

Chapter 1

Introduction

1.1 Background

Since ancient times the peculiar and complex phenomenon of geophagia, generally referred to as soil/earth-eating, has existed and intrigued society (Lacey, 1990; Fallon, 2006). Human geophagia occurs in many countries of the world, including African countries (Abrahams, 1997; Geissler, *et al.*, 1998; Saathoff, *et al.*, 2002; Young, *et al.*, 2007; Ekosse, *et al.*, 2010; Ngole, *et al.*, 2010). Geophagia is classified as a form of pica, which refers to the consumption of non-food materials (Lacey, 1990; Ellis and Schnoes, 2006; Fallon, 2006; Broomfield, 2007; Young, *et al.*, 2008). Although human geophagia is not limited by age, gender or race, it remains more prevalent amongst children and women of child-bearing age within developing countries (Vermeer and Frate, 1979; Hunter, 2003; Brand, *et al.*, 2009).

The practice of human geophagia can be an elaborate business, especially in rural communities such as those present in the district of Thabo Mofutsanyane, Free State Province (South Africa). Traditional miners collect geophagic material from various areas and sell the material to vendors (Ekosse, *et al.*, 2010). These geophagic materials are subsequently displayed as merchandise at local markets for geophagists to purchase. Therefore, geophagia may be viewed as a means of subsistence for many different people within geophagic communities.

A myriad of reasons for craving clayey soil is known. Most commonly, it is thought to supplement nutrient or mineral deficiencies and aid as a homeopathic remedy for a variety of ailments (Halsted, 1968; Knishinsky, 1998; Reilly and Henry, 2001). It is the general belief of the geophagic practitioner that soil-eating is either unequivocal, or beneficial to their health. Pregnant women find comfort in a variety of soothing effects resulting from clayey soil consumption, which include mineral and nutrient supplementation, antacid properties, anti-emetic properties and anti-diarrhoeal properties (Knishinsky, 1998; Hunter, 2003; Luoba, *et al.*, 2004; Kawai, *et al.*, 2009; Bisi-Johnson, *et al.*, 2010).

Studies have also shown the contrary, with serious health consequences resulting from human geophagia. Health hazards to consider include anaemia, microbiological infections, intestinal obstruction, dental abrasion and heavy metal poisoning (Geissler, *et al.*, 1998; Callahan, 2003; Hunter, 2003; Ellis and Schnoes, 2006). Two health risks which are continuously debated amongst scholars include the potential of geophagic clayey soil to induce or exacerbate iron deficiency and zinc deficiency (Halsted, 1968; Reid, 1992; Reilly and Henry, 2001; Trivedi, *et al.*, 2005; Ellis and Schnoes, 2006; Brand, *et al.*, 2009; Kawai, *et al.*, 2009). Furthermore, the deliberate or unintentional consumption of soil-dwelling human pathogenic nematodes, known as geohelminths, may result in the disease helminthiasis, which is one of the most prevalent human infections worldwide (Hotez, 2000; Holland and Kennedy, 2002; De Silva, *et al.*, 2003; Hotez, *et al.*, 2006; WHO, 2008). Helminthiasis includes infections caused by a range of different geohelminthic species, including *Ascaris lumbricoides*, *Trichuris trichiura* and the hookworms (Hotez, 2000; Bethony, *et al.*, 2006). These geohelminthic infections have

pronounced effects in particularly poor, developing countries with children suffering the most under heavy parasitic burdens (Hotez, 2000; Hotez, *et al.*, 2006; WHO, 2008). It is thus essential to determine the safety of geophagic clayey soil, so as to inform and advise communities on any potential health risks which may result from soil consumption (Brand, *et al.*, 2009; Ekosse, *et al.*, 2010).

Currently there is an ongoing, large-scale investigation in South Africa, Swaziland and Botswana to evaluate the health impact of human geophagic practices. This investigation is headed by Professor G-I.E. Ekosse, Director of Research Development at the Walter Sisulu University, Mthatha, South Africa. The Central University of Technology, Free State, has undertaken numerous research projects as part of the larger project under the guidance of Professor L. de Jager. A need was identified to characterize the parasitological content of geophagic clayey soil, including the presence of potentially pathogenic nematodes, which subsequently led to undertaking this study under the recommendation of Professors Ekosse and de Jager.

1.2 Aims and Objectives

The Thabo Mofutsanyane Municipality District in the Free State Province, South Africa, is known for its extensive practice of human geophagia and has therefore been identified as an area for research into the occurrence of potential pathological biological content such as parasites and bacteria, which may affect the health of the geophagic practitioners. The aim of this study was, therefore, to obtain a better understanding of

the types of human geophagic practices and nematode content of the clayey soil, more specifically the potentially pathogenic nematode content, consumed by geophagists in the district of Thabo Mofutsanyane.

The following objectives were devised to address this aim:

- To collect soil samples from the area and analyze samples for their parasitological content, specifically pathogenic nematodes (geohelminths) and non-pathogenic soil-inhabiting nematode larvae;
- To classify the soil samples according to soil colour in order to reveal possible soil colour preferences exhibited by geophagists from this area;
- To collect and process data from previously distributed vendor questionnaires on the demography, socio-economic and cultural characteristics, and indigenous knowledge of vendors on the practice of human geophagia; and
- To design and distribute an additional questionnaire to generate information on vendor's hygiene practices during the mining of soil.

Chapter 2

Literature review

2.1 Introduction

Geophagia or earth-eating is the intentional and sometimes accidental consumption of earth-like substances such as soil, clay or chalk and is classified as the most common form of pica (an eating disorder characterized by the persistent consumption of non-food substances for a period of one month or longer) (Halsted, 1968; Ellis and Schnoes, 2006; Broomfield, 2007; Young, *et al.*, 2008). It is observed in humans and animals worldwide, especially among people with low socio-economic status where poverty is rife, as well as in tropical regions of the world and in tribal societies (Halsted, 1968; Callahan, 2003; Wilson, 2003; Ellis and Schnoes, 2006).

In animals earth-eating is a common habit observed in herbivores and omnivores. In these species this behaviour is generally regarded as instinctive to either correct mineral deficiencies, or to detoxify their system after consumption of poisonous substances (Callahan, 2003; Hunter, 2003). Animals are very selective in choosing a geophagic site and seem to return to the same sites regularly (McGreevy, *et al.*, 2001; Hunter, 2003). Studies have shown that East African elephants, prone to iodine deficiency, often revisit specific hillsides to obtain deposits rich in iodine from that soil (Hunter, 2003). Similarly, Rwandan mountain gorillas regularly revisit sites on the side of a volcano where abundant iron and sodium is present in the soil (Hunter, 2003). Studies on the

geophagic tendencies of horses (wild as well as domesticated) in Australia have revealed that geophagic sites were rich in iron and copper, which may indicate micro-nutrient supplementation as a reason for eating the soil (McGreevy, *et al.*, 2001). Moustached tamarins in north-eastern Peru exhibit soil-eating from specifically ant hills as a method of mineral supplementation (Knishinsky, 1998). Furthermore, clay-eating tropical birds in New Guinea (cockatoos, parrots and pigeons) benefit from the detoxification properties of clay as their diet contains plant material that may be riddled with poisonous substances (Hunter, 2003). Consequently it is broadly accepted that non-human geophagia harbours various beneficial aspects to the animal species with a tendency for eating earth (Knishinsky, 1998; Hunter, 2003; Abrahams, 2005).

2.2 Historical perspectives of geophagia in humans

The history of human geophagia is thought to extend as far back as 3500 BC in ancient civilizations of Mesopotamia, India, Egypt and China (Abrahams, 2005). Africa is considered as the continent of origin for the practice of geophagia which subsequently spread to most other continents through migration of humans (Abrahams, 2005; Hooda and Henry, 2009). Evidence that human geophagia was borne from Africa has been presented from pre-historic sites at the Kalambo Falls (situated at the border of Zambia and Tanzania) where white powdered clay-like material was discovered adjacent to *Homo habilis* relics (Clark, 1969). The treatment of digestive ailments and facilitation of digestion is believed to be the reason for these pre-historic ancestors to have consumed clayey soils (Finkelman, 2006). The production and use of medicinal clay coins, known

as 'Terra sigillata' or the sacred 'sealed earth', was established more than two millennia ago in Greece and continues to be produced today (Halsted, 1968; Reinbacher, 2003; Finkelman, 2006).

The consumption of clay by tribal societies along the Orinoco River in South America has also been described in the early 1800's (Von Humboldt and Bonpland, 1853). Almost a century thereafter, a frantic desire, described as a mania, for the consumption of clay was observed amongst natives residing next to the upper Amazon, culminating in medical illness such as anaemia and oedema (Galt, 1872). Likewise, the explorer David Livingstone believed that clay-eating was the most probable cause of anaemia in many pregnant African women (Halsted, 1968). In summary it is apparent that the practice of geophagia is not a recent emergence, but has been intricately intertwined within society since pre-historic times, albeit to the benefit or health consequence of the geophagic practitioner (geophagist).

2.3 Current practices of human geophagia

2.3.1 Geophagia in Africa

Geophagia is not a confined phenomenon, but rather a commonly accepted and widespread occurrence across the globe, including the American continents, the British Isles, Europe and Africa (Ellis and Schnoes, 2006; Brand, *et al.*, 2009). Some African countries, where the consumption of earth-like substances have been documented, include the Cameroon (Von Garnier, *et al.*, 2008), Guinea (Glickman, *et al.*, 1999),

Kenya (Geissler, *et al.*, 1998; Luoba, *et al.*, 2004), South Africa (Saathoff, *et al.*, 2002; Ekosse, *et al.*, 2010; Ngole, *et al.*, 2010), Swaziland (Ngole, *et al.*, 2010), Tanzania (Young, *et al.*, 2007; Kawai, *et al.*, 2009), Uganda (Abrahams, 1997) and Zambia (Nchito, *et al.*, 2004). Although the practice of human geophagia is well-known in South Africa, it appears to be more prevalent in certain areas. South African areas currently known for this practice includes the Free State (Thabo Mofutsanyane and Mangaung), Limpopo (Polokwane and Sekhukhune) and KwaZulu-Natal Provinces (Saathoff, *et al.*, 2002; Ekosse, *et al.*, 2010; Ngole, *et al.*, 2010).

The practice of geophagia is evident amongst people of different races, ages, socio-economic classes and sexes worldwide (Hunter, 2003). However, inhabitants of poverty-stricken countries marred with low income, especially in Africa, are more commonly known for practicing geophagia, sometimes solely to relieve hunger (Young, *et al.*, 2007; Von Garnier, *et al.*, 2008). In contrast, humans who regularly consume animal-derived food, such as found in first world countries, are less likely to practice geophagia as the necessary nutrients are already supplied by their diet (Callahan, 2003). Although not limited by demographic and geographic boundaries, earth-eating remains more prevalent amongst children and women of child-bearing age (Vermeer and Frate, 1979; Geissler, 2000; Hunter, 2003; Brand, *et al.*, 2009).

2.3.2 Aetiology of geophagia

Reasons for why people crave soil are not clear, but seem to range from medicinal to religious in origin, as well as constituting part of a regular diet (Halsted, 1968;

Knishinsky, 1998; Reilly and Henry, 2001). Cultural, physiological and psychological grounds have been promulgated as the aetiology of this peculiar habit (Geissler, *et al.*, 1998; Callahan, 2003; Hunter 2003; Young, *et al.*, 2007).

Many geophagists believe in the beneficial qualities of clayey soil, which include diarrhoeal relief, detoxification, antimicrobial treatment, immune booster and mineral supplementation (Knishinsky, 1998; Hunter, 2003). Recently, the plight of famine-stricken Haitians was heightened after reports on their diet of mud cookies (made from dried yellow dirt, salt and vegetable fat) became public (Katz, 2008). Many third world countries are showing an increased consumption of clay-like substances as a dietary component (Knishinsky, 1998; Callahan, 2003; Abrahams, 2005; Ellis and Schnoes, 2006).

Children

Young children have a tendency to eat non-food substances, either through exploration of their surroundings (especially toddlers 18 to 24 months of age), or deliberate ingestion (Ellis and Schnoes, 2006; Broomfield, 2007; Bisi-Johnson, *et al.*, 2010). With increasing age, the frequency of soil consumption decreases especially in boys, whilst girls are not inclined to alter their earth-eating habit too much (Saathoff, *et al.*, 2002). In teenaged and adult males from developed countries geophagia has rarely been documented (Geissler, 2000; Ellis and Schnoes, 2006). Mineral deficiencies, such as calcium, iron or zinc deficiency, may serve as causative factors for children to develop

cravings for earth-like substances (Ellis and Schnoes, 2006). It has been discovered that children with mental retardation (such as autism) are more likely to present with this unusual eating practice, which may continue into adulthood (Decker, 1993; Ellis and Schnoes, 2006; Broomfield, 2007).

Women

The consumption of earth-like substances, such as clayey soils by women is closely related with pregnancy, especially in Africa, as this practice has proven to alleviate nausea and promote healthy foetal development (Knishinsky, 1998; Hunter, 2003; Luoba, *et al.*, 2004; Kawai, *et al.*, 2009; Bisi-Johnson, *et al.*, 2010). In addition, urban-dwelling South African women consider earth-eating to be somatologically advantageous in the development of a fairer complexion (Woywodt and Kiss, 2002). Often mothers, who practice geophagia due to cultural and familial aspects, will impart their habit to their children (Vermeer and Frate, 1979; Nchito, *et al.*, 2004; Ellis and Schnoes, 2006).

2.3.3 Geophagic clays: Properties and preferences

In literature geophagic earth materials are referred to by scholars as geophagic earth, soil, clay, dirt or chalk (Halsted, 1968; Ellis and Schnoes, 2006; Broomfield, 2007; Young, *et al.*, 2008). However, in the South African practice of human geophagia, clay minerals (alumino silicates) are present in the majority of the earth-like materials

consumed and therefore these geophagic substances are termed geophagic clays or clayey soils (Brand, *et al.*, 2009; Ekosse, *et al.*, 2010).

Physical properties

Geophagic clayey soil properties such as colour, texture, smell and taste are carefully considered before a geophagist will indulge (Reilly and Henry, 2001; Wilson, 2003; Nchito, *et al.*, 2004; Young, *et al.*, 2007; Ekosse, *et al.*, 2010; Ngole, *et al.*, 2010; Young, *et al.*, 2010). South African geophagists generally prefer geophagic clayey soils which have soft, smooth and powdery consistency (Ekosse, *et al.*, 2010). More specifically, the texture of most geophagic clayey soils from the Free State Province (South Africa) are silky, whilst those from the Limpopo Province (South Africa) are gritty and powdery (Ekosse, *et al.*, 2010).

The colouration of soils is dependent upon iron oxides/hydroxides and water drainage (Strydom, *et al.*, 2009). Subsequently, rusty coloured soil results from the presence of red iron oxides (hematite) and good water drainage, whilst yellowish coloured soil contains yellow iron oxides (goethite) (Young, *et al.*, 2008; Strydom, *et al.*, 2009). Geophagists exhibit a predilection for soils which may harbour noteworthy iron oxide/hydroxide content (Gomes, *et al.*, 2009; Ngole, *et al.*, 2010). As the practice of geophagia has been linked with iron deficiency anaemia through various studies (Halsted, 1968; Reilly and Henry, 2001; Nchito, *et al.*, 2004; Von Garnier, *et al.*, 2008), soil colour measurement may provide qualitative insights into the soil's iron content. In

addition, whitish clayey soils usually contain kaolin and smectite clay minerals (Kikouama, *et al.*, 2009), whilst blackish clayey soils contain higher levels of organic matter (Ekosse, *et al.*, 2010). The *Munsell Soil Color Chart* is routinely utilized for the determination of soil colour, measuring three distinctive parameters, namely hue (actual colour in relation to yellow and/or red), value (lightness of soil colour) and chroma (strength of soil colour) (*Munsell Soil Color Charts*, 2000; Young, *et al.*, 2008).

Soil colour diversity is evident amongst geophagic communities across Africa (Hooda, *et al.*, 2002; Ekosse, *et al.*, 2010; Ngole, *et al.*, 2010; Young, *et al.*, 2010). During a recent study conducted in the Limpopo and Free State Provinces of South Africa, geophagists indicated that they consumed red-, yellow-, white-, khaki- and black-coloured clays, with a preference for soft white and/or khaki coloured clays (Ekosse, *et al.*, 2010). However, when using the *Munsell Soil Color Chart*, the soil colour of geophagic samples from South Africa is generally classified as greyish to yellowish (Ngole, *et al.*, 2010). Geophagic samples from Swaziland tend to be greyish to reddish, which indirectly suggests the presence of iron (Ngole, *et al.*, 2010). In other countries, such as Zambia, geophagic school children seem to mainly favour brown earth and white clay (Nchito, *et al.*, 2004). Geophagic soils ranging from white, to brownish, to red are consumed by Pemban (Zanzibar, Tanzania) geophagists (Young, *et al.*, 2010). In Uganda, some of the geophagic soils are often dark brown in colour (Hooda, *et al.*, 2002).

Mining

Miscellaneous mining sites are identified by geophagists, usually in close proximity to human dwellings. In South Africa (Free State and Limpopo Provinces), geophagic clayey soils are mined from various areas such as hills, mountains, termitaria, riverbeds and excavation sites (Ekosse, *et al.*, 2010). Adult geophagists are very discriminating when selecting geophagic clayey soil. They tend to seek bands of clay-enriched soil and mine soil 25 – 75 cm below the surface, thus avoiding topsoil, which could possibly be contaminated by pathogens and other harmful substances (Vermeer and Frate, 1979; Hunter, 2003). Similar observations were made in a study conducted in Pemba where *ufue*, one of the earth types consumed by people, is obtained through burrowing a few centimetres under the earth's topsoil (Young, *et al.*, 2007). Children however, are less discriminating when selecting soil and often consume contaminated topsoil's resulting in health implications such as infectious diseases (Callahan, 2003; Young, *et al.*, 2008).

Types of geophagic materials favoured by the geophagists may vary considerably between different countries. In a single cross-continental study the geophagic soil and clay types included alluvium clay from ponds (India); brown earthy chunks (Tanzania); cylinder-shaped chalky clay (Turkey) and clay-oven linings (India) (Hooda, *et al.*, 2002). Geophagic Kenyan women favoured the consumption of soft stone (*odowa*) as well as earth from termitaria (Luoba, *et al.*, 2004). This finding was corroborated in a study where geophagic primary school children from Kenya gathered most (64 %) of their soil

from termitaria and from the soil collected by tree-living ants, whilst 19 % preferred to consume soft stone (*odowa*) (Geissler, *et al.*, 1998). Pemban geophagists consume a variety of soils. These include fine red-brown clayey soil from termitaria (*udongo*); sandy soil mined close to the surface (*mchanga*); large soft pale soil chunks also mined close to the surface; and white deeper embedded soil (*ufue*) (Young, *et al.*, 2010).

Various utensils are incorporated when mining geophagic clayey soil. These may include forks, machetes, pickaxes, crowbars, shovels, broken bottles, sharpened sticks, and discarded cans (Ekosse, *et al.*, 2010). Traditional mining techniques incorporated by 80 % of geophagists from rural Qwaqwa, located within the district of Thabo Mofutsanyane (Free State Province, South Africa), included digging and hand grabbing of geophagic clays from mainly hills, mountains and riverbeds (Ekosse, *et al.*, 2010). Geophagists from Pemba collect their soils from areas such as agricultural fields, hills and house walls (Young, *et al.*, 2010). After mining of soil, visible impurities and unwanted soil material are often removed from the mined samples using hand grabbing (Ekosse, *et al.*, 2010).

Mined geophagic clays are sometimes directly consumed, but often undergo some degree of processing before consumption. Types of processing include pounding, sieving, slurring and grinding (Ekosse, *et al.*, 2010). Furthermore, geophagists are also of the opinion that the texture, taste and colour of geophagic clay is improved upon heat treatments such as boiling, oven baking, sun drying and burning (Reilly and Henry, 2001; Ekosse, *et al.*, 2010; Young, *et al.*, 2010). Heat treatments may also decrease or

completely remove potential pathogens, such as bacteria, from these clays (Hunter, 2003; Ekosse, *et al.*, 2010).

