030	0,002 w
Phos (mmol/L)	43	-0.729	0,470 t
Mg (mg/dL)		-0.313	0,754 w
Ca (mg/dL)	43	1.076	0,288 t
Zinc (umol/L)	42	3.656	0,001 t

Table 4.17 Mean anthropometric and general health indicators changes of the group that continued with consumption of soil during different visits

VARIABLE	df	df	F	p value
Weight (kg)	1.509	34.716	0.143	0,808 gg
BMI	1.614	37.131	0.235	0,744 gg
Fat %	1.025	22.556	0.484	0,498 gg
Water %	1.114	24.507	0.589	0,468 gg
Muscle %	1.032	22.694	0.507	0,489 gg
Cal. r/d	1.142	25.118	0.165	0,721 gg
Bust (cm)	1.059	22.235	0.306	0,710 gg
Waist (cm)	1.150	25.299	0.180	0,381 gg
Hip (cm)	1.167	25.675	0.853	0.593
WHR	1.712	37.673	0.481	0,599 gg
SBP	1.793	37.660	0.620	0,527 gg
DBP	1.903	39.957	0.695	0,498 gg
Pulse	3	63	2.791	0,063

Table 4.18 Mean blood cell count changes of the group that continued with consumption of soil at different visits

VARIABLE	df	df	F	p value
RBC	1.361	44.914	0.318	0.645 gg
Hb (g/dL)	1.220	39.026	4.110	0,042 gg
HCT (%)	1.428	47.116	3.236	0,064 gg
MCV (fl)	1.148	37.898	3.361	0,069 gg
MCH (pg)	1.078	35.588	3.352	0.073 gg
MCHC	1.363	44.988	2.127	0,145 gg
RDW (%)	1.838	60.643	10.592	<0,001 g
PLT	2.128	70.227	0.801	0,460 gg
WBC	1.896	62.583	1.408	0,252 gg
Neutrophil #	2.152	71.002	1.846	0,163 gg
Lymphocyte	1.708	56.371	0.669	0,494 gg
Monocyte #	2.333	77.002	2.412	0,088 gg
Eosinophil #			(0.531)	0,912 f
Basophil #	2.220	73.260	2.054	0,130 gg

(Chi square)

Table 4.20 Mean iron study, pregnancy and inflammatory changes of the groups that continued with consumption of soil during at different visits

VARIABLE	df	df	F	p value
S Fe			(8.436)	0,038 f w
Trans. (g/L)			(2,491)	0,477 f
Ferritin			(4,003)	0,261 f
ICBT (µg/L)			(14.767)	0,002 f w
ISAT (%)			(17.233)	<0,001fw
hCG (IU/L)			(41.082)	<0,001fw
cRP (mg/L)			(2.283)	0,516 f

(Chi square)

Table 4.19 Mean general clinical chemistry changes of the group that continued with consumption of soil at different visits

VARIABLE	df	Z t	p value
BUN (mg/dL)	34	-2.992	0,005 t
S Creat. (mg/dl)	30	-1.216	0,233 t
		-1.071	0,284 w
TP (g/L)	34	2.726	0,010 t
		-2.303	0,021 w
Albumin (g/L)	34	2.185	0,036 t
ALP (U/L)	30	3.631	0,001 t
		-3.244	0,001 w
AST (U/L)	30	-0.770	0,447 t
		-0.462	0,644 w
ALT (U/L)	35	-1.370	0,179 t
		-2.028	0,043 w
K (mmol/L)	35	1.755	0,088 t
Na (mmol/L)		-3.503	<0,001 w
	35	4.210	<0,001 t
Phos (mmol/L)		-2.967	0,003 w
	33	-3.302	0,002 t
Mg (mg/dL)	35	-0.576	0,568 t
Ca (mg/dL)	35	1.105	0,277 t
Zinc (umol/L)	34	3.692	0,001 t

Table 4.21 Mean anthropometric and general health indicators changes the entire group at different visits

VARIABLE	df	df	F	p value
Weight (kg)	1.108	72.011	0.012	0.931 gg
BMI			(2.526)	0.283 f
Fat %			(3.341)	0.188 f
Water %	1.353	87.918	0.305	0.651 gg
Muscle %	1.381	89.756	0.365	0.616 gg
Cal. r/d	1.238	80.472	0.013	0.943 gg
Bust (cm)			(5.331)	0.558 f
Waist (cm)	1.307	86.243	0.446	0.792 gg
Hip (cm)	1.183	78.082	0.102	0.080 f
WHR			(5.041)	0.070 f
SBP	1.753	103.432	0.425	0.628 gg
DBP	1.687	99.547	0.760	0.450 gg
Pulse	1.593	90.826	6.134	0.006 gg

(Chi square)

Table 4.22 Mean blood cell count changes of the entire study group at different study visits