2.3.4 Business traits of geophagia

Commercialization of geophagic soils and clays has long been established and is apparent in especially developing countries across the world. In South Africa, the livelihood of some vendors depends upon the purchasing of geophagic clayey soils from traditional miners and the subsequent selling thereof to geophagists at local markets (Ekosse, *et al.*, 2010). The selling of geophagic soils is also evident on Zambian streets and in Tanzanian village markets (Hooda, *et al.*, 2002; Nchito, *et al.*, 2004). Ugandan and Turkish markets vend geophagic materials for treatment of a variety of medical illnesses, ranging from asthma to syphilis and anaemia, as well as the management of pregnancy-related anxiety (Abrahams, 1997; Hooda, *et al.*, 2002). Apart from the African continent, ethnic shops in Britain commonly engage in the trading of Bengalese geophagic soils sourced from southern Asia (Abrahams, *et al.*, 2006). Moreover, kaolin/kaolinite (present in many types of geophagic clay) is commercially produced and sold in health food stores globally as a treatment for diarrhoea (Knishinsky, 1998; Finkelman, 2006). From a religious perspective, human geophagia has also become a profitable business through the selling of Arabic inscribed clay in Mecca, where these clay consumers believe to share their spirit with that of Allah (Knishinsky, 1998).

2.4 Health benefits of human geophagia

Non-western societies generally consider the practice of geophagia as beneficial to their health and, to a certain extent this belief has been substantiated through various studies related to the health effects of eating earth (Knishinsky, 1998; Hooda, *et al.*, 2002; Dominy, *et al.*, 2004; Haydel, *et al.*, 2008; Gomes, *et al.*, 2009; Young, *et al.*, 2010). In addition, an increasing prevalence of geophagia is found in westernized societies, such as the United States of America, where companies produce and market a more refined health product derived from geophagic clays. These products are considered safe as companies ensure compliance with the United States federal purity standards to reduce any potential hazard to the consumer (Knishinsky, 1998).

It is the belief of geophagic women that the consumption of clayey soil prior to and during pregnancy, as well as post-partum, harbours numerous positive effects. Some of the many enhancing effects of geophagia include an increased likelihood of conception; enhanced virility; provision of sufficient calcium to develop strong bones and teeth in the foetus; assisting in an uneventful pregnancy and hastening delivery (Vermeer and Frate, 1979; Knishinsky, 1998). Research on pregnant and lactating women in western Kenya showed that the mean daily earth intake declined significantly between mid-term and six months postpartum (Luoba, *et al.*, 2004). Pregnant women crave earthy substances to combat related nausea, heartburn, and as nutritional supplementation (Luoba, *et al.*, 2004; Young, *et al.*, 2010). Ptyalism (increased production of saliva) experienced during pregnancy is countered by consumption of clayey soil rich in kaolinite (a clay mineral

compound) as it protects the gastro-intestinal tract (GIT) through adherence to mucous membranes (Kikouama, *et al.*, 2009; Young, *et al.*, 2010).

Gastrointestinal adsorption through kaolinite and smectite, is arguably the most functional health benefit resulting from geophagia (Dominy, *et al.*, 2004; Ekosse, *et al.*, 2010). Manifold advantageous applications stem from the *in vivo* functioning of these clays, which include anti-diarrhoeal treatment; detoxification through microbial, viral and toxic plant compound adsorption; and general GIT protection through alteration of the intestinal lining (Wilson, 2003; Dominy, *et al.*, 2004; Gomes, *et al.*, 2009; Bisi-Johnson, *et al.*, 2010; Young, *et al.*, 2010).

The use of geophagic clays to treat various microbiological infections is advocated by many scholars (Knishinsky, 1998; Wilson, 2003; Haydel, *et al.*, 2008; Gomes, *et al.*, 2009). Bacteriological infections caused by *Escherichia coli*, *Salmonella*, *Shigella*, *Klebsiella* and *Mycobacterium ulcerans*, may be managed using specific mineral products in clay, namely iron-rich smectite and illite, kaolinite and also fibrous clays containing palygorskite or sepiolite. These clay minerals harbour inherent, heat-stable antibacterial properties (Haydel, *et al.*, 2008; Gomes, *et al.*, 2009). This is substantiated by literature where some island communities in the South use an herbal clay brew to treat cholera (Knishinsky, 1998). In addition, anti-helminthic and anti-viral properties of geophagic clays have also been documented (Knishinsky, 1998; Wilson, 2003).

Naturally occurring antimicrobial agents are largely produced by soil-dwelling micro-organisms (Abrahams, 2002). Fungal populations within soil harbour a wealthy array of these substances, more notably penicillin (Abrahams, 2002; Salyers and Whitt, 2005). Similarly, bacterial populations present in soil, especially *Streptomyces*, contribute to the production of natural antimicrobials (Abrahams, 2002; Salyers and Whitt, 2005). The consumption of soil-borne commensal flora under the precept of a probiotic, is also indicated for maintaining a healthy GIT (Bisi-Johnson, *et al.*, 2010).

The consumption of clayey soil by especially children and pregnant women, but also geophagic tropical inhabitants, is viewed as an intuitive attempt to supplement essential mineral nutrients such as iron (Fe), zinc (Zn) and calcium (Ca) (Abrahams, 1997; Smith, *et al.*, 2000; Hooda, *et al.*, 2002; Wilson, 2003; Dominy, *et al.*, 2004; Luoba, *et al.*, 2004). To date, some research has shown that iron supplementation by means of geophagia may contribute to the recommended daily intake (RDI) of iron as found in a study on trace elements in soils consumed by geophagists in the Mukono District of Uganda (Smith, *et al.*, 2000). Furthermore, there are convincing studies which indicate that calcareous geophagic material may augment dietary calcium intake (Hooda, *et al.* 2002).

2.5 Health risks of human geophagia

2.5.1 Introduction

Human geophagia is generally viewed as unhygienic and inappropriate by many western cultures and the more cultivated societies around the globe (Knishinsky, 1998; Reilly and Henry, 2001). In contrast, geophagists do not perceive the consumption of soil or clay as improper and malevolent to their health (Knishinsky, 1998; Hooda, *et al.*, 2002; Young, *et al.*, 2010). However, many scholars have voiced concerns regarding the potential health risks, which human geophagia may pose. Hazardous health implications result from the presence of toxins, potentially harmful elements (PHE), pathogenic microorganisms, the abrasiveness of some geophagic soils and also an inherent ability to reduce micro-nutrient absorption (Halsted, 1968; Abrahams, 1997; Geissler, *et al.*, 1998; Glickman, *et al.*, 1999; Abrahams, 2002; Hooda, *et al.*, 2002; Callahan, 2003; Luoba, *et al.*, 2004; Abrahams, 2005; Luoba, *et al.*, 2005; Ellis and Schnoes, 2006; Ngozi, 2008; Von Garnier, *et al.*, 2008; Bisi-Johnson, *et al.*, 2010; Ekosse, *et al.*, 2010). Subsequently, further investigations into the health implications associated with geophagia are necessitated.

2.5.2 Soil particles

The abrasiveness of geophagic materials harbours several health repercussions. Especially sandy geophagic materials contain quartz, an exceptionally hard mineral, which is able to cause considerable dental injury upon ingestion (Geissler, *et al.*, 1998;

Hunter, 2003; Ngole, *et al.*, 2010). Accumulation of ingested geophagic soil particles, more specifically quartz particles, results in an increased risk to develop abdominal complications, such as intestinal perforations (Geissler, *et al.*, 1998; Hunter, 2003; Ellis and Schnoes, 2006; Ngole, *et al.*, 2010). Indigestible intestinal contents may also cause intestinal obstruction, constipation and ulceration (Ellis and Schnoes, 2006).

2.5.3 Mineral nutrients

Geophagia has also been implicated as a causative agent/or accelerant of iron (Fe), zinc (Zn) and potassium (K) deficiency in geophagists (Halsted, 1968; Reid, 1992; Reilly and Henry, 2001; Ellis and Schnoes, 2006; Trivedi, *et al.*, 2005; Kawai, *et al.*, 2009). Contrary to the belief of some scholars, studies have proven that these clayey soils promote reduced absorption of bio-available Fe, Zn and K, resulting in deficiencies and associated illness, which includes anaemia, hypogonadism and dwarfism (Prasad, *et al.*, 1961; Abrahams, 2002; Hooda, *et al.*, 2002; Hooda, *et al.*, 2004; Ghorbani, 2008; Kawai, *et al.*, 2009). A study amongst western Kenyan school children has shown that anaemia and iron deficiency were more prevalent in those children who were geophagic (Geissler, *et al.*, 1998). Kaolinite, which has a negatively charged surface, adsorb Fe^{2+} and Fe^{3+} cations readily in the duodenum, preventing iron absorption and consequently leads to iron deficiency anaemia (Von Garnier, *et al.*, 2008).

Human geophagia is also viewed by some as a psychiatric disease (Ghorbani, 2008). A study among Zambian school children in Lusaka indicated that geophagic behaviour did

not subside upon administration of iron supplements to those that were iron deficient (Nchito, *et al.*, 2004). It is further substantiated by a paediatric case report of pica, where the behaviour was unresponsive to iron therapy, but subsided upon treatment with fluoxetine (trade name *Prozac*), a selective serotonin reuptake inhibitor (SSRI) used to treat depression and obsessive-compulsive spectrum disorders (OCSD) (Hergüner, *et al.*, 2008). Hergüner, *et al.* (2008) implied that certain cases of pica, especially those exhibiting iron deficiency anaemia but unresponsive to iron therapy, are likely borne from OCSD and may be treated successfully with SSRI's.

2.5.4 Potentially harmful elements (PHE's)

The ingestion of toxic soil constituents, such as pesticides and heavy metals, that result in, for example lead poisoning, is considered a health hazard especially in geophagic children (Abrahams, 2002; Hunter, 2003; Ellis and Schnoes, 2006). Soil within urban areas are believed to contain larger amounts of PHE's than rural areas, as a result of atmospheric pollution and traffic aggregation prominent in these larger cities (Mielke, 1999; Ljung, *et al.*, 2006). Some PHE's to consider include aluminium, cadmium, chromium, lead and mercury, all of which may impart deleteriously on especially neurologic developmental aspects of children (Geissler, *et al.*, 1998; Mielke, 1999; Ekosse, *et al.*, 2010).

2.5.5 Fungi

The role that soil-borne fungi portray in the initiation of disease as a result of the consumption of fungal contaminated soil is debatable. However, geophagists are potentially at risk to inhale soil borne fungal spores such as those of *Aspergillus* and *Coccidioides immitis*, which may initiate pulmonary and systemic disease (Abrahams, 2002; Murray, *et al.*, 2009).

2.5.6 Bacteria

Soil consumption may have dire consequences as soil contains an array of bacterial populations of which some are human pathogens (Callahan, 2003; Ekosse, *et al.*, 2010). Generally, soils rich in organic matter are capable of sustaining diverse bacterial taxa, although populations decrease as the depth of soil increases (Callahan, 2003; Hunter, 2003; Srivastava, 2007; Ngole, *et al.*, 2010). Halsted (1968) quoted a case study from 1934 where Booth reported tetanus as a result of human geophagia through oral ingestion of viable spores in soil. Human and animal faecal contamination of geophagic soil may result in the deposition of more virulent *Escherichia coli* strains within the soil (Abrahams, 2002). Faecally deposited *E. coli* may remain infectious for several months, resulting in numerous clinical illnesses upon oral ingestion, including diarrhoea (Abrahams, 2002; Murray, *et al.*, 2009). Interestingly, geophagic soils from South Africa and Swaziland are thought to carry low levels of pathogenic bacteria as it should correlate with the low levels of organic matter present in these soils (Ngole, *et al.*, 2010).

2.5.7 Parasites

The practice of geophagia has been linked to a multitude of soil-borne parasitic infections (Hunter, 2003; Ellis and Schnoes, 2006). Some parasitic infections resulting from the consumption of faecally contaminated soil (human or animal derived) include toxocariasis, toxoplasmosis, cryptosporidiosis, giardiasis and soil-transmitted helminthiasis (Abrahams, 2002; Callahan, 2003; Hunter, 2003; Ellis and Schnoes, 2006). Often these soil-acquired parasites generate disease which may sometimes be fatal, as seen with the death of one child and severe neurological damage of another post-ingestion of soil contaminated with raccoon roundworm (*Baylisascaris procyonis*) (Hunter, 2003).

Geophagia, as a vector for geohelminth infection, has long been considered by many scientists (Callahan, 2003; Ellis and Schnoes, 2006; Young, *et al.*, 2008). Soil transmitted helminths are frequently referred to collectively as geohelminths (Hotez, 2000). These pathogenic parasitic nematodes consist of four specific nematode species, namely *Trichuris trichiura* (whipworm), *Ascaris lumbricoides* (large roundworm) and *Necator americanus* and *Ancylostoma duodenale* (jointly known as hookworms) (Hotez, 2000). Geohelminths pose a considerable health threat to especially children, as a high parasite load results in severe morbidity with consequent physical, intellectual and cognitive impairment (Hotez, 2000; De Silva, *et al.*, 2003; Bethony, *et al.*, 2006). Heavy geohelminth infection may initialize or exacerbate malnutrition in already malnourished children in poor communities worldwide (Ozumba and Ozumba, 2002).

Numerous studies have indicated that geophagic soils may contain geohelminths transferred to humans through soil consumption. Due to their soil-eating habits, geophagic pregnant women from Kenya have an increased risk for geohelminth infection and especially *A. lumbricoides* reinfection, which may impart negatively on their health (Luoba, *et al.*, 2004; Luoba, *et al.*, 2005). This is corroborated by a study on Tanzanian pregnant women where an association between geophagia and *A. lumbricoides* infection was discovered (Kawai, *et al.*, 2009). Furthermore, African children are at an increased risk of acquiring geohelminth infection, commonly *A. lumbricoides* and *T. trichiura*, as a result of eating soil (Glickman, *et al.*, 1999). In a study conducted among primary school children in western Kenya, researchers established a significant association between geophagia and the infection intensity of *A. lumbricoides*, as well as *T. trichiura* (Geissler, *et al.*, 1998). Of note is that these communities would regularly defecate behind termitaria of which soil from the mound crust was then later consumed by the children, increasing the risk of ingesting faecal contaminants (Geissler, *et al.*, 1998). A higher prevalence of *A. lumbricoides* infection in geophagic school children from KwaZulu-Natal (South Africa), who collect their soil from termitaria, was also demonstrated (Saathoff, *et al.*, 2002).

Contrary to other research, no detectable geohelminths of risk to human health were found in any geophagic soil samples during a study on pregnant geophagic women from Pemba (Tanzania) (Young, *et al.*, 2007). These women selected soil only from areas that they deemed 'good and clean' (Young, *et al.*, 2007). As this finding is in contrast to

other research, a void is left which requires further in-depth study on soil samples and geophagists in other sites.

2.6 Nematodes

2.6.1 Introduction

Nematodes, or roundworms, are the most abundant multicellular organisms found in most environments and belong to the Phylum *Nematoda*. This Phylum is further subdivided into two classes, namely *Adenophorea* and *Secernentae*, which are again subdivided into various orders, superfamilies and families (New World Encyclopaedia, 2008; Schistosomiasis Research Group, 2009). Characteristically, nematodes are uniformly worm-like and possess hallmark features such as a smooth cylindrical, spindle-shaped body, separate sexual entities and a complete digestive tract (Poinar, 1983; Murray, *et al.*, 2009).

A vast majority of nematodes are non-pathogenic to humans and exist as soil-inhabiting nematodes, which pass harmlessly through the GIT if accidentally ingested (Viglierchio, 1991). Due to their feeding habits, these nematodes are classified into various trophic levels (also known as feeding groups), which include bacteriovores, herbivores, fungivores, omnivores and predators (Yeates, 1998). Furthermore, these nematode feeding groups may also serve as useful biological indicators of soil health in natural and managed ecosystems (Poinar, 1983; Yeates, 1998; Yeates and Bongers, 1999; Van Bruggen and Semenov 2000; Neher, 2001). Noteworthy soil-inhabiting nematodes are

free-living species, which are beneficial to the decomposition of organic matter in soil, and also plant parasitic species (herbivores) (New World Encyclopaedia Contributors, 2008). Potential health consequences stemming from plant parasitic nematodes lie in their ability to encumber the quality and yield of crops (Viglierchio, 1991; Coyne, *et al.*, 2007).

Nematodes commonly parasitic to humans and capable to induce disease upon entering the human host are sometimes found in soils and directly transmitted to humans in the absence of an intermediate host (Peters and Gilles, 1989; Hotez, 2000; Winn, *et al.*, 2006). The most frequently encountered pathogenic nematodes associated with human geophagia, are the geohelminths, *T. trichiura*, *A. lumbricoides*, and the hookworms, *N. americanus* and *A. duodenale* (Hotez, 2000; Bethony, *et al.*, 2006). *T. trichiura* is classified under the superfamily *Trichuroidea*, *A. lumbricoides* under the family *Ascarididae* and the hookworms under the superfamily *Strongyloidea* (Schistosomiasis Research Group, 2009).

2.6.2 Pathogenic nematode life cycle

Geohelminths are responsible for the most prevalent human infections globally, having a significant effect on the impoverished in developing countries (Hotez, 2000; Holland and Kennedy, 2002; De Silva, *et al.*, 2003; Hotez, *et al.*, 2006; WHO, 2008). Especially the African continent seems to be heavily burdened with geohelminth infections (De Silva, *et al.*, 2003).

The life cycles of geohelminths necessitates dispersion of fertilized ova within soil through faecal contamination. Favourable soil conditions (warm and humid) allow these ova to develop into an infective stage (Maier, *et al.*, 2000; Winn, *et al.*, 2006, Murray, *et al.*, 2009). *Trichuris* and *Ascaris* ova are well-protected and able to survive extreme environmental temperatures (Murray, *et al.*, 2009). Both *T. trichiura* and *A. lumbricoides* enter the host through oral ingestion of infective ova from contaminated soil (Figure 2.1a, 2.1b). In contrast, the hookworms (*A. duodenale* and *N. americanus*) mature into infective filariform larvae within the soil and commonly enter the host through larval penetration of the skin, although oral ingestion of *A. duodenale* filariform larvae may also initiate infection (Figure 2.1c) (Winn, *et al.*, 2006; Ash and Orihel, 2007; Murray, *et al.*, 2009).

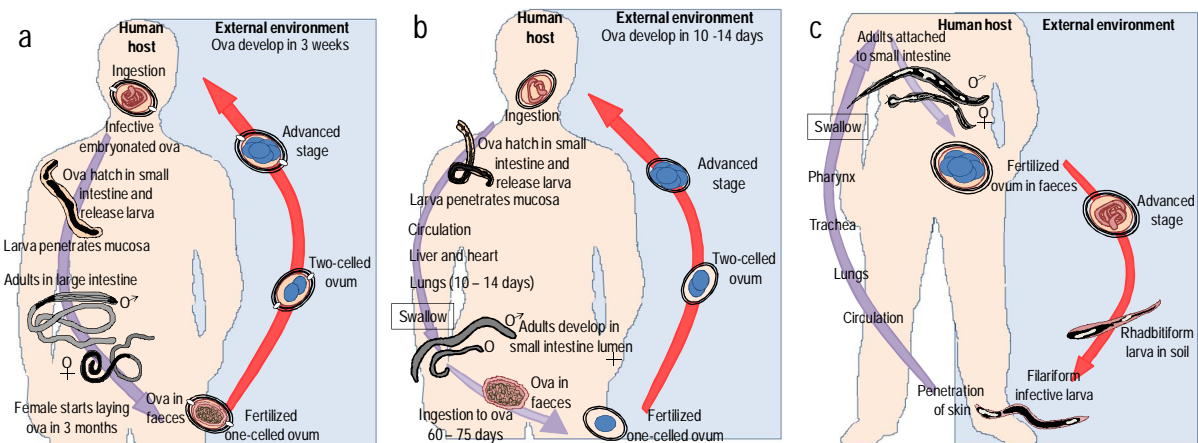


Figure 2.1 Life cycles of pathogenic nematodes (geohelminths): a. *T. trichiura*; b. *A. lumbricoides*; and c. *A. duodenale* and *N. americanus* (hookworms) (Courtesy of Fossey and Perridge, 2010).

Adult geohelminths reside primarily in the intestinal lumen of their host where copulation and ovi-production takes place; *T. trichiura* in the large intestine, and *A. lumbricoides* and the hookworms in the small intestine (Winn, *et al.*, 2006; Ash and Orihel, 2007; Murray, *et al.*, 2009). However, the pathway of these parasitic geohelminths to the intestinal lumen, differ notably. After ingestion, *T. trichiura*, hatches in the small intestine and the hatched larvae migrate to the large intestine (Winn, *et al.*, 2006; Ash and Orihel, 2007; Murray, *et al.*, 2009). *A. lumbricoides* also hatches in the small intestine and undergoes obligatory migration through the liver and lungs, after which larvae migrate to the small intestine *via* the respiratory tract, coughing and swallowing (Winn, *et al.*, 2006; Ash and Orihel, 2007; Murray, *et al.*, 2009). In contrast, hookworm filariform larvae enter through the skin, migrate *via* the circulation to the lungs, after which larvae also migrate to the small intestine *via* the respiratory tract, coughing and swallowing (Winn, *et al.*, 2006; Ash and Orihel, 2007; Murray, *et al.*, 2009).

In the final stages of the life cycles in human hosts, ova are passed into the faeces and thereafter the degree of sanitation and hygiene practiced by the community determine how ova are spread and deposited into the environment (Peters and Gilles, 1989). More common vectors of geohelminth ova dispersment throughout the environment include indiscriminate defecation by humans and animals, the use of raw human faecal matter as crop fertilizer and unrefined human habits (Peters and Gilles, 1989).

2.6.3 Pathogenic nematode epidemiology

Trichuris trichiura

T. trichiura is present in up to one quarter of the world's population (Donkor and Lundberg, 2009). However, trichuriasis remains more prevalent in developing tropical and sub-tropical regions globally and is associated with improper sanitation and sub-standard hygiene practices, as well as the use of human faecal matter as crop fertilizer (Farrar and Wood, 1992; Murray, *et al.*, 2009). *T. trichiura* is often found in combination with other intestinal helminth infections, especially *A. lumbricoides* (Heelan and Ingersoll, 2002; Donkor and Lundberg, 2009). For example, both *T. trichiura* and *A. lumbricoides* were detected in geophagic Kenyan primary school children (Geissler, *et al.*, 1998). *T. trichiura* has a higher incidence under children, especially boys, due to their habit of consuming soil directly or indirectly (Heelan and Ingersoll, 2002; Donkor and Lundberg, 2009).

Ascaris lumbricoides

Ascariasis remains the most prevalent geohelminth infection worldwide, afflicting more than one billion of the population (Hotez, *et al.*, 2006; Winn, *et al.*, 2006; WHO, 2008; Murray, *et al.*, 2009). Increased incidence is found in more temperate tropical regions with poor sanitation and the use of raw human faeces as fertilizer for crops (Farrar and Wood, 1992; Murray, *et al.*, 2009). Interestingly children, and in particular boys, are more often infected, due to their increased tendency to consume soil (Dora-Laskey and

Ezenkwele, 2009). In contrast, a low incidence of ascariasis is apparent in areas with proper irrigation and waste treatment (Winn, *et al.*, 2006). No known animal reservoir exists for *A. lumbricoides*, thus making humans singly responsible for environmental contamination with this parasite (Murray, *et al.*, 2009).

***Ancylostoma duodenale* and *Necator americanus* (Hookworms)**

The two hookworms, *A. duodenale* (Old World hookworm) and *N. americanus* (New World hookworm), are very similar intestinal parasites, only distinguishable in geographic distribution and different mouth structures (Murray, *et al.*, 2009). *N. americanus* incidence is mainly confined to the western hemisphere, central and South Africa, South Asia, India, Melanesia and Polynesia, while *A. duodenale* is essentially distributed throughout southern Europe, northern Africa, China, Japan and also India (Ash and Orihel, 2007). Worldwide travelling has resulted in a more widespread emergence of both hookworms globally, although the poorest countries continue to present with the highest incidence of hookworm infections (Heelan and Ingersoll, 2002; De Silva, *et al.*, 2003).

Hookworm infections are prevalent in the humid tropical and subtropical regions of the world as ova require warm, moist soil to develop into infective larvae (Murray, *et al.*, 2009). These larvae actively penetrate the skin of a human host who comes into contact with the contaminated soil (Murray, *et al.*, 2009). Subsequently, an increased incidence of hookworm infection is documented in rural settlements and amongst

agricultural labourers (Haburchak, 2008). In contrast to trichuriasis and ascariasis, adults (notably agricultural workers) are more susceptible to hookworm infection due to the nature of their working environment and carry heavier worm loads than children (Haburchak, 2008). However, the age group curve for hookworm infection starts in children, ascends into adulthood and then reaches a plateau (Watson and Hickey, 2008).

2.6.4 Pathogenic nematode clinical manifestations

Trichuris trichiura

The manifestation of clinical syndromes of trichuriasis usually relies upon the concentration of the parasitic load (Murray, *et al.*, 2009). Patients are generally asymptomatic, becoming symptomatic with increased load (Donkor and Lundberg, 2009). Clinical syndromes mainly associated with GIT complications include loose stools, bloody diarrhoea due to mechanical injuries of the intestinal mucosa, frequent passing of stools at night time, electrolyte imbalances, weight loss, weakness, abdominal pain, secondary bacterial infections at the site of penetration by the worms, appendicitis, intestinal leakiness, anaemia, eosinophilia and finger nail clubbing (Heelan and Ingersoll, 2002; Winn, *et al.*, 2006; Donkor and Lundberg, 2009; Murray, *et al.*, 2009).

In children, trichuriasis may prohibit proper nutrient absorption, causing a vitamin A deficiency, malnutrition and growth impediment (Donkor and Lundberg, 2009).

Furthermore, tenesmus (straining) upon defecation may lead to rectal prolapse (Winn, *et al.*, 2006; Murray, *et al.*, 2009). The prolapse can be termed 'coconut cake' prolapse, as the tiny white whipworms are visualised macroscopically (Winn, *et al.*, 2006).

Ascaris lumbricoides

The manifestation of clinical syndromes of ascariasis may vary considerably, although most infections are asymptomatic. Syndromes can be attributed to either pulmonary larval migration (early stage) or mechanical exploitation of mature roundworms in the GIT (late stage) (Heelan and Ingersoll, 2002; Dora-Laskey and Ezenkwele, 2009). Symptoms of early stage infections include coughing, possible haemoptysis (blood-flecked sputum) and wheezing (Shoff, *et al.*, 2008). Further early stage complications may arise, such as *Ascaris* pneumonitis (also known as Loeffler syndrome) with symptoms including eosinophilia, dyspnea, oxygen desaturation and fever (Ash and Orihel, 2007; Dora-Laskey and Ezenkwele, 2009; Murray, *et al.*, 2009).

During the late stage mainly GIT syndromes prevail, such as appendicitis, pancreatitis and cholecystitis (Shoff, *et al.*, 2008; Dora-Laskey and Ezenkwele., 2009). Intestinal obstruction and occlusion of the appendix can precipitate due to an internal conglomerate of mature worms, known as a bolus (Murray, *et al.*, 2009). Intestinal perforation, subsequent secondary bacterial infection at the puncture site(s) and peritonitis may result if the intestinal lumen is breached by the resilient, flexible body of

the roundworm (Murray, *et al.*, 2009). Occasionally, when worms die off, inflammation, necrotic processes and the development of abscesses occur (Shoff, *et al.*, 2008).

In children malnutrition is pre-eminent, because adult worms feed on the digested intestinal contents of the host (Shoff, *et al.*, 2008). Furthermore, in children growth and cognitive impairment and mental retardation have also been linked to ascariasis (Shoff, *et al.*, 2008).

Adult worms may exhibit hazardous, potentially life-threatening migratory patterns to extra-intestinal regions, as well as passage through bodily orifices (Dora-Laskey and Ezenkwele, 2009). Pharyngeal globus (tingling throat) and frequent throat clearing are symptoms occasionally noted (Dora-Laskey and Ezenkwele, 2009). Extra-intestinal complications may result from even a single adult worm that migrates into the bile-duct, liver and other anatomical areas where extensive tissue damage takes place (Ash and Orihel, 2007; Murray, *et al.*, 2009). Opportunistic migration of adult worms may be initiated through fever and in response to drugs administered for treatments other than ascariasis (Murray, *et al.*, 2009)

***Ancylostoma duodenale* and *Necator americanus* (Hookworms)**

The manifestation of clinical syndromes related to hookworm infection depends on the worm burden, age and diet of the patient (Haburchak, 2008). At the site of larval skin penetration, a vesicular or reddish pruritic rash (ground itch) may develop (Haburchak,

2008; Murray, *et al.*, 2009). As larvae undergo pulmonary migration, Loeffler syndrome may present, characterized with a cough, fever, malaise and sore throat and possibly eosinophilia (Haburchak, 2008; Murray, *et al.*, 2009). In the gastro-intestinal tract mature hookworms may result in non-specific symptoms, which include diarrhoea, abdominal pain, nausea and vomiting, although secondary bacterial infections may develop at the intestinal site of attachment (Haburchak, 2008; Murray, *et al.*, 2009).

In children, hookworm infection is of particular concern. Impaired mental and physical development may result from such infections (Winn, *et al.*, 2006; Haburchak, 2008; Murray, *et al.*, 2009). Furthermore, immune-suppression as a result of protein-losing enteropathy has been documented in children, which may be ascribed to the loss of immunoglobulins (Haburchak, 2008). Consequently, increased susceptibility to infections ensues with accelerated progression of the Human Immuno-deficiency Virus (HIV) in HIV-positive individuals (Haburchak, 2008).

Of the one billion people worldwide that are infected with hookworms, approximately 0.2 % present with severe iron deficiency anaemia and hypo-albuminaemia as a result of intestinal blood loss (Farrar and Wood, 1992; Winn, *et al.*, 2006). Mild to severe hypochromic iron deficiency anaemia develops in especially young women of child-bearing age, as a result of chronic blood loss (Haburchak, 2008). In severe cases, symptoms include weakness, pallor, nail-spooning, oedema and tachycardia (Haburchak, 2008). Two serious complications include osteoporosis and bone cysts that are produced in response to intense erythroid hyperplasia of the bone marrow in

response to the anaemia (Winn, *et al.*, 2006). Congestive heart failure is another complication seen as a result of severe anaemia (Winn, *et al.*, 2006).

2.7 Summary

Literature has shown that geophagia is a rather common phenomenon in the world today and also plays an important role in the subsistence of vendors who sell geophagic soils (Halsted, 1968; Callahan, 2003; Wilson, 2003; Ellis and Schnoes, 2006; Ekosse, *et al.*, 2010). Generally, geophagists are of the opinion that their soil-eating habit is not detrimental to their health, often consuming soil for the treatment of an illness, or in the belief that it will benefit their wellbeing in some way (Knishinsky, 1998; Hunter, 2003; Luoba, *et al.*, 2004; Kawai, *et al.*, 2009; Bisi-Johnson, *et al.*, 2010). However, scholars have shown that geophagia may pose certain health risks to the geophagist, including heavy metal poisoning and the consumption of potentially pathogenic microorganisms, such as geohelminths (Halsted, 1968; Abrahams, 1997; Geissler, *et al.*, 1998; Glickman, *et al.*, 1999; Mielke, 1999; Abrahams, 2002; Hooda, *et al.*, 2002; Callahan, 2003; Luoba, *et al.*, 2004; Abrahams, 2005; Luoba, *et al.*, 2005; Ellis and Schnoes, 2006; Ljung, *et al.*, 2006; Ngozi, 2008; Von Garnier, *et al.*, 2008; Bisi-Johnson, *et al.*, 2010; Ekosse, *et al.*, 2010). Therefore, more focused research is warranted in this area to ascertain the exact health implications of geophagia.

Chapter 3

Materials and methods

3.1 General overview

The phenomenon of human geophagia is found in many countries across the globe, including South Africa. Knowledge and research into this practice in South Africa remains limited and mainly uncharted. A large investigation is currently underway in South Africa, Swaziland and Botswana under the leadership of Professor Georges Ekosse from the Walter Sisulu University, to establish the health impact of geophagia on geophagists.

The geophagia investigation is fragmented into smaller studies of which this specific study focuses on the Thabo Mofutsanyane District Municipality where the inhabitants practice geophagia extensively and without condemnation. Furthermore, the rural landscape and unhygienic practices favour contamination of geophagic soils with potential pathogens.

This investigation focused mainly on the determination and characterization of the biological content of geophagic soils used in the practice of human geophagia in the district of Thabo Mofutsanyane. In particular, the pathogenic and non-pathogenic nematode content of these soils were investigated.

3.2 Geophagic study area

Thabo Mofutsanyane District Municipality is located in the east of the Free State Province of South Africa and covers an area of approximately 28 400 square kilometers (Figure 3.1). This municipality encompasses five wards of which two, namely the Dihlabeng Local Municipality and the Maluti a Phofung Local Municipality were included in this study. Towns located within Dihlabeng include Bethlehem, Bohlokong, Clarens, Fouriesburg, Paul Roux and Valsrivier. Towns located within Maluti a Phofung Local Municipality include Harrismith, Kestell, Makwane, Matsegeng, Moeding, Phuthaditjhaba and Witsieshoek. A local interpreter and guide suggested the selection of the rural town of Phuthaditjhaba and the small town of Clarens, because of the existence of prior knowledge of existing mines and practicing geophagists in these towns.

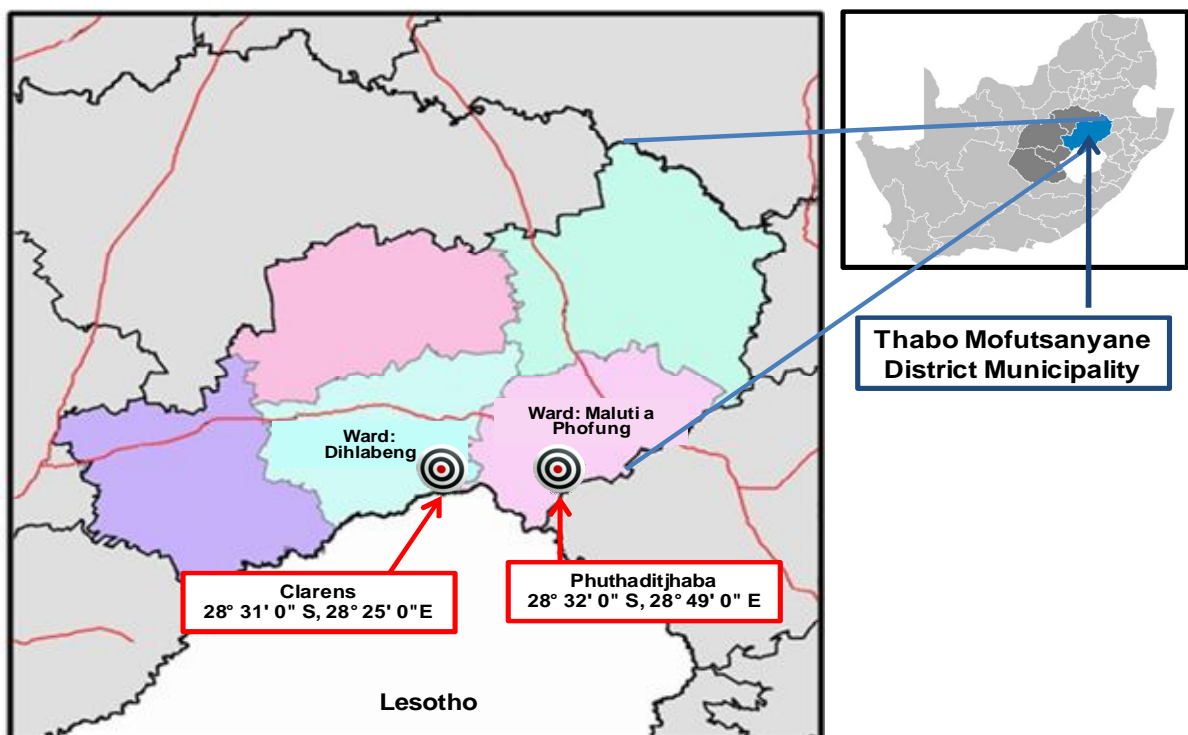


Figure 3.1 Locations of Clarens and Phuthaditjhaba in South Africa.

3.3 Development and distribution of questionnaires

A questionnaire (named *Human Geophagia* for this study) was developed as part of the larger investigation to obtain information about the practice of human geophagia. This questionnaire was used to gather data about the demographic, socio-economic and cultural information of vendors who sell geophagic clayey soil, as well as the customers who purchase geophagic clayey soil. Simultaneously, information was gathered on the importance of selling geophagic clayey soil and the vendors' indigenous knowledge pertaining to the practice of geophagia. This questionnaire was distributed to eleven soil vendors in the Setsing-Phuthaditjhaba local market, willing to participate in this study and completed with the assistance of an interpreter, Jack Mokoena, who hails from this area. An example of this questionnaire has been presented in Appendix B.

This *Human Geophagia* questionnaire, being part of the larger investigation, did not contain data on the vendors' methods of soil mining, nor did it request data on the hygienic practices of vendors when obtaining geophagic soil as merchandise. Because hygienic practices, or the lack thereof, may influence the bacteriological and parasitological content of geophagic soil, an additional supplementary questionnaire was developed (refer to Figure 4.1, p. 62) specifically for this study to determine vendors' methods and hygiene practices during the mining of soil.

The focus of the supplementary questionnaire was to obtain data specifically about the following (also refer to Table 4.5, p 60):

- Whether vendors mined their own soil;
- Where soil was mined by vendors and the environmental conditions prevalent at the mining site;
- How soil was mined by vendors;
- Hygienic practices of vendors during the mining of soil; and
- Type of containers used for soil collection and transport.

The supplementary questionnaire was circulated to nine Setsing-Phuthaditjhaba market soil vendors, who were willing to participate, during soil collection in October 2009. The interpreter facilitated accurate completion of the questionnaire.

3.4 Collection of soil samples

3.4.1 Introduction

A convenience sampling method (not based on random selection or probability) was applied during soil sample collection in the Thabo Mofutsanyane District Municipality. This method entails the collection of samples at the convenience of the researcher and is an economical estimation of the truth (Keyton, 2006). The indigenous knowledge obtained from the interpreter (native), was employed to decide upon which mining sites should be included in the study. The interpreter provided valuable information regarding potential collection sites. Soil samples were thus collected in areas known for human geophagic practices.

Indigenous knowledge and the ongoing investigations into the practice of human geophagia, has shown that particular areas are selected and revisited for geophagic practices. Vendors in Phuthaditjhaba collect their soil from areas similar to those visited by the local inhabitants who practice geophagia, or purchase their soil from traditional miners. Collection of soil samples in Clarens and Phuthaditjhaba took place from the 5th to the 8th of October 2009.

The geophagic soils for this study were divided into three soil sample groups: a *topsoil sample group* collected from existing mines; an *excavated soil sample group* collected from existing mines, usually more than 20 cm from the surface; and a *vendor-purchased soil sample group*. A fourth soil sample group consisted out of non-geophagic soil collected from areas not used by geophagists and was used as a *control soil sample group*. A total of 52 soil samples ($n = 52$) were collected for this study. The details of the sampling areas have been provided in Table 3.1.

3.4.2 Soil collection method

Soil samples for this study were collected from the preselected areas and kept cool in cooler boxes whilst collection was taking place and during transport to the laboratory, where they were refrigerated until analyzed (Sandor and Estrada, 2008).

Table 3.1 Soil sample collection, origin, sites and description.

| Soil sample groups | Sample group size (n) | Origin of sample groups | Coordinates of sample groups | Description of sample groups |
|---|-----------------------|--|-----------------------------------|---|
| <i>Vendor soil sample group (V)</i> | 13 | Setsing-Phuthaditjhaba local market: various origins within the Thabo Mofutsanyane District Municipality | 28° 32' 0" South, 28° 49' 0" East | Geophagic soil purchased from vendors |
| <i>Topsoil sample group (T)</i> | 17 | Clarens | 28° 31' 0" South, 28° 25' 0" East | Geophagic topsoil collected from known and established mines |
| | | Phuthaditjhaba | 28° 32' 0" South, 28° 49' 0" East | |
| <i>Excavated soil sample group (E)</i> | 17 | Clarens | 28° 31' 0" South, 28° 25' 0" East | Geophagic soil excavated from known and established mines |
| [mined 5-10 cm below surface] | | Phuthaditjhaba | 28° 32' 0" South, 28° 49' 0" East | |
| <i>Control soil sample group (C)</i> | 5 | Clarens | 28° 31' 0" South, 28° 25' 0" East | Non-geophagic soil collected from areas not known for geophagia mines |
| [mined 5-10 cm below surface] | | Phuthaditjhaba | 28° 32' 0" South, 28° 49' 0" East | |
| Total size | 52 | | | |

Collection of the vendor soil sample group

The *vendor soil sample group* was collected from a selection of vendors who set up stall at the Setsing-Phuthaditjhaba local market, which is located within the rural settlement of Phuthaditjhaba. These vendors sell geophagic clayey soil, generally pre-packed in plastic bags, to geophagists. Collection of the *vendor soil sample group* from these vendors took place on the 5th and 7th of October 2009.