VARIABLE	df	df	F	p value
RBC	1.312	82.675	16.333	<0.001 gg
Hb (g/dL)	1.193	75.161	53.436	<0.001 gg
HCT (%)	1.261	79.465	73.360	<0.001 gg
MCV (fl)			(48.925)	<0.001 f
MCH (pg)	1.186	74.730	19.265	<0.001 gg
MCHC	1.389	87.519	0.738	0.435 gg
RDW (%)			(22.574)	<0.001 F w
PLT			(20.614)	<0.001 F w
WBC			(2.310)	0.315 f
Neutrophil #			(0.661)	0.718 F
Lymphocyte	1.228	77.334	6.369	0.009 gg
Monocyte #			(3.530)	0.171 f
Eosinophil #			(0.715)	0.699 f
Basophil #			(10.157)	0.006 f

(Chi square)

Table 4.24 Mean iron study, pregnancy and inflammatory indicators changes of the entire study groups at different study visits

VARIABLE	df	df	F	p value
S Fe			(47.348)	<0.001 f w
Trans. (g/L)	1.672	105.331	71.016	<0.001 gg
Ferritin			(108.863)	< 0.001 f w
ICBT (µg/L)	1.834	128.354	90.990	<0.001 gg
ISAT (%)			(59.521)	< 0.001 f w
hCG (IU/L)			(59.660)	< 0.001 f w
cRP (mg/L)			(3.642)	0.162 f

(Chi square)

Table 4.23 Mean general health clinical chemistry changes of the entire study group during different study visits

VARIABLE	df	Z t	p value
BUN (mg/dL)	73	-0.181	0.857 t
S Creat. (mg/dl)	72	-3.181	0.002 t
TP (g/L)		-2.652	0.008 w
Albumin (g/L)		-4.583	<0.001 w
ALP (U/L)		-0.544	0.587 w
AST (U/L)		-2.108	0.035 w
ALT (U/L)		-0.370	0.711 w
K (mmol/L)		-2.440	0.015 w
Na (mmol/L)		-3.945	<0.001 w
Phos (mmol/L)		-4.662	<0.001 w
Mg (mg/dL)		-2.999	0.003 f
Ca (mg/dL)	73	-5.395	<0.001 t
Zinc (umol/L)	68	-1.249	0.216 t

Table 4.25 Mean anthropometric and general health indicators changes of the group that abstained from consumption of soil at different visits

VARIABLE	df	df	F	p value
Weight (kg)	1.169	44.430	0.005	0.963 gg
BMI	2	78	0.178	0.811
Fat %	1.425	55.557	0.041	0.913 gg
Water %	1.424	2385.926	0.250	0.703 gg
Muscle %	1.296	49.233	0.510	0.525 gg
Cal. r./day	1.295	50.494	0.017	0.941 gg
Waist (cm)	1.376	53.645	0.223	0.716 gg
Hip (cm)	1.229	47.932	0.590	0.479 gg
WHR			(2.126)	0.345 f
Bust (cm)			(0.416)	0.812 f
SBP			(0.735)	0.692 f
DBP	1.696	64.442	0.405	0.635 gg
Pulse	1.674	58.597	1.141	0.319 gg

Table 4.26 Mean blood cell count changes of the group that abstained from consumption of soil at different visits

VARIABLE	df	df	F	p value
RBC	1.331	49.239	8.393	0.003 gg
Hb (g/dL)	1.291	47.776	33.268	<0.001 gg
HCT (%)	1.303	48.193	36.680	<0.001 gg
MCV (fl)	1.294	47.885	7.692	0.004 gg
MCH (pg)	1.344	49.721	7.135	0.006 gg
MCHC	1.440	53.297	5.108	0.017 gg
RDW (%)	1.566	57.939	15.992	<0.001 gg
PLT	1.419	52.519	5.353	0.015 gg
WBC	1.293	47.828	0.991	0.346 gg
Neutrophil #			(0.693)	0.707 f
Lymphocyte	1.319	48.788	2.306	0.128 gg
Monocyte #	1.343	49.708	1.914	0.170 gg
Eosinophil #			(4.582)	0.101 f
Basophil #			(1.050)	0.592 f

Table 4.28 Mean iron study, pregnancy and inflammatory indicators changes of the groups that abstained from consumption of soil at different visits

VARIABLE	df	df	F	p value
S Fe			(31.801)	<0.001fw
Trans. (g/L)	1.602	52.880	37.029	<0.001 gg
Ferritin			(55.192)	<0.001 f w
ICBT (µg/L)	1.746	64.598	53.459	<0.001
ISAT (%)			(33.211)	<0.001 f w
hCG (IU/L)			(36.000)	<0.001 f w
cRP (mg/L)			(7.600)	0,022

Table 4.27 Mean general health clinical chemistry indicators changes of the group that abstained from consumption of soil at different visits