Macroscopic evaluation of more notably the sample colour and texture, determined whether more than one sample was obtained from an individual vendor, so as to include all possible soil sample types available for purchase at the time of sample collection. Purchased sample bags were labeled with a permanent marker and also documented using a pre-constructed vendor data collection sheet of which an example has been presented in Appendix C1. Attention was given to the following information, modified from (Young, *et al.*, 2008):

- Labels contained soil sample number and soil sample group (sample 1-V (V for vendor));
- Collection date and time recorded;
- Name of vendor recorded;
- Location of vendor recorded;
- The cost per bag of geophagic clayey soil purchased from a vendor recorded;
- Processing method of geophagic clayey soil by the vendor recorded;
- Macroscopic appearance of the vendor soil sample recorded; and
- Digital image of vendor soil sample(s) obtained photographically.

Collection of the topsoil and excavated soil sample group

The *topsoil* and *excavated soil sample groups* were collected from existing human geophagia mines located within the rural settlement of Phuthaditjhaba, as well as the small town of Clarens. These mines were previously identified and known by the

interpreter, or newly identified by local inhabitants. Collection of these *topsoil* and *excavated soil sample groups* took place on the 6th and the 8th of October 2009.

It was envisaged that excavated soil beneath the topsoil at different mining sites would be obtained as far as practically possible from the same depth of 25 - 30 cm, as suggested by Hunter (2003), who observed that individuals plagued by geophagic cravings tend to obtain deeper embedded soil. During the excursion it was found, however, that existing mines were already excavated at variable depths and therefore 'excavated' soil for this study was obtained at a further depth of 5 - 10 cm from these mines. Approximately 250 grams of topsoil, as well excavated soil were collected using a hand trough. Hands and utensils were cleaned between sampling using 70 % alcohol. Mined samples were placed into appropriate re-sealable plastic bags or screw-cap collection bottles.

From each identified mine a topsoil sample was first collected and then an excavated sample. These soil samples were then labeled and documented using a similar pre-constructed data collection sheet to that of the vendor's data collection sheet (refer to Appendix C2), with attention given to the following:

- Labels contained soil sample number and soil sample group (sample 1-T (T for topsoil) and sample 1-E (E for excavated));
- Exterior of container labeled using a permanent marker/adhesive;
- Labeled paper tag placed inside the container;

- Date and time of collection recorded;
- Area name where soil was collected recorded;
- Description of mining site recorded;
- Digital image of the mining sites obtained photographically;
- Macroscopic appearance of soil; and
- Weather conditions of the day of collection.

Collection of the control soil sample group

The *control soil sample group* was collected from areas not used by geophagists for the mining of soil located within the rural settlement of Phuthaditjhaba and the small town of Clarens. Collection of the *control soil sample group* took place on the 6th and the 8th of October 2009.

Control soil samples were collected, labeled and also documented using the same pre-constructed data collection sheet as for the *topsoil* and *excavated soil sample group*. The collection methodology for the *control soil sample group* was also similar to that described for the *topsoil* and *excavated soil sample group*, with the exception of the sample group designator which changed to “C” (sample 1-C (C for control)) (Appendix C2).

3.5 Detection of the presence of soil nematodes

3.5.1 Introduction

No standard method exists for the detection of nematode ova, including geohelminth ova, in soil-based samples (Buckley, *et al.*, 2008). However, a wide variety of recovery methods for nematode ova from soil-based samples has been used over the years (Buckley, *et al.*, 2008). For example, geohelminth identification from Pemban geophagic soils were conducted using a basic technique which involved sieving and the microscopic examination of the resultant sediment (Young, *et al.*, 2007). However, flotation techniques have shown several advantages during soil sample analysis, including cost-effectiveness and time-efficiency (Kuczynska and Shelton, 1999). Generally, poor geohelminth ova recovery occurs with flotation techniques, as ova inherently adhere to clay and silt soil particles (Kuczynska and Shelton, 1999). For this study, the recommended Ammonium Bicarbonate (AMBIC) protocol was chosen and adapted for nematode ova recovery, as the method entails disruption of ova-soil particle bonds and consequently, a higher ova yield (Buckley, *et al.*, 2008).

3.5.2 Identification of parasitic geohelminth ova

Soil samples were analyzed for the presence of potentially pathogenic geohelminth ova, using the Ammonium Bicarbonate (AMBIC) protocol, which incorporates a modified zinc sulphate flotation method, described by the Water Research Commission (WRC) of South Africa (Buckley, *et al.*, 2008).

The AMBIC protocol consists of four analytical procedures, namely, sample preparation, sample washing with AMBIC solution, nematode ova recovery through a modified zinc flotation method and microscopic analysis (Buckley, *et al.*, 2008). The volumes used were adjusted according to the amount of soil sample analyzed. All soil samples were analyzed in duplicate. Recipes for solutions have been presented in Appendix A.

Procedure 1: Sample preparation comprised of the following:

1. One gram of the soil sample was placed into a 50 ml Falcon conical test tube (Lasec).

Procedure 2: Sample washing with AMBIC solution comprised of the following:

2. The sample was then washed through addition of saturated AMBIC solution up to the 15 ml mark on the tube and vortexed (Vortex mixer-300) for 3 min.
3. During mixing, larger particles were freed from the bottom of the tube using applicator sticks if necessary.
4. Tubes were then capped and left to stand for one hour.
5. Thirty minutes into the standing period, tubes were vortexed for 3 min. and then shaken (Labcon shaker) for an additional 2 min.
6. After an hour, samples were centrifuged (Heraeus Biofuge Primo centrifuge) at 940 g for 3 min.
7. The supernatant was then discarded into waste buckets.
8. Deionised water (about 15 ml) was added to the sediment and vortexed for 2 min.
9. The sample was again centrifuged at 940 g for 3 min. and the

supernatant discarded.

Procedure 3: Modified zinc flotation method for nematode ova recovery comprised of the following:

1. About 12 ml of zinc sulphate solution was added to the tube and vortexed for 2 min.
2. Tubes were then centrifuged at 600 g for 3 min.
3. The supernatant was poured into a clean 50 ml conical test tube and thereafter each tube was filled with deionised water to decrease the specific gravity.
4. Tubes were then centrifuged at 1850 g for 3 min.
5. Wet preparations were prepared from the pellet of each tube and then examined under $\times 10$ and $\times 40$ objectives of a light microscope (Olympus CX21).

Procedure 4: Microscopic analysis comprised of the following:

1. Pellet examination of each tube was conducted in duplicate and visualization under the light microscope aided through the addition of Lugol's iodine to accentuate any ova present in the wet preparations.
2. The presence of nematode ova was recorded and the ova identified using parasitological knowledge coupled to unique characteristics of each species' ova in conjunction with the Atlas of Human Parasitology (Ash and Orihel, 2007).

Formalin-fixed suspensions containing nematode ova (*A. lumbricoides*, *T. trichiura*, *A. duodenale*, *N. americanus*) were used to create qualitative positive control

specimens. These formalin-fixed concentrated ova suspensions (product codes: SDL-CONT-483, SDL-CONT-511, SDL-CONT-522 and SDL-CONT-515) were obtained from Davies Diagnostics. Non-geophagic soil samples, obtained from different areas as the geophagic samples, were spiked with the concentrated ova immediately prior to the first step of processing. This positive control specimen was then processed simultaneously with every batch of samples analyzed in order to validate the accuracy of the method.

3.5.3 Identification of non-ova biological content

In addition to the isolation of geohelminth ova, the AMBIC protocol also detected the presence of non-ova biological contents such as mesofauna, fungal structures and nematode larvae. The non-ova biological contents were recorded and digitally documented using a digital microscopic eyepiece (Premier model MA88), courtesy of Van Rensburg Pathology Microbiology laboratory in Bloemfontein.

3.5.4 Extraction, classification and enumeration of nematode larvae

Differentiation between non-pathogenic soil-inhabiting nematode larvae and pathogenic hookworm larvae is imperative as deposited hookworm ova may develop into infective filariform larvae within the soil (Murray, *et al.*, 2009). Therefore, all soil samples that yielded nematode larvae under the light microscope during the AMBIC protocol, were sent to Dr Mariette Marais at the Agricultural Research Council Plant Protection Research Institute (ARC/PPRI) in Pretoria for nematode extraction, taxa classification

and population enumeration. The ARC/PPRI routinely uses a sieving-centrifugation-flotation method, modified from Kleynhans (1997), for nematode extraction from soil samples as follows:

Procedure 1: Nematode extraction comprised of the following:

1. Using tap water, each soil sample (of about 250 cm³) was washed through a 2 mm aperture coarse mesh sieve (Spar/Checkers) into a 5 l bucket.
2. The bucket was filled up to the 5 l mark with tap water.
3. The suspension was stirred and allowed to settle for 30 sec. and then poured through a 45 µm aperture sieve (Lasec) into another 5 l bucket.
4. This procedure was repeated two more times, decreasing the settling time to 20 sec., and then 10 sec.
5. The resulting residue on the mesh was carefully transferred to four 100 ml tubes (Lasec), using water to gently wash the residue into tubes.
6. One teaspoon of kaolin powder (Arcos Organics) was added to each tube and stirred.
7. Tubes were then centrifuged (Hereaus Thermo Scientific centrifuge) at 1750 revolutions per minute (rpm) for 7 min. and the supernatant discarded.
8. A saturated sugar solution was added to each tube and gently mixed. Tubes were then centrifuged again at 1750 rpm for 3 min.
9. The supernatant was then poured through the 45 µm sieve and residue carefully washed into a suitable container using water.

Procedure 2: Nematode classification comprised of the following:

1. Skilled nematologists classified nematode taxa after mounting temporary slides and examining the preparations using dissection (Nikon SMZ645) and research microscopes (Nikon Eclipse E400/Zeiss Axioskop40/ Nikon Labophot-2).

Procedure 3: Nematode population enumeration comprised of the following:

1. Nematode populations were enumerated by placing a sub-sample obtained from the modified sieving-centrifugation-flotation method, into a De Grisse counting dish and counting each genus level using a Laboratory DC Counter.

3.6 Classification of soil types according to colour

Evidence exists that geophagists may use soil colour as a selection tool when obtaining geophagic clayey soil. In turn, the soil colour may directly translate to the principal type of iron oxide present within the soil (Young, *et al.*, 2008; Gomes, *et al.*, 2009; Ngole, *et al.*, 2010). As a relationship between iron deficiency anaemia and geophagia has already been established and some scholars hypothesize that geophagia serves as a method of iron supplementation, the evaluation of soil colour allows qualitative establishment of the iron oxides present in the geophagic soil (Halsted, 1968; Knishinsky, 1998; Reilly and Henry, 2001; Nchito, *et al.*, 2004; Von Garnier, *et al.*, 2008, Young, *et al.*, 2008).

Soil sample colour was documented using the user-friendly *Munsell Soil Color* classification charts as a comparative standard to maintain objectivity (Young, *et al.*, 2008). A portion of each sample in its natural state was spread onto white paper and, under white light, compared to the *Munsell Soil Color Charts*, selecting the closest resembling colour chip. Different characteristics of the soil colour were noted: the *hue* referring to the colour of the sample related to red, yellow, green, blue and purple; the *value* portraying the lightness of the colour and the *chroma* pertaining to colour strength (*Munsell Soil Color Charts*, 2000).

Soil colour classification was determined through the *Munsell Soil Color* notation in combination with a colour name. The Munsell notation is always documented in the order of *hue*, *value* and then *chroma*, with the final reading appearing, for example, as 5YR 4/6. The *hue* (5YR) consists of a number ranging from 0 to 10, followed by letter abbreviations of colours (Y for yellow, YR for yellow-red, etc.). The *value* (4) consists of a number ranging from 0 (absolute black) to 10 (absolute white). Lastly, the *chroma* also consist out of a number ranging from 0 (neutral greys) to 20 (*Munsell Soil Color Charts*, 2000).

3.7 Statistical analysis

Data from the vendor questionnaires and soil samples were captured in Microsoft Excel 2007. Further statistical analyses were conducted with the assistance of a bio-statistician, Maryn Viljoen, using SAS Version 9.1.3. Descriptive statistics, namely

frequencies and percentages were calculated for categorical data. Means and standard deviations, or medians and percentiles were calculated for numerical data. Analytical statistics, namely the Chi-Square statistic or Fisher's exact test for differences between categorical data, were used to calculate significant differences amongst the different soil sample groups (control, excavated, topsoil and vendor). The Kruskal-Wallis test was used to compare median values within the different soil sample groups. A significance level of 0.05 was used.

Chapter 4

Results of questionnaires

4.1 Introduction

A questionnaire (named *Human Geophagia* for this study, refer to Appendix B) developed by Professor Georges Ekosse to characterize human geophagic habits and geophagic clayey soils, was completed by the vendors at the Setsing-Phuthaditjhaba market in the Thabo Mofutsanyane district, Free State, South Africa. This questionnaire, however, did not address the methods of mining and hygiene practices incorporated by vendors during collection of geophagic clayey soils. Therefore, a supplementary questionnaire (named *Mining and Hygiene Practices* for this study, refer to pp. 60 - 62) was developed by the researcher and also completed by Setsing-Phuthaditjhaba market vendors.

4.2 Data from questionnaire: Human geophagia

Data pertaining to human geophagic practices was obtained upon completion of the questionnaire by vendors at the Setsing-Phuthaditjhaba market. This comprehensive questionnaire included categories such as the demography, socio-economic and cultural aspects of geophagia, businesses of geophagia, and indigenous knowledge of geophagia (Appendix B).

4.2.1 Human aspects of geophagia: Vendors

One section of the questionnaire related to human geophagia that specifically addressed the demographic, socio-economic and cultural aspects of geophagia related to the vendors who sell geophagic clayey soil. The percentage of vendors who selected the different options of the different components of the questionnaire, is reflected in Table 4.1. Generally, vendors who sell geophagic clayey soil at the Setsing-Phuthaditjhaba market were mostly married Sesotho-speaking females, over the age of 30 years, from the surrounding rural settlements who have undergone some degree of secondary schooling. The majority of these vendors depended upon the income generated from their vendor shops and stated that the selling of clayey soil for consumption was not negatively perceived by their customers. In addition, more than 70 % of these vendors practiced geophagia themselves and cited that taste was the main reason for consuming clayey soil.

4.2.2 Business aspects of geophagia

Another section of the questionnaire specifically addressed the business aspects of geophagia as viewed by the vendors who sell geophagic clayey soil (Table 4.2). Although other items were also sold by these vendors, all of the vendors indicated that the selling of clayey soil remained a very important component of their business. Generally, twenty rand income was generated through the sale of ten to fifty bags of geophagic clayey soil to customers per day.

Table 4.1 Demographic, socio-economic and cultural aspects of geophagic vendors ($n = 11$).

| | | | | | |
|---|---------------------|------------------|--------------------|-------------------|-------------------------|
| Location | Rural | Suburban | Urban | | |
| % | 90.9 | 9.1 | 0.0 | | |
| Gender | Male | Female | | | |
| % | 18.2 | 81.8 | | | |
| Age in years | ≤ 20 | 21-30 | 31-40 | 41-50 | ≥ 51 |
| % | 9.1 | 9.1 | 36.4 | 18.2 | 27.3 |
| Ethnic group | Sesotho | isiZulu | | | |
| % | 72.7 | 27.3 | | | |
| Marital status | Married | Single | | | |
| % | 72.7 | 27.3 | | | |
| Income besides shop? | Yes | No | | | |
| % | 18.2 | 81.8 | | | |
| Education | No schooling | Primary | Secondary | Tertiary | Literacy program |
| % | 18.2 | 18.2 | 63.6 | 0.0 | 0.0 |
| Do you eat soil/clay? | Yes | No | | | |
| % | 72.7 | 27.3 | | | |
| If yes, how often? | 1/ wk | 1/day | Taste | | |
| % ($n = 8$) | 37.5 | 50.0 | 12.5 | | |
| If yes, for how long? | < 1yr | 1 yr | 2 yr | > 2yr | |
| % ($n = 7$; 1 missing) | 28.6 | 0.0 | 57.1 | 14.3 | |
| Your reason(s) for eating soil/clay? | Craving | Medicinal | Taste | | |
| % ($n = 8$) | 25.0 | 12.5 | 100.0 | | |
| How do others perceive the sale of soil/clay? | Positive | Negative | Indifferent | Don't know | |
| % | 90.9 | 0.0 | 9.1 | 0.0 | |
| How do others perceive the consumption of soil/clay? | Positive | Negative | Indifferent | Don't know | |
| % | 100.0 | 0.0 | 0.0 | 0.0 | |

Table 4.2 Importance/size of business in selling soil/clay ($n = 11$).

| Period of selling | < 1 yr | 1 yr | 2 yrs | 3 yrs | > 3 yrs |
|---|-----------------------|------------------|----------------------|----------------|--------------------|
| % | 18.2 | 18.2 | 45.5 | 9.1 | 9.1 |
| How much is sold? | 10 bags | 12 bags | 15 bags | 20 bags | 50 bags |
| % | 27.3 | 9.1 | 9.1 | 36.4 | 18.2 |
| Income per day | < R20.00 | R20.00 | R30.00 | R40.00 | > R40.00 |
| % | 9.1 | 54.5 | 18.2 | 9.1 | 9.1 |
| Sell other items? | Yes | No | | | |
| % | 100.0 | 0.0 | | | |
| Importance of selling soil/clay? | Very important | Important | Not important | | |
| % | 100.0 | 0.0 | 0.0 | | |

4.2.3 Human aspects of geophagia: Customers

A third section of the questionnaire specifically addressed the customers' demographic, socio-economic and cultural aspects of geophagia as viewed by the vendors. Table 4.3 reflects the percentage of vendors who selected the different options of the different components of the questionnaire. The majority of the customers who purchased clayey soil were females of all income groups and age groups, notably 21 to 30 years of age, from the surrounding rural settlements. More than 80 % of these customers purchase two bags of clayey soil per day, generally priced at one rand per bag.

Table 4.3 Demographic, socio-economic and cultural aspects of geophagic customers ($n = 11$).

| Age | Under 20 | 21-30 | 31-40 | 41-50 | 51-60 | 60+ |
|------------------|-------------|---------------|------------|------------|---------|------|
| % | 63.3 | 100.0 | 72.7 | 54.5 | 27.3 | 27.3 |
| Gender | Male | Female | Both | | | |
| % | 0.0 | 72.7 | 27.3 | | | |
| Location | Urban | Rural | Suburban | | | |
| % | 18.2 | 100.0 | 9.1 | | | |
| Economic status | High income | Middle income | Low income | All income | | |
| % | 0.0 | 36.4 | 0.0 | 63.6 | | |
| Quantity bought? | One bag/day | Two bags/day | | | | |
| % | 18.2 | 81.8 | | | | |
| Cost per bag? | < R1.00 | R1.00 | R1.50 | R2.00 | > R2.00 | |
| % | 9.1 | 54.5 | 27.3 | 36.4 | 9.1 | |

4.2.4 Indigenous knowledge of geophagia

The final section of the questionnaire specifically addressed the indigenous knowledge of vendors who collect, prepare and sell geophagic clayey soil. The percentage of vendors who selected the different options of the different components of the questionnaire, is reflected in Table 4.4. Geophagic clayey soil sold by vendors at Setsing-Phuthaditjhaba market is traditionally known as *mobu*, but sometimes also referred to as *sweets*, *dipompong* or *rama*. Although some vendors purchased clayey soils from suppliers (traditional miners), most collected the soils themselves from the wild, mountain- and riversides. Customers of vendors mostly preferred whitish clayey

soil, because of its taste, perceived by some as being sour. Vendors, therefore, tend to collect mostly whitish clayey soil to satisfy customer

Table 4.4 Vendors' indigenous knowledge on type of geophagic material collected, processed and sold ($n = 11$).

| | | | | | | | |
|--|--------------------------|-------------------------------|--------------------------|----------------------|------------------------------------|----------------|-----------------------|
| What kind of soil/clay do you sell? | Soil/clay | Clay | From termitaria | Other | | | |
| % | 100.0 | 9.1 | 0.0 | 0.0 | | | |
| How do you obtain soil/clay? | Buy from supplier | Buy from other vendors | From termitaria | From the wild | Other: mountain & river | | |
| % | 9.1 | 0.0 | 0.0 | 72.7 | 81.8 | | |
| Colour of soil/clay? | Reddish | Blackish | Khaki | Brownish | Yellowish | Whitish | Other: greyish |
| % | 0.0 | 0.0 | 9.1 | 18.2 | 36.4 | 90.9 | 9.1 |
| Customer colour preference? | Yes | No | | | | | |
| % | 100.0 | 0.0 | | | | | |
| If yes, what colour? | Reddish | Blackish | Khaki | Brownish | Yellowish | Whitish | Other: greyish |
| %($n = 11$) | 0.0 | 0.0 | 9.1 | 18.2 | 27.3 | 63.6 | 9.1 |
| Why is colour preferred? | Taste | Medicinal value | Other: sourness | | | | |
| % | 100.0 | 9.1 | 36.4 | | | | |
| For whom do they buy? | Themselves | Members of family | | | | | |
| % | 100.0 | 63.6 | | | | | |
| Advise on use? | Yes | No | | | | | |
| % | 27.3 | 72.7 | | | | | |
| Administration | Swallow | | | | | | |
| %($n = 7$) | 100.0 | | | | | | |
| Traditional name | Mobu | Sweets/ dipompong | Rama | | | | |
| % | 90.9 | 9.1 | 9.1 | | | | |
| Storage | Maize meal bag | Plastic bag | Plastic container | | | | |
| % | 18.2 | 54.5 | 36.4 | | | | |

| | | | | | | | |
|--------------------------------|-----------------|---------------------|---------------|---------------|----------------|--|--|
| Storage period? | 2 days | 4 days | 5 days | 7 days | 14 days | | |
| % | 18.2 | 9.1 | 36.4 | 27.3 | 9.1 | | |
| Expected consumption? | Wet | Dry | | | | | |
| % | 0.0 | 100.0 | | | | | |
| Process before selling? | Yes | No | | | | | |
| % | 100.0 | 0.0 | | | | | |
| Who does processing? | Vendor | Customer | Both | Other | | | |
| % | 100.0 | 0.0 | 0.0 | 0.0 | | | |
| Method of processing? | Pounding | | | | | | |
| % | 100.0 | | | | | | |
| Heat treatment | Yes | No | | | | | |
| % | 100.0 | 0.0 | | | | | |
| If yes, how? | Baking | Sun exposure | | | | | |
| % (<i>n</i> = 11) | 63.6 | 72.7 | | | | | |

preferences. These clayey soils are purchased by customers for their own oral consumption, although they often purchase clayey soil for family members as well.

Vendors process and store geophagic clayey soil before it is sold to customers. Processing of geophagic clayey soil by the vendors include pounding and heat treatment, which comprises baking and/or sun drying. The clayey soils are then stored in a variety of containers, more often plastic bags, for periods ranging from two days to two weeks prior to them being purchased by customers.

4.3 Data from questionnaire: Mining and hygiene practices

The supplementary questionnaire was designed to generate data about the mining and hygiene practices of vendors in the Setsing-Phuthaditjhaba market. The questions of this questionnaire were based upon a set of objectives as set out in Table 4.5.

Table 4.5 Objectives and questions of the mining and hygiene practices questionnaire.

| Objective | Question |
|---|---|
| Determine if vendor mines own soil | Do you mine your own soil? |
| Establish area and surrounding conditions of mines | Where do you mine your soil samples? Rural, suburban, urban or other? |
| | Do you take environmental factors, for example cattle grazing, pollution, sewage, contamination, etc. into consideration when mining your samples in a specific area? |
| Determine tools used for mining | What do you use to mine your samples with? Bare hands, utensils or other? |
| Establish hygiene practices during mining | Is what you use to mine your samples clean/unclean? |
| | Is what you use to transfer your samples clean/unclean? |
| | Is your collection container clean/unclean? |
| Determine type of collection containers used | In what type of collection container do you put your mined samples? Plastic bag, box or other? |

The mining and hygiene practices questionnaire covered nine questions focusing on generating information pertaining specifically to the mining and hygiene practices of vendors (Figure 4.1).

| Name: | | Date: | |
|-------|--|---|-----------------------------|
| 1.1 | Do you mine your own soil samples? | <input type="checkbox"/> Yes | <input type="checkbox"/> No |
| 1.2 | If YES, where do you mine your samples? | <input type="checkbox"/> Rural | |
| | | <input type="checkbox"/> Suburban | |
| | | <input type="checkbox"/> Urban | |
| | | <input type="checkbox"/> Other, please specify: | |
| 2 | Do you take environmental factors, for example cattle grazing, pollution, sewage contamination, etc. into consideration when mining your samples in a specific area? | <input type="checkbox"/> Yes | <input type="checkbox"/> No |
| 3.1 | What do you use to mine your samples with? | <input type="checkbox"/> Bare hands | |
| | | <input type="checkbox"/> Utensils | |
| | | <input type="checkbox"/> Other, please specify: | |
| 3.2 | Is what you use to mine your samples: | <input type="checkbox"/> Clean? | |
| | | <input type="checkbox"/> Unclean? | |
| 4.1 | What do you use to transfer your mined sample to your collection container? | <input type="checkbox"/> Bare hands | |
| | | <input type="checkbox"/> Utensils | |
| | | <input type="checkbox"/> Other, please specify: | |
| 4.2 | Is what you use: | <input type="checkbox"/> Clean? | |
| | | <input type="checkbox"/> Unclean? | |
| 5.1 | In what type of collection container do you put your mined samples? | <input type="checkbox"/> Plastic bag | |
| | | <input type="checkbox"/> Box | |
| | | <input type="checkbox"/> Other, please specify: | |
| 5.2 | Is what you use: | <input type="checkbox"/> Clean? | |
| | | <input type="checkbox"/> Unclean? | |

Figure 4.1 Mining and hygiene practices questionnaire.

The supplementary vendor questionnaire was completed by nine soil vendors at the Setsing-Phuthaditjhaba market on the 5th and 7th of October 2009. All of these vendors indicated that they mined their own clayey soil from mainly rural, but also suburban areas using clean utensils and taking environmental factors, such as soil pollution, into consideration. Thereafter, the mined clayey soil was transferred to clean plastic bags or plastic containers by these vendors using clean bare hands (Table 4.6).

Table 4.6 Vendor mining and hygiene practices ($n = 9$).

| | | | |
|---|--------------------|-----------------|---------------------------------|
| Mine own samples? | Yes | No | |
| % | 100.0 | 0.0 | |
| Where do you mine? | Rural | Suburban | Urban |
| % | 55.6 | 44.4 | 0.0 |
| Consider environmental factors | Yes | No | |
| % | 100.0 | 0.0 | |
| With what do you mine? | Bare hands | Utensils | Other |
| % | 0.0 | 100.0 | 0.0 |
| For above is it: | Clean | Unclean | |
| % | 100.0 | 0.0 | |
| With what is sample transferred? | Bare hands | Utensils | Other |
| % | 88.9 | 11.1 | 0.0 |
| For above is it: | Clean | Unclean | |
| % | 100.0 | 0.0 | |
| Collection container? | Plastic bag | Box | Other: plastic container |
| % | 66.7 | 0.0 | 33.3 |
| For above is it: | Clean | Unclean | |
| % | 100.0 | 0.0 | |

Chapter 5

Soil characterization and soil nematode content

5.1 Introduction

In this investigation, the 52 soil samples obtained from the district of Thabo Mofutsanyane were analyzed for their biological content – focusing on the geohelminth and other nematode contents. Macroscopic soil characteristics and presence of fungal structures were also noted.

5.2 Characterization of soil samples

The soil samples comprised of geophagic soil obtained from vendors at the Setsing-Phuthaditjhaba local market (*vendor soil sample group*), geophagic soil mined from existing mines in Phuthaditjhaba and Clarens (*topsoil* and *excavated soil sample groups*), and control soils collected from areas not regarded as mining sites (*control sample group*).

5.2.1 Vendor soils

The Setsing-Phuthaditjhaba local market was visited on two occasions (5th and 7th of October 2009). A total of thirteen vendor soil samples were purchased from eight

different soil vendors during this time. All the vendor soil samples that were purchased for this study were already aliquoted and pre-packed by each respective vendor into plastic bags, in general weighing approximately 200 grams per bag (Figure 5.1).



Figure 5.1 Setsing-Phuthaditjhaba local market:

a. – c. Vendors with their geophagic soil merchandise; d. Bulk merchandise stored in a used 10 kg plastic bag; and e. – i. Geophagic soil in the process of being pre-packed, or already pre-packed by the vendor.

The information that was gathered on all the geophagic soils obtained from vendors revealed data on the pricing of the soil per bag sold, whether soil was processed before selling, the method of processing the soil, and the macroscopic description of the soil (Table 5.1).

Table 5.1 Characterization of vendor soils ($n = 13$).

| Price per bag | R 1.50 | R 2.00 | R 3.00 | |
|-------------------------------------|--------------------------------------|----------------------------|---------------------|---------------|
| % | 7.7 | 84.6 | 7.7 | |
| Processed? | Yes | No | | |
| % | 92.3 | 7.7 | | |
| Method of processing | Coal stove oven (10 – 15 min) | Sun and oven drying | Sun drying | |
| % ($n = 12$) | 8.3 | 83.3 | 8.3 | |
| Macroscopic soil description | Small chunks | Medium chunks | Large chunks | *Other |
| % | 23.1 | 46.2 | 7.7 | 30.8 |

*Other = finer soil, grainy smaller chunks, coarser chunks and angular chunks.

Vendor soils were generally priced at two rand per bag and mostly processed by the vendor, which involved a combination of oven and sun drying. Soil configuration varied considerably. Some soils could be categorized as small chunky (approximately of 1 to 2 cm), medium chunky (approximately of 2.5 to 3 cm) and large chunky (approximately of 3.5 to 5 cm). Other soils that did not fit into these categories comprised of finer soils mixed with various sizes of chunks.

5.2.2 Mining site soils

A total of seventeen geophagia mining sites were visited in the Thabo Mofutsanyane district on the 6th and 8th of October 2009. Five of these mining sites were located in Clarens, situated within the Dihlabeng Local Municipality, while the remaining twelve mining sites were located in Phuthaditjhaba, situated within the Maluti a Phofung Local Municipality. The geographic coordinates of each of the mining sites are listed in Table 5.2.

All of the mining sites had been extensively mined by geophagists, therefore, soil samples were collected from the loose-lying soil within each of the mines (topsoil), as well as 10 cm deeper from the topsoil level in each mine (excavated) (Figure 5.2).

Generally, the mining sites were located in areas that were easily accessible (Table 5.3). Garbage lay strewn at the mouths of six of the seventeen mining sites and cattle dung was noted in front of one mining site. The weather during the collection of these soil samples was sunny and windy; although it was evident that the area received rainfall during the past few days as was noted from the moistness of some excavated soil samples, as well as a few water puddles near some of the mining sites.

Table 5.2 Geographic coordinates of the mining sites within the Thabo Mofutsanyane District Municipality used for sample collection.

| Mine | Location | Coordinates |
|---------|------------------------------------|----------------------------|
| Mine 1 | Matsikeng, Phuthaditjhaba | S 28.51413° E 28.85304° |
| Mine 2 | Matsikeng, Phuthaditjhaba | S 28.51396° E 28.85303° |
| Mine 3 | Qhelaqhe, Phuthaditjhaba | S 28.51401° E 28.85285° |
| Mine 4 | Qhelaqhe, Phuthaditjhaba | S 28.51408° E 28.85285° |
| Mine 5 | Namahadi, Phuthaditjhaba | S 28.51402° E 28.85289° |
| Mine 6 | Namahadi, Phuthaditjhaba | S 28.55926° E 28.82986° |
| Mine 7 | Namahadi, Phuthaditjhaba | S 28.55729° E 28.83263° |
| Mine 8 | Namahadi, Phuthaditjhaba | S 28.56030° E 28.84315° |
| Mine 9 | Phahameng, Clarens | S 28.56027° E 28.84316° |
| Mine 10 | Phahameng, Clarens | S 28.52952° E 28.42185° |
| Mine 11 | Outskirts of Phahameng, Clarens | S 28.53830° E 28.42515° |
| Mine 12 | Outskirts of Phahameng, Clarens | S 28.52937° E 28.43517° |
| Mine 13 | Outskirts of Phahameng, Clarens | S 28.52934° E 28.43527° |
| Mine 14 | Mangaung, Phuthaditjhaba | S 28.52926° E 28.43538° |
| Mine 15 | Mangaung, Phuthaditjhaba | S 28.57685° E 28.83201° |
| Mine 16 | Madikwe, Phuthaditjhaba | S 28.58712° E 28.83615° |
| Mine 17 | Madikwe, Phuthaditjhaba | S 28.58557° E 28.83837° |

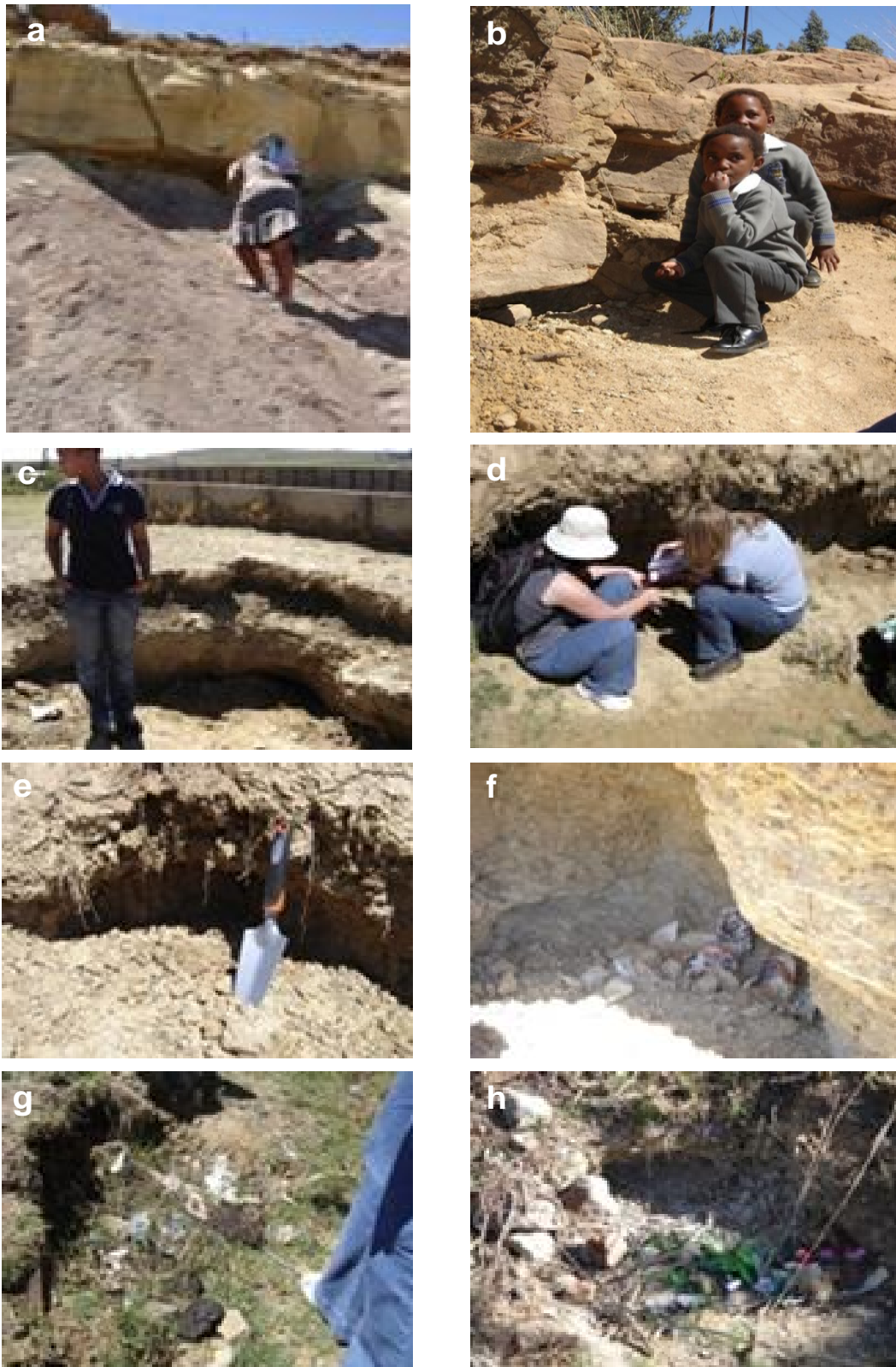


Figure 5.2 Geophagia mining sites in the Thabo Mofutsanyane District:

a. Local Clarens inhabitant indicating a mining site up an embankment situated on the outskirts of Clarens; b. Two school children at a mining site in Phuthaditjhaba; c. An extensively excavated Phuthaditjhaba mining site; d. Soil sample collection at a Phuthaditjhaba mining site; e. Phuthaditjhaba mining site; f. – h. Pollutants (municipal waste and cattle dung) in the vicinity of mining sites.

Table 5.3 Description of the seventeen mining sites.

| Description of mining site | Frequency | Percentage |
|--|-----------|------------|
| Embankment next to public road | 2 | 11.8 |
| Footpath next to public road, shallow, garbage nearby | 1 | 5.9 |
| Loose chunks underneath sandstone cliffs next to public road | 3 | 17.6 |
| Next to footpath | 1 | 5.9 |
| Next to footpath close to public road | 1 | 5.9 |
| Next to public road, garbage and cattle dung noted | 1 | 5.9 |
| Next to public road, garbage noted | 3 | 17.6 |
| Sandstone-like embankment next to public road; 3 picking utensils: concrete shard; broken bottle neck; and a stick | 1 | 5.9 |
| Sandstone-like embankment next to public road; metal plate to pick | 1 | 5.9 |
| Side embankment next to footpath | 1 | 5.9 |
| Side embankment of mountain, next to footpath | 1 | 5.9 |
| Footpath, garbage and broken bottles noted | 1 | 5.9 |

All the mining sites were accessible to geophagists from footpaths and/or public roads in the immediate vicinity of the mining sites. More than 75 % of these mining sites were directly adjacent to a public road, while one third were located next to a footpath (Table 5.4).

Table 5.4 Mining sites near footpaths and/or public roads ($n = 17$).

| | Footpath (%) | Public road (%) | Footpath and public road (%) |
|----------------|-----------------|--------------------|---------------------------------|
| Present | 35.3 | 76.5 | 11.8 |
| Absent | 64.7 | 23.5 | 88.2 |

5.3 Soil sample colour

The soil colour for all 52 soil samples collected in the Thabo Mofutsanyane District for this study were classified using the *Munsell Soil Color Chart* (2000). The colours of the geophagic soils comprised of three main colour groupings, namely greyish, yellowish and brownish, with brownish the most prevalent (Figure 5.3). Within these three colour groups a range of different colours was identified using the *Munsell Soil Color Chart*, totaling 15 specific soil colours, excluding the *control soil sample group*.

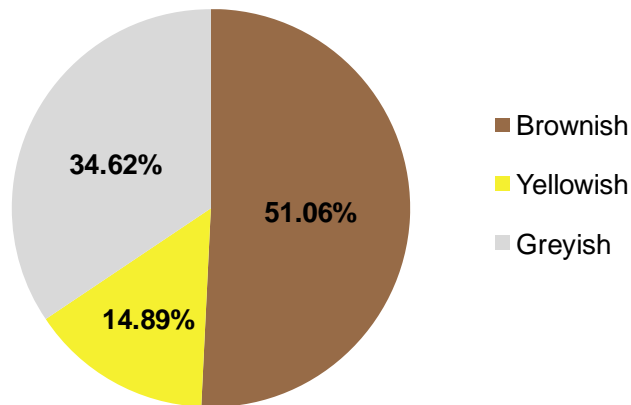


Figure 5.3 Percentages of geophagic soil samples categorized into the main colour groups.