VARIABLE	df	Z t	p value
BUN (mg/dL)	39	-1.305	0.200 t
S Creat. (mg/dl)	38	-1.778	0.083 t
TP (g/L)		-2.651	0.008 w
Albumin (g/L)	39	-5.008	<0.001 t
ALP (U/L)		-1.533	0.125 w
AST (U/L)		-1.075	0.282 w
ALT (U/L)		-0.156	0.876 w
K (mmol/L)		-2.263	0.024 w
Na (mmol/L)	39	-3.646	0.001 t
Phos (mmol/L)	39	-3.199	0.003 t
Mg (mg/dL)	39	-3.096	0.004 t
Ca (mg/dL)	39	-3.999	<0.001 t
Zinc (umol/L)	38	-1.003	0.322 t

Table 4.29 Mean anthropometric and general health indicators changes of the group that continued with consumption of soil at different visits

VARIABLE	df	df	F	p value
Weight (kg)	1.005	26.138	0.018	0,894 gg
BMI	1009	26.229	0.264	0,614 gg
Fat %	1.027	26.699	0.195	0,669 gg
Water %	1.133	29.448	0.253	0,419 gg
Muscle %	1.423	36.986	0.256	0,699 gg
Cal. r./day	1.095	27.375	0.007	0,946 gg
Waist (cm)	1.133	29.448	0.253	0,648 gg
Hip (cm)	1.077	28.007	0.466	0,524 gg
WHR	1.538	39.994	0.827	0.416 gg
Bust (cm)	1.033	26.869	0.889	0.358 gg
SBP	1.477	29.550	0.085	0,863 gg
DBP	2	40	0.476	0,625 b
Pulse	1.330	27.938	10.381	0,002 gg

Table 4.30 Mean blood cell count changes of the group that continued with consumption of soil at different visits

VARIABLE	df	df	F	p value
RBC	1.265	31.613	8.061	0,005 gg
Hb (g/dL)	1.107	27.683	23.175	<0,001 gg
HCT (%)	1.247	31.178	47.692	<0,001 gg
MCV (fl)	1.133	28.329	38.727	<0,001 gg
MCH (pg)	1.035	25.864	13.485	0,001 gg
MCHC	1.376	34.405	1.543	0,228 gg
RDW (%)	1.554	38.860	5.986	0,009 gg
PLT	1.496	37.404	6.181	0,009 gg
WBC	1.374	34.341	0.777	0.423 gg
Neutrophil #			(5.154)	0.076 f
Lymphocyte	1.127	28.182	4.397	0.041 gg
Monocyte #	1.399	34.965	1.179	0.304 gg
Eosinophil #			(3.347)	0.188 f
Basophil #	2	50	9.238	<0.001 b

Table 4.32 Mean iron study, pregnancy and inflammatory indicators changes of the groups that continued with consumption of soil at different visits

VARIABLE	df	df	F	p value
S Fe			(16.534)	<0.001 f w
Trans. (g/L)	2	58	33.215	<0.001 b
Ferritin			(54.189)	<0.001 f w
ICBT (µg/L)	2	64	39.539	<0.001 b
ISAT (%)	2	64	29.058	<0.001 b
hCG (IU/L)			(24.043)	<0.001 f w
cRP (mg/L)			(1.000)	0.607 f

Table 4.31 Mean general health clinical chemistry indicators changes of the group that continued with consumption of soil at different

VARIABLE	df	Z t	p value
BUN (mg/dL)	33	1.014	0,318 t
S Creat. (mg/dl)	33	-2.661	0,012 t
		-2.386	0.017 w
TP (g/L)		-1.243	0,214 w
Albumin (g/L)		-2.510	0,012 w
ALP (U/L)	28	0.737	0,467 t
		-0.790	0,430 w
AST (U/L)		-1.964	0,050 w
ALT (U/L)	33	0.498	0,622 t
		-0.701	0,483 w
K (mmol/L)	32	-1.472	0,151 t
Na (mmol/L)		-2.075	0,038 w
Phos (mmol/L)	33	-4.281	<0,001 t
		-3.530	<0,001 w
Mg (mg/dL)	33	-1.241	0.223 t
		-1.215	0,224 w
Ca (mg/dL)		-3.036	0.002 w
Zinc (umol/L)	29	-0.733	0.469 t

Factorial ANOVA statistics' summary

VARIABLE	df	df	F	p value
RBC	1.323	86.026	0.713	0.438 gg
Hb (g/dL)	1.304	85.753	0.545	0.512 gg
MCV (fl)	1.171	76.103	80.513	<0,001 gg
MCH (pg)	1.122	72.909	1.988	0.161 gg
RDW (%)	1.561	98.104	0.237	0.726 gg
PLT	1.699	106.410	1.876	0.165 gg
Eosinophil #	2	130	1.435	0.242
Serum iron	2	140	0.079	0.924
Ferritin	1.064	73.432	0.218	0.657 gg
Transferrin	1.513	98.237	0.587	0.507 gg
ICBT	2	140	1.179	0.304 gg
ISAT	1.817	129.032	0.165	0.848
CRP	2	138	0.182	0.834