5.3.1 Vendor soil sample group

The soil colour for each of the thirteen vendor soil samples was compared to the *Munsell Soil Color Chart* (2000). Many of the thirteen vendor soil samples ($n = 7$) were classified into different shades of grey (Table 5.5). The soil colour with the most frequent occurrence within the *vendor soil sample group* was light grey ($n = 5$).

Table 5.5 Soil colours of the *vendor soil sample group*.

| Soil sample number | Hue/Value/Chroma | Soil colour name |
|--------------------|------------------|-----------------------|
| 1V | 10YR 8/4 | Very pale brown |
| 2V | 2.5Y 7/3 | Pale yellow |
| 3V | 2.5Y 7/2 | Light grey |
| 4V | 2.5Y 7/3 | Pale yellow |
| 5V | 10YR 8/4 | Very pale brown |
| 6V | 2.5Y 6/3 | Light yellowish brown |
| 7V | 5Y 7/1 | Light grey |
| 8V | 5Y 7/1 | Light grey |
| 9V | 10YR 7/2 | Light grey |
| 10V | 7.5YR 6/2 | Pinkish grey |
| 11V | 2.5Y 7/2 | Light grey |
| 12V | 2.5Y 8/2 | Pale yellow |
| 13V | 2.5Y 6/2 | Light brownish grey |

YR = yellow-red; Y = yellow; and R = red.

5.3.2 Topsoil sample group

The soil colour for each of the seventeen topsoil samples was also compared to the *Munsell Soil Color Chart* (2000). Generally, the majority of the topsoil samples ($n = 10$) presented with different shades of a brown (Table 5.6). The most frequently documented soil colours for *the topsoil sample group* were pale brown ($n = 3$) and grey ($n = 3$); whereas the remaining samples were of a range of colours varying from light yellow to dark brown.

Table 5.6 Soil colours of the *topsoil sample group*.

| Soil sample number | Hue/Value/Chroma | Soil colour name |
|--------------------|------------------|-----------------------|
| 1T | 10YR 6/4 | Light yellowish brown |
| 2T | 10YR 6/3 | Pale brown |
| 3T | 10YR 6/3 | Pale brown |
| 4T | 10YR 6/3 | Pale brown |
| 5T | 2.5Y 7/2 | Light grey |
| 6T | 2.5Y 7/2 | Light grey |
| 7T | 10YR 7/3 | Very pale brown |
| 8T | 7.5YR 5/1 | Grey |
| 9T | 7.5YR 4/6 | Strong brown |
| 10T | 7.5YR 4/4 | Brown |
| 11T | 2.5Y 6/1 | Grey |
| 12T | 2.5Y 5/1 | Grey |
| 13T | 2.5Y 6/2 | Light brownish grey |
| 14T | 10YR 5/4 | Yellowish brown |
| 15T | 10YR 6/4 | Light yellowish brown |
| 16T | 2.5Y 7/3 | Pale yellow |
| 17T | 10YR 5/4 | Yellowish brown |

YR = yellow-red; Y = yellow; and R = red.

5.3.3 Excavated soil sample group

A comparison of the colours of the seventeen excavated soil samples with the *Munsell Soil Color Chart* (2000) revealed that the majority of the soils were different shades of a brown, similar to what was noted for topsoil samples ($n = 11$) (Table 5.7). The

remaining six samples were equally divided between the yellow and grey soil colour spectrum. The two colours, light yellowish brown and pale yellow, occurred most frequently and equally in the *excavated soil sample group* ($n = 3$ for each colour).

Table 5.7 Soil colours of the *excavated soil sample group*.

| Soil sample number | Hue/Value/Chroma | Soil colour name |
|--------------------|------------------|-----------------------|
| 1E | 2.5Y 6/4 | Light yellowish brown |
| 2E | 2.5Y 5/4 | Light olive brown |
| 3E | 2.5Y 7/3 | Pale yellow |
| 4E | 10YR 7/3 | Very pale brown |
| 5E | 2.5Y 7/1 | Light grey |
| 6E | 2.5Y 6/2 | Light brownish grey |
| 7E | 10YR 7/3 | Very pale brown |
| 8E | 2.5Y 4/1 | Dark grey |
| 9E | 7.5YR 4/4 | Brown |
| 10E | 7.5YR 4/6 | Strong brown |
| 11E | 2.5Y 5/2 | Greyish brown |
| 12E | 2.5Y 6/3 | Light yellowish brown |
| 13E | 2.5Y 4/2 | Dark greyish brown |
| 14E | 2.5Y 7/4 | Pale yellow |
| 15E | 2.5Y 8/2 | Pale yellow |
| 16E | 2.5Y 6/3 | Light yellowish brown |
| 17E | 10YR 5/3 | Brown |

YR = yellow-red; Y = yellow; and R = red.

5.3.4 Control soil sample group

The soil colour for each of the five control soil samples of the *control soil sample group* ranged from pale brown to grey when compared to the *Munsell Soil Color Chart* (2000) (Table 5.8). The soil colour of three of the control soil samples was of different shades of brown (1C, 4C and 5C), while two were shades of grey (samples 2C and 3C).

Table 5.8 Soil colours of the *control soil sample group*.

| Soil sample number | Hue/Value/Chroma | Soil colour name |
|--------------------|------------------|------------------|
| 1C | 7.5YR 4/4 | Brown |
| 2C | 2.5Y 6/1 | Grey |
| 3C | 5YR 5/1 | Grey |
| 4C | 10YR 5/4 | Yellowish brown |
| 5C | 10YR 6/3 | Pale brown |

YR = yellow-red; Y = yellow; and R = red.

5.3.5 Comparison between the different soil sample groups

When the soil colours of the different sample groups were compared, it was found that the *control*, *excavated* and *topsoil sample groups* were generally brownish in colour, whereas the samples of the *vendor soil sample group* were mostly different shades of grey.

A comparison between different pairs of soil sample groups with regard to the soil colour indicated only a significant difference between the *control* and *vendor soil sample group* pair ($p < 0.05$) (Table 5.9).

Table 5.9 Statistical comparison between soil colours of soil sample group pairs.

| Soil sample group pairs | | <i>p</i> -value |
|-------------------------|-----------|-----------------|
| Control | Excavated | 0.154 |
| Control | Topsoil | 1.000 |
| Control | Vendor | 0.008 |
| Excavated | Topsoil | 0.391 |
| Excavated | Vendor | 0.464 |
| Vendor | Topsoil | 0.173 |

5.4 Biological material in soil samples

The 52 soil samples collected in this study were analyzed for the presence of biological material. An analysis of the soil samples using the AMBIC protocol, revealed the absence of parasitic nematode ova. Furthermore, numerous other non-ova biological materials were visualized microscopically, demonstrating the rich biodiversity within all the samples. These biological materials comprised fungal structures and mesofauna, for example mites and various nematode larvae (Figure 5.4).

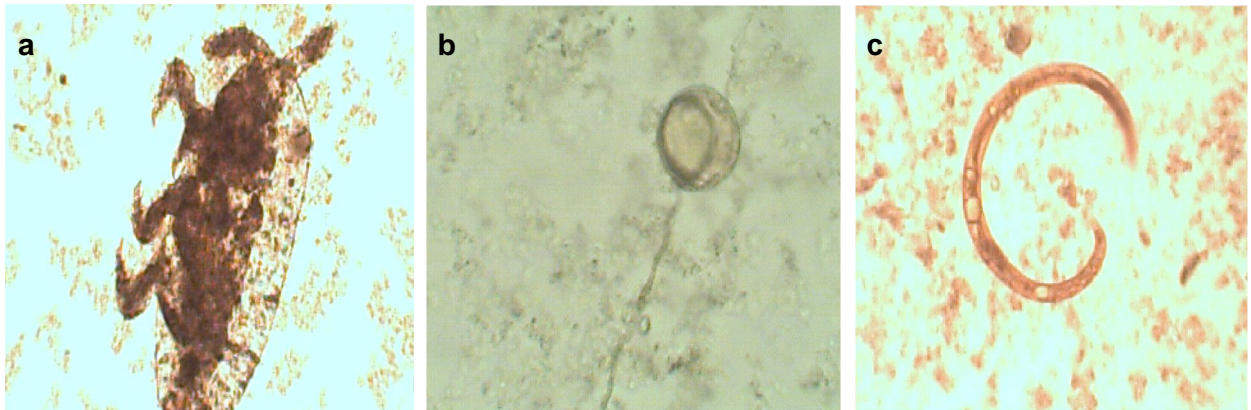


Figure 5.4 Examples of biological materials present in soil samples: a. Soil mite; b. Fungal structure; and c. Nematode larvae.

The biological material with the highest incidence was nematode larvae (38.5 %), whilst the lowest incidence was parasitic ova (0.0 %). Approximately one third (32.7 %) of all the soil samples yielded fungal structures, which indicated that these geophagic and non-geophagic soil samples were rich in organic matter.

The general trends of the presence of biological materials in the geophagic and non-geophagic soil samples demonstrated only the presence of nematode larvae and fungal structures in reasonable amounts (Figure 5.5). Other biological materials such as mites were represented in lesser amounts.

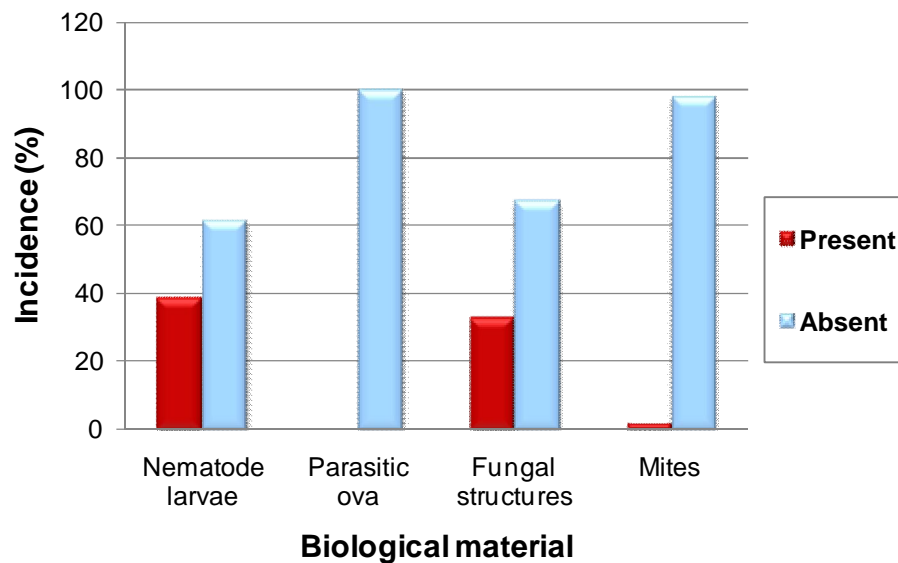


Figure 5.5 Status of biological material in soil samples ($n = 52$).

5.5 Fungal structures

Fungal structures were present in several of the soil samples. Filamentous mycelium, individual hyphae and sac-like sporangiophores were commonly noted within the soil samples (Figure 5.6). However, the scope of this study did not include the specific identification of these fungal structures.

For all the soil sample groups, less than 50 % of the samples contained fungal structures, with the *vendor soil sample group* marginally less than 50 %, at approximately 46 % (Table 5.10). However, only about one fifth of the soil samples of the *control* and *excavated soil sample groups* contained fungal structures. The presence of fungal structures in mined geophagic soil, namely the *excavated* and *topsoil*



Figure 5.6 Examples of fungal structures present in soil samples.

Table 5.10 Prevalence of fungal structures in soil samples.

| Fungal structures | Soil sample groups | | | | Total (n) |
|--------------------------------|--------------------|---------------|-------------|------------|-----------|
| | Control (%) | Excavated (%) | Topsoil (%) | Vendor (%) | |
| Present | 1 (20.0) | 4 (23.5) | 6 (35.3) | 6 (46.2) | 17 |
| Absent | 4 (80.0) | 13 (76.5) | 11 (64.7) | 7 (53.9) | 35 |
| Total number of samples | 5 | 17 | 17 | 13 | 52 |

sample groups, varied with slightly more than 10 %, showing a higher incidence of fungal structures in the *topsoil sample group*, compared to that of the *excavated soil sample group*.

When these data are presented graphically, a distinct trend for the presence of fungal structures could be identified (Figure 5.7). The *control soil sample group* presented with

the least amount of fungal structures, followed by the *excavated soil sample group*, *topsoil sample group* and *vendor soil sample group*.

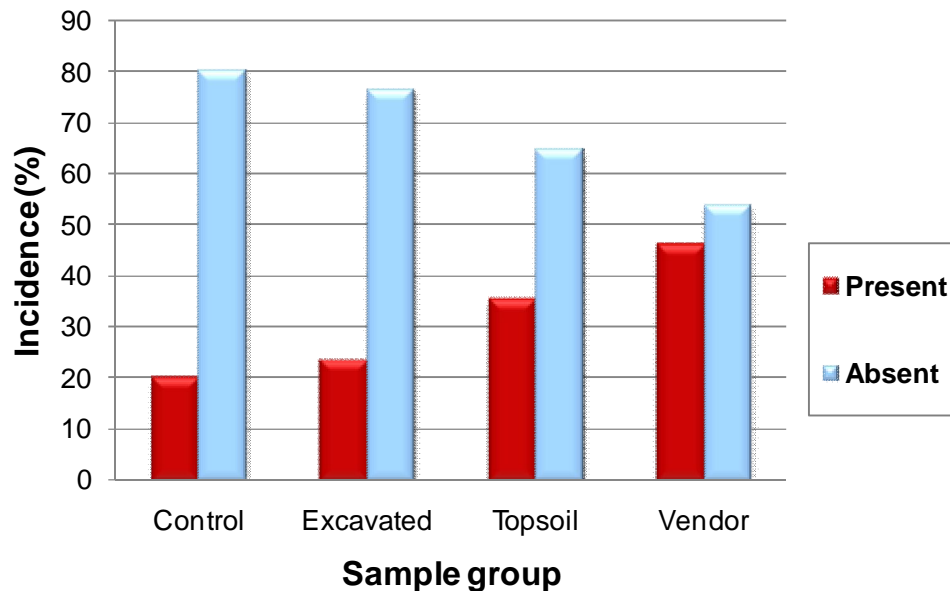


Figure 5.7 Graphic representation of fungal structures present in soil sample groups ($n = 52$).

5.6 Nematode larvae

Analysis of soil samples using the AMBIC protocol yielded several nematode larvae with distinguishing features under the light microscope, such as complete digestive tracts and body cavities. Twenty of the 52 soil samples contained nematode larvae. Examples of nematode larvae isolated are pictured in Figure 5.8.

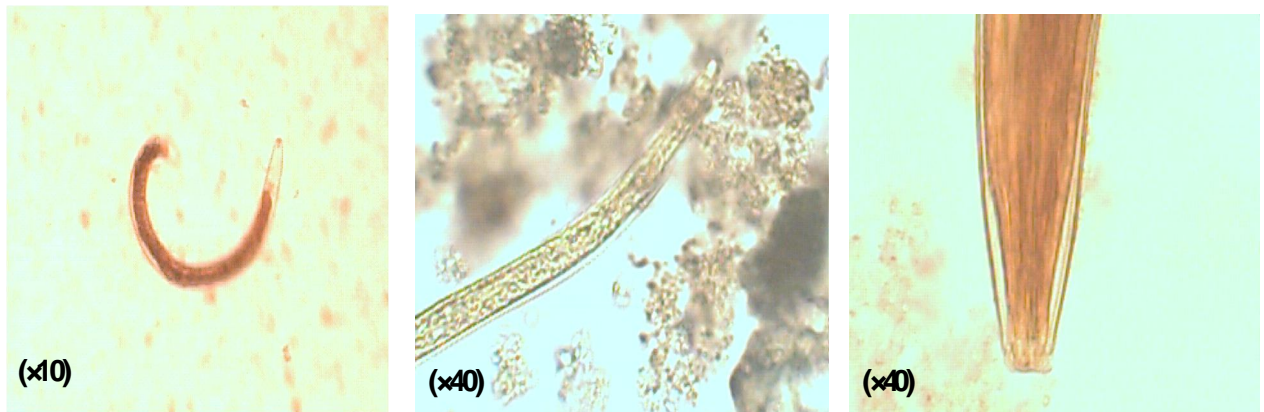


Figure 5.8 Examples of unidentified nematode larvae present in soil samples.

5.6.1 Nematode larvae content

Contrary to the fungal structure content, the highest incidence of nematode larvae was found in the *control soil sample group*, whilst the lowest incidence was recorded for the *vendor soil sample group* (Table 5.11). The incidence of nematode larvae in the *topsoil sample group*, on the other hand, was more than 10 % greater than that of the *excavated soil sample group*.

Table 5.11 Prevalence of nematode larvae in soil sample groups.

| Nematode larvae | Soil sample groups | | | | Total |
|--------------------------------|--------------------|---------------|-------------|------------|-------|
| | Control (%) | Excavated (%) | Topsoil (%) | Vendor (%) | |
| Present | 3 (60.0) | 6 (35.3) | 8 (47.1) | 3 (23.1) | 20 |
| Absent | 2 (40.0) | 11 (64.7) | 9 (52.9) | 10 (76.9) | 32 |
| Total number of samples | 5 | 17 | 17 | 13 | 52 |

When presenting these data graphically, it is clearly discernable that all soil sample groups, except for the *vendor soil sample group*, displayed relatively high levels of nematode larvae (Figure 5.9).

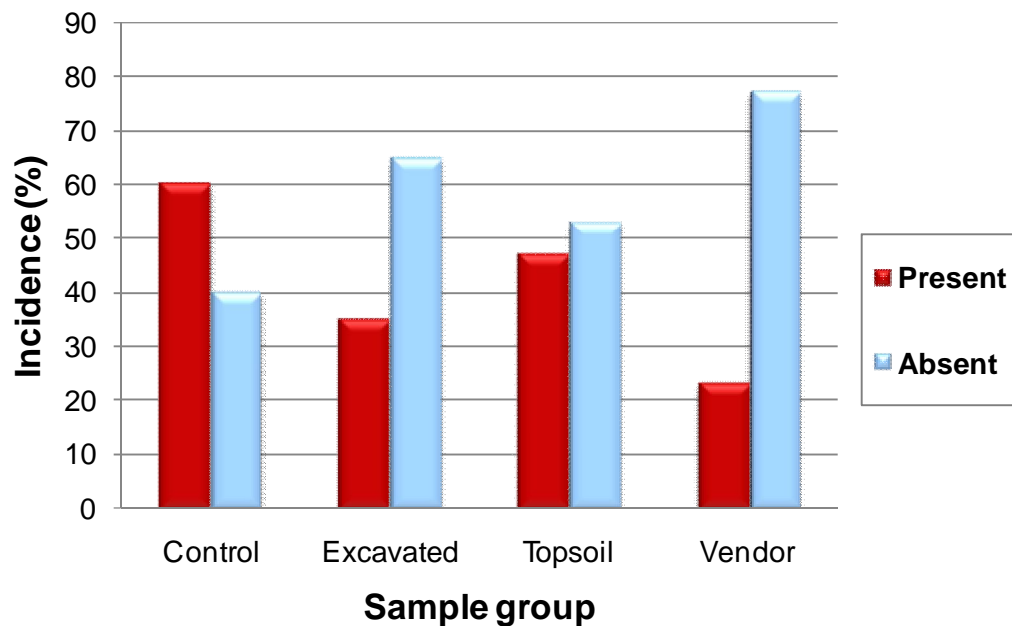


Figure 5.9 Graphic representation of nematode larvae in different soil sample groups ($n = 52$).

A comparison between different pairs of soil sample groups with regard to the presence of nematode larvae indicated no significant differences between any of the pairs ($p > 0.05$) (Table 5.12).

Table 5.12 Statistical comparison of nematode larval content.

| Soil sample group pairs | | <i>p</i> -value |
|-------------------------|-----------|-----------------|
| Control | Excavated | 0.609 |
| Control | Topsoil | 1.000 |
| Control | Vendor | 0.268 |
| Excavated | Topsoil | 0.728 |
| Excavated | Vendor | 0.691 |
| Vendor | Topsoil | 0.260 |

5.6.2 Nematode taxa content

The nematode taxa, taxon diversity and population enumeration of larvae-containing samples ($n = 20$) were determined to ascertain whether parasitic nematode larvae, such as hookworm larvae, were present. A total of 34 different nematode taxa, all non-pathogenic to humans, were identified and each taxon categorized into one of five different trophic levels.

Vendor soil sample group

Three of the 20 nematode larvae-containing soil samples belonged to the *vendor soil sample group*. Of the nine different nematode taxa found in the *vendor soil sample group*, none were found to be larvae pathogenic to humans. Nematode taxa present in sample 9V and 11V represented only two different trophic levels, whereas sample 10V, in contrast, showed three different trophic levels (Table 5.13). *Tylenchus* sp. was the only taxon that was present in all three vendor soil samples. Within these few samples

the standard deviation (SD), as an indicator of how dispersed the data are, revealed that the taxon population sizes varied moderately.

Table 5.13 Nematode taxa present in the *vendor soil sample group*.

| Sample number | Nematode taxa* | Taxon population enumeration | SD | Trophic level |
|---------------|--------------------------------------|------------------------------|-------|---------------|
| 9V | <i>Scutellonema brachyurus</i> | 25 | 0 | Herbivores |
| | <i>Tylenchus</i> sp. | 25 | | Fungivores |
| 10V | <i>Acrobeles</i> sp. | 8 | 15.39 | Bacteriovores |
| | <i>Acrobelloides</i> sp. | 50 | | Bacteriovores |
| | <i>Aphelenchoides</i> sp. | 8 | | Fungivores |
| | <i>Ditylenchus</i> sp. | 8 | | Fungivores |
| | <i>Dorylaiminae</i> sp. | 17 | | Omnivores |
| | <i>Mesorhabditis</i> sp. | 8 | | Bacteriovores |
| | <i>Tylenchus</i> sp. | 13 | | Fungivores |
| 11V | <i>Criconemoides sphaerocephalus</i> | 25 | 12.02 | Herbivores |
| | <i>Tylenchus</i> sp. | 8 | | Fungivores |
| TOTAL | 9 | 195 | | 4 |

*Genera with undefined species and genera with undefined juveniles were categorized separately for the purposes of this study.

Topsoil sample group

Eight of the 20 nematode larvae-containing soil samples belonged to the *topsoil sample group*. Of the 24 different nematode taxa found in the *topsoil sample group*, none were found to be larvae pathogenic to humans. One of the samples contained no nematodes (13T); however the taxon *Tylenchus* sp. was present in half of the topsoil samples. Six

nematode taxa, namely *Cephalobus* sp., *Scutellonema unum*, *Criconemoides sphaerocephalus*, *Rotylenchulus parvus*, *Ditylenchus* sp. and *Dorylaiminae* sp. were recorded in two of the eight *topsoil samples*. The nematode taxon *Acrobeloides* sp. was present in three of the eight *topsoil samples* (Table 5.14).

Five of the eight topsoil samples contained nematodes that were representative of three different trophic levels, which included mainly bacteriovores, fungivores and herbivores. However, sample 16T revealed nematode taxa, which were classified under all five possible trophic levels including predators and omnivores (Table 5.14). The standard deviations (SDs) of these taxon population sizes varied moderately, similarly to that of the *vendor soil sample group*.

Table 5.14 Nematode taxa present in the *topsoil sample group*.

| Sample number | Nematode taxa* | Taxon population enumeration | SD | Trophic level |
|---------------|---|------------------------------|-------|---------------|
| 2T | <i>Cephalobus</i> sp. | 13 | 4.24 | Bacteriovores |
| | <i>Helicotylenchus indicus</i> | 19 | | Herbivores |
| 3T | <i>Eucephalobus</i> sp. (j) | 6 | 0 | Bacteriovores |
| | <i>Helicotylenchus</i> sp. (j) | 6 | | Herbivores |
| | <i>Pratylenchus</i> sp. (j) | 6 | | Herbivores |
| | <i>Psilenchus</i> sp. (j) | 6 | | Herbivores |
| | <i>Tylenchus</i> sp. | 6 | | Fungivores |
| 4T | <i>Acrobeloides</i> sp. | 11 | 12.13 | Bacteriovores |
| | <i>Geocenamus</i> sp. | 5 | | Herbivores |
| | <i>Scutellonema unum</i> | 5 | | Herbivores |
| | <i>Tylenchorhynchus brevilineatus</i> & <i>T. capitus</i> | 33 | | Herbivores |
| | <i>Tylenchus</i> sp. | 5 | | Fungivores |

| | | | | |
|--------------|--------------------------------------|------------|-------|----------------------------------|
| 5T | <i>Cephalobus</i> sp. | 16 | 17.74 | Bacteriovores |
| | <i>Criconemoides sphaerocephalus</i> | 48 | | Herbivores |
| | <i>Rotylenchulus</i> sp. (j) | 32 | | Herbivores |
| | <i>Tylenchus</i> sp. | 8 | | Fungivores |
| 7T | <i>Acrobeloides</i> sp. | 19 | 22.82 | Bacteriovores |
| | <i>Criconemoides sphaerocephalus</i> | 44 | | Herbivores |
| | <i>Rotylenchulus parvus</i> | 56 | | Herbivores |
| | <i>Tylenchus</i> sp. | 6 | | Fungivores |
| 13T | No nematodes observed | 0 | - | |
| 14T | <i>Acrobeloides</i> sp. | 50 | 16.80 | Bacteriovores |
| | <i>Acrobeles</i> sp. | 8 | | Bacteriovores |
| | <i>Mesorhabditis</i> sp. | 8 | | Bacteriovores |
| | <i>Aphelenchoides</i> sp. | 8 | | Fungivores |
| | <i>Ditylenchus</i> sp. | 8 | | Fungivores |
| | <i>Dorylaiminae</i> sp. | 17 | | Omnivores |
| 16T | <i>Alaimus</i> sp. | 6 | 14.81 | Bacteriovores |
| | <i>Ditylenchus</i> sp. | 50 | | Fungivores |
| | <i>Dorylaiminae</i> sp. | 6 | | Omnivores |
| | <i>Hemicriconemoides brachyurus</i> | 19 | | Herbivores |
| | <i>Mermithidae</i> sp. | 6 | | Predator (insect parasite) |
| | <i>Rotylenchulus parvus</i> | 25 | | Herbivores |
| | <i>Scutellonema unum</i> | 19 | | Herbivores |
| | <i>Thada</i> sp. | 6 | | Fungivores |
| | <i>Tylenchrohynchus</i> sp. (j) | 6 | | Herbivores |
| TOTAL | 24 | 592 | | 5 |

j = juvenile; classified only to genus level.

*Genera with undefined species and genera with undefined juveniles were categorized separately for the purposes of this study.

Excavated soil sample group

Six of the 20 nematode larvae-containing soil samples belonged to the *excavated soil sample group*. Of the 16 different nematode taxa found in the *excavated soil sample group*, none were found to be larvae pathogenic to humans. Interestingly, two of these excavated soil samples (1E and 3E) contained only a single taxon. Similarly to the *vendor and topsoil sample groups*, the nematode taxon *Tylenchus* sp. was identified in two different excavated soil samples (1E and 16E). A number of instances were identified where taxa occurred in more than one soil sample, for example, *Criconemoides sphaerocephalus* was present in 3E and 7E; *Scutellonema unum* in 7E and 9E; and *Scutellonema* sp. (j) present in 12E and 16E (Table 5.15).

Nematode larvae in this sample group also represented five trophic levels. Nematode taxa isolated from a third of these excavated samples were representative of only one trophic level, whilst another third of these excavated soil samples contained nematode taxa representative of two trophic levels. Samples 9E and 12E revealed three and four trophic levels respectively (Table 5.15). The standard deviations (SDs) of the taxon population sizes of this group varied considerably, in contrast to that of the *vendor and topsoil sample groups*.

Table 5.15 Nematode taxa present in the *excavated soil sample group*.

| Sample number | Nematode taxa* | Taxon population enumeration | SD | Trophic level |
|---------------|---|------------------------------|--------|----------------------------|
| 1E | <i>Tylenchus</i> sp. | 10 | - | Fungivores |
| 3E | <i>Criconemoides sphaerocephalus</i> | 6 | - | Herbivores |
| 7E | <i>Cephalobus</i> sp. | 8 | 38.41 | Bacteriovores |
| | <i>Criconemoides sphaerocephalus</i> | 75 | | Herbivores |
| | <i>Rotylenchulus parvus</i> | 92 | | Herbivores |
| | <i>Scutellonema unum</i> | 33 | | Herbivores |
| 9E | <i>Discolanium</i> sp. | 38 | 611.62 | Predator |
| | <i>Dorylaiminae</i> sp. | 6 | | Omnivores |
| | <i>Scutellonema unum</i> & <i>Helicotylenchus</i> sp. | 1081 | | Herbivores |
| 12E | <i>Cruznema</i> sp. | 10 | 2.00 | Bacteriovores |
| | <i>Ditylenchus</i> sp. | 5 | | Fungivores |
| | <i>Panagrolaimus</i> sp. | 5 | | Bacteriovores |
| | <i>Scutellonema</i> sp. (j) | 6 | | Herbivores |
| | <i>Steinemematidae</i> sp. | 5 | | Predator (insect parasite) |
| | <i>Tobrilus</i> sp. | 5 | | Predator |
| 16E | <i>Aphelenchus</i> sp. (j) | 16 | 85.45 | Fungivores |
| | <i>Helicotylenchus</i> sp. (j) | 63 | | Herbivores |
| | <i>Scutellonema</i> sp. (j) | 31 | | Herbivores |
| | <i>Tylenchus</i> sp. | 203 | | Fungivores |
| TOTAL | 16 | 1698 | | 5 |

j = juvenile; classified only to genus level.

*Genera with undefined species and genera with undefined juveniles were categorized separately for the purposes of this study.

Control soil sample group

Three of the 20 nematode larvae-containing soil samples belonged to the *control soil sample group*. Of the 14 different nematode taxa found in the *control soil sample group*, none were found to be larvae pathogenic to humans. Analysis of these soil samples revealed that sample 1C contained seven different nematode taxa of which only one nematode taxon, classified as *Tylenchus* sp., was also identified in another control soil sample, namely within sample 5C. However, the same three different nematode taxa were identified within samples 4C and 5C, namely *Aphelenchus* sp. (j), *Ditylenchus* sp. and *Scutellonema* sp. Generally, the nematode larvae contained within these three *control group soil samples* were classified as either herbivores or fungivores according to their trophic levels. Only sample 5C revealed a third trophic level not present in the other two soil samples, that of bacteriovores (Table 5.16). Similarly to the *excavated soil sample group*, the standard deviations (SDs) of the taxon population sizes of the *control soil sample group* also displayed considerable variation.

Table 5.16 Nematode taxa present in the *control soil sample group*.

| Sample number | Nematode taxa* | Taxon population enumeration | SD | Trophic level |
|---------------|--------------------------------------|------------------------------|--------|---------------|
| 1C | <i>Boleodorus</i> sp. | 9 | 15.96 | Herbivores |
| | <i>Criconemoides sphaerocephalus</i> | 54 | | Herbivores |
| | <i>Helicotylenchus</i> sp. (j) | 27 | | Herbivores |
| | <i>Pratylenchus</i> sp. (j) | 27 | | Herbivores |
| | <i>Psilenchus</i> sp. (j) | 36 | | Herbivores |
| | <i>Tylenchorhynchus</i> sp. (j) | 18 | | Herbivores |
| | <i>Tylenchus</i> sp. | 9 | | Fungivores |
| 4C | <i>Aphelenchoides</i> sp. | 17 | 165.21 | Fungivores |
| | <i>Aphelenchus</i> sp. (j) | 17 | | Fungivores |
| | <i>Ditylenchus</i> sp. | 25 | | Fungivores |
| | <i>Scutellonema unum</i> | 350 | | Herbivores |
| 5C | <i>Acrobeloides</i> sp. | 260 | 148.23 | Bacteriovores |
| | <i>Aphelenchoides</i> sp. | 417 | | Fungivores |
| | <i>Aphelenchus</i> sp. (j) | 83 | | Fungivores |
| | <i>Ditylenchus</i> sp. | 167 | | Fungivores |
| | <i>Scutellonema</i> sp. (j) | 10 | | Herbivores |
| | <i>Tylenchus</i> sp. | 83 | | Fungivores |
| TOTAL | 13 | 1609 | | 3 |

j = juvenile; classified only to genus level.

*Genera with undefined species and genera with undefined juveniles were categorized separately for the purposes of this study.

5.6.3 Comparison of soil sample groups

The 20 nematode larvae-containing soil samples demonstrated a moderate number of different nematode taxa, referred to as taxon richness in this study, present within all the different soil sample groups. The *topsoil sample group* contained the highest number of

taxa at approximately 40 %, and the *vendor soil sample group* the lowest number of taxa at approximately 15 % (Table 5.17). Population enumeration of the identified nematode taxa indicated the highest nematode numbers within the *excavated soil sample group*. The *vendor soil sample group*, in addition to containing the lowest number of taxa, also showed the lowest number of nematode populations. The two mined geophagic soil sample groups, namely the *excavated* and *topsoil sample group*, illustrated nematode taxa belonging to five trophic levels, namely herbivores, fungivores, bacteriovores, omnivores as well as predators. Generally, the trophic level most commonly reported was that of herbivores. Herbivores, bacteriovores and omnivores were more prevalent within the *topsoil sample group* than within the other soil sample groups. Fungivores were equally abundant within *control* and *topsoil sample groups* and bacteriovores and omnivores were generally rather few.

The population size of the smallest nematode populations in all the soil sample groups were very similar, ranging from five to nine, whereas the size of the largest nematode populations of the *excavated* and *control soil sample groups*, were greater than noted in the other two soil sample groups (Table 5.18). When the sample groups were compared in a pair wise fashion to test for differences among median values, all pairs excluding the *control soil sample group* did not differ significantly, however all pairs that included the *control soil sample group* did differ significantly: control and vendor $p = 0.0070$; control and topsoil $p = 0.0003$; and control and excavated $p = 0.0590$.

Table 5.17 Comparison between nematode taxon richness, population enumeration and trophic levels of different soil sample groups.

| | Vendor (%) | Topsoil (%) | Excavated (%) | Control (%) | Total | Mean |
|-------------------------------|------------|-------------|---------------|-------------|-------|--------|
| Taxon richness | 9 (14.5) | 24 (38.7) | 16 (25.8) | 13 (21.0) | 62 | 15.5 |
| Population enumeration | 195 (4.8) | 592 (14.5) | 1698 (41.5) | 1609 (39.3) | 4094 | 1023.5 |
| Trophic levels: | 4 (23.5) | 5 (29.4) | 5 (29.4) | 3 (17.6) | 17 | 4.3 |
| 1. Herbivores | 2 (6.1) | 15 (45.5) | 8 (24.2) | 8 (24.2) | 33 | 8.3 |
| 2. Fungivores | 5 (20.0) | 8 (32.0) | 4 (16.0) | 8 (32.0) | 25 | 6.3 |
| 3. Bacteriovores | 3 (18.8) | 9 (56.3) | 3 (18.8) | 1 (6.3) | 16 | 4 |
| 4. Omnivores | 1 (25.0) | 2 (50.0) | 1 (25.0) | 0 (0.0) | 4 | 1 |
| 5. Predators | 0 (0.0) | 1 (25.0) | 3 (75.0) | 0 (0.0) | 4 | 1 |

Table 5.18 Summary statistics of nematode larvae of all four soil sample groups.

| Soil sample group | Taxon richness (n)* | Number of populations (n)* | Median size of populations | Size of largest population | Size of smallest population |
|-------------------|---------------------|----------------------------|----------------------------|----------------------------|-----------------------------|
| Vendor | 9 | 11 | 13 | 50 | 8 |
| Topsoil | 24 | 35 | 8 | 56 | 5 |
| Excavated | 16 | 19 | 10 | 1081 | 5 |
| Control | 13 | 17 | 27 | 417 | 9 |

*Number of nematode populations is greater than number of taxa per soil sample group, as the same taxa may appear in different soil samples within each soil sample group.

Tylenchus sp., *Criconemoides sphaerocephalus* and *Ditylenchus* sp. were the only nematode taxa to be identified in all the different sample groups, with the taxon *Tylenchus* sp. notably more abundant overall.

Soil samples which revealed the presence of nematode larvae using the AMBIC analyses ($n = 20$), were further analyzed for nematode taxa classification and population enumeration. The colour of these 20 soil samples was compared to the number of nematode trophic levels identified within each soil sample group (Figure 5.10). Trophic levels ranged between three and five, with the topsoil and excavated soils containing five different trophic levels each. Only the *topsoil sample group* represented all three main colour groupings identified in this study, namely brownish, greyish and yellowish. Vendor soils analyzed for nematode taxa, were greyish in colour, whilst the control soils analyzed were brownish in colour.

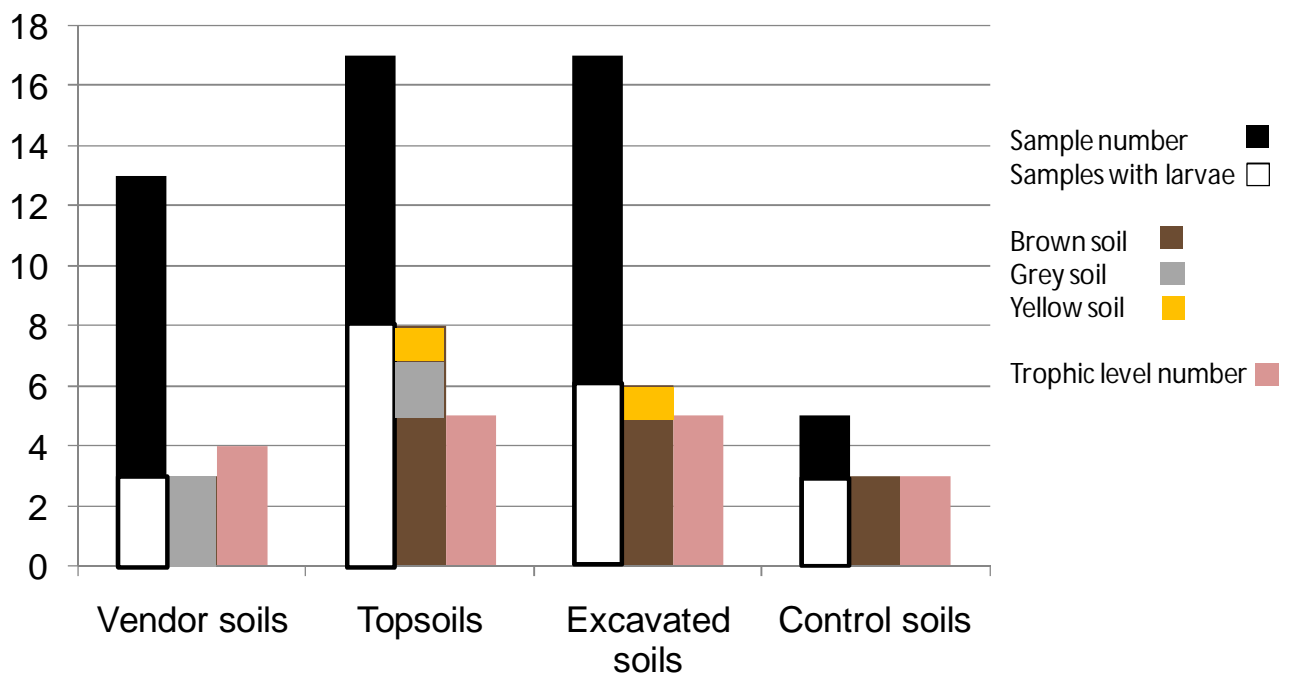


Figure 5.10 Summary of number of samples containing nematode larvae, soil colours and trophic levels.

Chapter 6

Discussion and Conclusion

6.1 Introduction

The phenomenon of human geophagia, the most common subgroup of pica, is evident in numerous communities globally, but remains more prevalent in countries with a poor socio-economic status, including many Sub-Saharan African countries (Abrahams, 1997; Geissler, *et al.*, 1998; Glickman, *et al.*, 1999; Luoba, *et al.*, 2004; Nchito, *et al.*, 2004; Young, *et al.*, 2007; Von Garnier, *et al.*, 2008; Kawai, *et al.*, 2009). Some South African communities are also known to practice geophagia (Saathoff, *et al.*, 2002; Ekosse, *et al.*, 2010; Ngole, *et al.*, 2010), including communities from the poorer rural settlements within the district of Thabo Mofutsanyane, Free State Province. This district was the subject of this investigation, in particular the towns of Clarens and several wards of Phuthaditjhaba.

This study of human geophagia in the Thabo Mofutsanyane district, revealed interesting aspects of vendor perceptions and that the geophagists of this district are apparently not at risk of pathogenic geohelminth infections as a result of their soil-eating habit.

6.2 General geophagic practices

Literature has shown that especially children and women exhibit geophagic behaviour (Vermeer and Frate, 1979; Hunter, 2003; Brand, *et al.*, 2009). This finding is further substantiated in some of the South African Thabo Mofutsanyane communities studied, where notably women of child-bearing age (aged 21-30 years) tend to consume geophagic clayey soil.

Geophagists quote diverse reasons for consuming geophagic soils, which may range from its medicinal value, to religious grounds, as well as hunger relief (Halsted, 1968; Knishinsky, 1998; Reilly and Henry, 2001). Geophagists from the investigated district of Thabo Mofutsanyane generally consume clayey soil because of its taste, sometimes specifically acknowledged as being sour, but also for medicinal purposes. The sourness of geophagic clayey soils results from their slightly acidic pH and is believed to alleviate nausea and ptyalism in geophagic pregnant women (Kikouama, *et al.*, 2009; Ngole, *et al.*, 2010; Young, *et al.*, 2010).

Different people of a community are usually involved in the practice of geophagia, often as a means of subsistence. The existence of traditional miners, vendors and geophagists within a single community has led to many business opportunities sprouting from geophagia. Several vendors at local markets in African countries (Uganda, Tanzania and Zambia) generate income through the sale of geophagic soils to geophagists (Abrahams, 1997; Hooda, *et al.*, 2002; Nchito, *et al.*, 2004). Similarly, the

shop merchandise of different Setsing-Phuthaditjhaba vendors, from the district of Thabo Mofutsanyane, includes pre-packed bags of geophagic clayey soil, weighing approximately 200 grams per bag. Daily, these vendors may sell ten to fifty of these bags. When the investigation was initiated the mean price of these bags was one rand per bag and vendors subsequently earned an average daily income of twenty rand from these sales alone. However, upon subsequent visits to the Setsing-Phuthaditjhaba market, vendors sold these bags at a mean price of two rand per bag, indicating price fluctuation. Setsing-Phuthaditjhaba vendors are notably married females with no tertiary education. Therefore, the sale of clayey soil, which forms an integral business component of their vendor shops, contributes to sustaining their livelihood.

Geophagic materials across the African continent are diverse in origin. Vendors from the district of Thabo Mofutsanyane usually mine geophagic clayey soil themselves from especially the mountain- and riversides in the wild, whereas geophagists from, for example, Kenya and Tanzania may select soils from termitaria (Geissler, *et al.*, 1998; Luoba, *et al.*, 2004, Young, *et al.*, 2010). Existing geophagic mining sites visited in the district of Thabo Mofutsanyane were mostly located within small embankments in close proximity to footpaths and/or public roads. These Thabo Mofutsanyane clayey soils are traditionally named *mobu* (sometimes also affectionately referred to as *sweets*, *dipompong* or *rama*) by local geophagists. In contrast, geophagic soils from other African countries are traditionally known by different names, including *odowa* (Kenya), *ufue*, *mchanga* or *udongo* (Tanzania) (Geissler, *et al.*, 1998; Luoba, *et al.*, 2004, Young, *et al.*, 2010).

In a number of studies, the soil colour preference demonstrated by geophagists when they select clayey soils for consumption, was recorded (Nchito, *et al.*, 2004; Ekosse, *et al.*, 2010; Ngole, *et al.*, 2010). Iron in the form of goethite and hematite is present in reddish and yellowish soils (Strydom, *et al.*, 2009; Young, *et al.*, 2010) and may contribute to dietary iron intake when consumed (Smith, *et al.*, 2000). Free State Province geophagists indicated a preference for white- and khaki-coloured clayey soils (Ekosse, *et al.*, 2010). This was further substantiated through the sale of mainly whitish geophagic clayey soils by the Setsing-Phuthaditjhaba market vendors interviewed in this study. The whitish colour seems to impart a desired palatability preferred by the majority of the geophagic customers. Interestingly, whitish clayey soils contain kaolin and smectite clay minerals, which harbour numerous gastro-intestinal benefits (Wilson, 2003; Dominy, *et al.*, 2004; Gomes, *et al.*, 2009; Bisi-Johnson, *et al.*, 2010; Young, *et al.*, 2010). However, after comparison to the *Munsell Soil Color Charts*, these whitish-perceived clayey soils sold by the Setsing-Phuthaditjhaba vendors, were classified as being mostly greyish in colour, whilst the colour of all the soil samples collected in this study ranged from greyish, to yellowish, to brownish. This is supported by earlier findings where South African geophagic soils were classified as yellowish to greyish in colour (Ngole, *et al.*, 2010). The Munsell classification of freshly mined topsoil and excavated soil from geophagic sites in the district of Thabo Mofutsanyane generally revealed a brownish colouration, which may be attributed to the fact that these soils have not undergone any processing or heat treatment.

Mining techniques and hygiene practices incorporated by geophagists when collecting geophagic clayey soil may vary. Often, very basic utensils are incorporated for soil collection and may include broken bottles, sharpened sticks and shovels (Ekosse, *et al.*, 2010). A metal plate, concrete shard, broken bottle neck and sticks were some of the picking utensils observed at a few of the geophagic mining sites studied in the district of Thabo Mofutsanyane. Generally, most of these mines were already excavated at an average depth of approximately 20 cm, which confirms the discriminating criteria (such as burrowing underneath the soil surface) adult geophagists apply when seeking clayey soil (Vermeer and Frate, 1979; Hunter, 2003; Young, *et al.*, 2007). Setsing-Phuthaditjhaba market vendors interviewed, maintained that detrimental environmental factors were considered when selecting appropriate mining sites, and that hygiene practices, such as using clean hands and mining utensils, were also applied during the mining of clayey soil. Contrary to this information, it was clear from this study that the immediate surroundings of many existing geophagic mining sites were contaminated with municipal waste and even cattle dung.

Geophagic clayey soil often undergoes some degree of processing prior to consumption, which may include pounding, grinding, slurring and various heat treatments (Ekosse, *et al.*, 2010). The interviews with the Setsing-Phuthaditjhaba vendors revealed that the majority of them processed geophagic clayey soil before selling it, through pounding and heat treatment, which involved oven baking and/or sun drying. Heat treatment of clayey soil is believed to enhance physical properties, including taste and colour and also reduce potentially pathogenic temperature-sensitive

micro-organisms present in these soils (Reilly and Henry, 2001; Hunter, 2003; Ekosse, *et al.*, 2010; Young, *et al.*, 2010). After processing, the Setsing-Phuthaditjhaba vendors stored their clayey soils in plastic containers for two to fourteen days before it was purchased by geophagic customers.

6.3 Biological content

For this study the biological content of geophagic clayey soils from the district of Thabo Mofutsanyane was limited to mainly nematode larvae. Fungi present in these soils were identified in a related study to this project as *Penicillium* sp., *Aspergillus flavus* and *fumigatus*, *Alternaria* sp., *Mucor* sp., *Paecilomyces* sp., *Trichophyton rubrum* and *Candida albicans* (unpublished Masters' dissertation data: Nellie Smit). Notably, no pathogenic parasitic nematodes, including geohelminths, which may be of potential risk to human health, were detected in these soils. Similarly, in a study conducted on geophagic pregnant women from Pemba, no geohelminths were detected in any of the geophagic materials analyzed (Young, *et al.*, 2007). In contrast, various studies have shown an association between geophagia and geohelminth infection, for example, pregnant women from Kenya and Tanzania were found to be at an increased risk for acquiring especially *A. lumbricoides* infection as a result of their soil-eating habits (Luoba, *et al.*, 2004; Luoba, *et al.*, 2005; Kawai, *et al.*, 2009). In related studies, a significant association between geophagia and mainly *A. lumbricoides*, but also *T. trichiura* infection, was demonstrated in geophagic school children from South Africa and Kenya (Geissler, *et al.*, 1998; Saathoff, *et al.*, 2002).

The AMBIC method used in this study specifically for geohelminth ova isolation, also provided information about other biological contents in the soil, such as fungal structures, mites and nematode larvae. However, it should be noted that very small quantities of soil are used in this method, which may lead to an underestimation of the extent of the biological content. Further studies that employ more sensitive technologies may provide additional and more comprehensive revelations on the parasitological content of geophagic soils.

Data generated in this study clearly revealed that the processing of geophagic clayey soil through sun exposure and/or oven baking diminishes the nematode content, as observed in the vendor soils when compared to the freshly mined soils. Although these nematodes were determined to be non-pathogenic to humans, it was evident that heat treatment may reduce microbial populations present in geophagic clayey soils, including those which are potentially pathogenic to humans (Hunter, 2003; Ekosse, *et al.*, 2010). Contrary to the nematode content, the fungal content in vendor geophagic clayey soils were notably higher in comparison to the other soils and may be attributed to the favourable storage environments of these soils in plastic containers/bags prior to being purchased.

The characterization of the nematode larval content of geophagic clayey soils from the Thabo Mofutsanyane District is the first known study of this kind to be conducted. The number of nematode taxa was extensive in many clayey soil samples with a total of 34 different taxa classified into five different trophic levels. These data are, however, mere

underestimates of the potential range of different nematode taxa in these geophagic samples due to the small sample population. Interestingly, from the diverse range of nematode taxa, only three taxa were present in all four sample groups, namely, *Tylenchus* sp., *Criconemoides sphaerocephalus* and *Ditylenchus* sp., of which only one taxon, namely *Tylenchus* sp., was the most abundant. Vendor soils contained the least number of nematode taxa as well as nematode population numbers, which again may be attributed to processing using heat treatment. In contrast, the freshly mined and unprocessed topsoil samples contained the highest number of nematode taxa and the excavated soil samples the highest nematode population numbers.

Overall, herbivores (plant parasitic nematodes) were the most prevalent trophic level in the geophagic clayey soils from the district of Thabo Mofutsanyane, with the highest incidence in topsoil samples from geophagic mines. This may be indicative of some degree of human intervention/disturbance of the soil, consequently altering the nematode communities present within those soils (Freckman and Ettema, 1993; Yeates and Bongers, 1999). The mining actions of geophagists may probably be regarded as a method of human intervention/disturbance causing the presence of a dominant trophic level.

Utilization of nematode communities as biological indicators of general soil health has long been practiced (Doran and Zeiss, 2000; Ferris, *et al.*, 2001; Neher, 2001). When soil is exposed to a disturbance, some biological communities may be sensitive to such a disturbance, and subsequently decrease in numbers, or may be insensitive and

subsequently increase in numbers (Neher, 2001). Soils polluted with for example heavy metals, such as cadmium, copper, chrome, lead, mercury and zinc, often results in plant death, decomposition and increased organic matter in the soil. This increased abundance of organic matter could result in the proliferation of nematode populations of all trophic levels in an ecosystem (Yeates and Bongers, 1999). Therefore, when considering the health impact of geophagia on the geophagists, the abundance of the various nematode functional groups in the geophagic soils may serve as an indicator of the possible contamination of these soils with environmental pollutants, such as heavy metals. In turn, heavy metal ingestion may lead to many sequelae in the geophagist, which also includes heavy metal poisoning and neurologic impairment (Geissler, *et al.*, 1998; Mielke, 1999; Abrahams, 2002; Hunter, 2003; Ellis and Schnoes, 2006; Ekosse, *et al.*, 2010).

The soil colours of the 20 nematode-yielding soil samples in this study were classified as brownish, greyish and yellowish. Interestingly, the freshly mined topsoils and excavated soils revealed the highest number of nematode trophic levels present, in conjunction with being predominantly brownish in colour. In contrast, the vendor soils contained fewer nematode trophic levels and were all classified as greyish in colour, which may be attributed to the degree of processing and heat treatment undergone by these vendor soils, subsequently altering the soil colour and nematode populations.

6.4 Conclusion

Scholars are showing a renewed interest in the phenomenon of human geophagia (Finkelman, *et al.*, 2005; Ekosse, *et al.*, 2010). More specifically, a need to identify potential health hazards which may arise from this habit has emerged (Young, *et al.*, 2007; Ekosse, *et al.*, 2010). Geophagists in the Thabo Mofutsanyane region of South Africa are probably not at risk for acquiring geohelminth infections through consumption of clayey soils. However, as this study consisted out of a small sample population and sample volume, a more extensive study on geophagic clayey soils from the same region will provide significant information regarding the geohelminth content thereof. In addition, the prevalence of geohelminth infections in geophagic humans from the district of Thabo Mofutsanyane may present further insights into the relationship between geophagia and the presence of geohelminths in geophagic soils.

Although this study focused on the nematode content of geophagic soils, geophagists may be exposed to various other potentially hazardous biological and non-biological soil contents. Some of these health hazards include heavy metals and also human pathogenic bacteria, viruses and other parasites which often originate from faecally contaminated water and soils. Consumption of these contaminated soils may impart serious health consequences on especially immune-compromised individuals in poor communities. Therefore, the assessment of the geophagic soil composition in combination with the free-living soil nematode communities, which serve as biological

indicators of soil health, may provide explicit knowledge into the risks that these soils potentially harbour.

References

Abrahams, P.W. 1997. Geophagy (soil consumption) and iron supplementation in Uganda. *Tropical Medicine and International Health*. 2(7): 617 – 623.

Abrahams, P.W. 2002. Soils: their implications to human health. *The Science of the Total Environment*. 291: 1 – 32.

Abrahams, P. W. 2005. Chapter 17: The involuntary and deliberate (geophagy) ingestion of soil by humans and other members of the animal kingdom. In: *Essentials of Medical Geology*. Editors: Selinus, O., Alloway, B., Centeno, J., Finkelman, R.B., Fuge, R., Lindh, U., Smedley, P. Elsevier Academic Press, Amsterdam.

Abrahams, P.W., Follansbee, M.H., Hunt, A., Smith, B. and Wragg, J. 2006. Iron nutrition and possible lead toxicity: An appraisal of geophagy undertaken by pregnant women of UK Asian communities. *Applied Geochemistry*. 21: 98 – 108.

Ash, L.R. and Orihel, T.C. 2007. *Atlas of Human Parasitology*. Fifth edition. American Society of Clinical Pathologists Press, Chicago, Illinois.

Bethony, J., Brooker, S., Albonic, M., Gelger, S.M., Loukas, A., Dlemert, D. and Hotez, P.J. 2006. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *The Lancet*. 367(9521): 1521 - 1532.

Bisi-Johnson, M.A., Obi, C.L. and Ekosse, G-I.E. 2010. Microbiological and health related perspectives of geophagia: An overview. *African Journal of Biotechnology*. 9(19): 5784 – 5791.

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

Broomfield, J. 2007. Pica. *Diseases and Conditions Encyclopedia: Discovery Health*. [Accessed 30 March 2009].

<http://health.discovery.com/encyclopedias/illnesses.html?article=1699>

Buckley, C.A., Foxon, K.M., Hawksworth, C.A., Pillay, S., Appleton, C., Smith, M. and Rodda, N. 2008. Prevalence and die-off of *Ascaris* Ova in Urine Diversion Waste. TT 356/08 to Water Research Commission. TT 356/08.

Callahan, G.N. 2003. Eating Dirt. *Emerging Infectious Diseases*. 9(8) [Accessed 31 March 2009]. *<http://www.cdc.gov/ncidod/EID/vol9no8/03-0033.htm>*

Clark, J.D. 1969. Kalambo Falls Prehistoric Site. Vol.1. Cambridge University Press, London.

Coyne, D.L., Nicol, J.M. and Claudius-Cole, B. 2007. *Practical plant nematology: a field and laboratory guide*. SP-IPM Secretariat, International Institute of Tropical Agriculture (IITA), Cotonou, Benin.

Decker, C.M. 1993 Pica in the mentally handicapped: a 15-year surgical perspective. *Canadian Journal of Surgery*. 36(6): 551 - 4.

De Silva, N.R., Brooker, S., Hotez, P.J., Montresor, A., Engels, D. and Saviol, L. 2003. Soil-transmitted helminth infections: updating the global picture. *Trends in Parasitology*. 19(12): 547 – 551.

Dominy, N.J., Davoust, E. and Minekus, M. 2004. Adaptive function of soil consumption: an *in vitro* study modeling the humanstomach and small intestine. *The Journal of Experimental Biology*. 207: 319 – 324.

Donkor, K.A-P. and Lundberg, S. 2009. *Trichuris trichiura*. eMedicine. [Accessed 12 May 2010]. <http://emedicine.medscape.com/article/788570-overview>

Dora-Laskey, A. and Ezenkwele, U.A. 2009. *Ascaris lumbricoides*. eMedicine. [Accessed 12 May 2010]. <http://emedicine.medscape.com/article/788398-overview>

Doran, J.W. and Zeiss, M.R. 2000. Soil health and sustainability: managing the biotic component of soil quality. *Applied Soil Ecology*. 15: 3 – 11.

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

Ellis, C.R. and Schnoes, C.J. 2006. Eating Disorder: Pica. *eMedicine*.

[Accessed 30 March 2009]. <http://www.emedicine.medscape.com/article/914765-overview>

Fallon, L.F. 2006. Pica. *Gale Encyclopedia of Children's Health: Infancy through Adolescence*. [Accessed 27 March 2009]. <http://www.encyclopedia.com>

Farrar, W.E. and Wood, M.J. 1992. *Atlas of Gastrointestinal and Hepatobiliary Infections*. First edition. Gower Medical Publishing, London.

Ferris, T., Bongers, T. and De Goede, R.G.M. 2001. A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. *Applied Soil Ecology*. 18: 13 – 29.

Finkelman, R.B., Centeno, J.A. and Selinus, O. 2005. The Emerging Medical and Geological Association. *Transactions of the American Clinical Climatological Association*. 116: 155 - 165.

Finkelman, R.B. 2006. Health Benefits of Geologic Materials and Geologic Processes. *International Journal of Environmental Research and Public Health*. 3(4): 338 – 342.

Freckman, D.W. and Ettema, C.H. 1993. Assessing nematode communities in agro-ecosystems of varying human intervention. *Agriculture, Ecosystems and Environment*. 45: 239 - 261.

Galt, F.L. 1872. Medical Notes on the Upper Amazon. *American Journal of the Medical Sciences*. 63(128): 395 – 416.

Geissler, P.W., Mwaniki, D., Thiong'o, F. and Friis, H. 1998. Geophagy as a risk factor for geohelminth infections: a longitudinal study of Kenyan primary schoolchildren. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 92 (1): 7 - 11.

Geissler, P.W. 2000. The significance of earth-eating: social and cultural aspects of geophagy among Luo children. *Africa: Journal of the International African Institute*. 70(4): 653-682.

Ghorbani, H. 2008. Environmental related disease. *International Meeting on Soil Fertility Land Management and Agroclimatology*. 957 – 967.

Glickman, L.T., Camara, A.O., Glickman, N.W. and McCabe, G.P. 1999. Nematode intestinal parasites of children in rural Guinea, Africa: prevalence and relationship to geophagia. *International Journal of Epidemiology*. 28: 169 – 174.

Gomes, C.S.F., Hernandez, R., Sequeira, M.C., and Silva, J.B.P. 2009. Characterization of clays used for medicinal purposes in the Archipelago of Cape Verde. *Geochimica Brasiliensis*. 23(3): 315 - 331.

Haburchak, D.R. 2008. Hookworms. *eMedicine*. [Accessed 17 May 2010].
<http://emedicine.medscape.com/article/218805-overview>

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

Haydel, S.E., Remenih, C.M. and Williams, L.B. 2008. Broad-spectrum in vitro antibacterial activities of clay minerals against antibiotic-susceptible and antibiotic-resistant bacterial pathogens. *Journal of Antimicrobial Chemotherapy*. 61: 353 - 361.

Heelan, J.S. and Ingersoll, F.W. 2002. *Essentials of Human Parasitology*. First Edition. Delmar, Thomas Learning Inc., Albany.

Hergüner, S., Hastanesi, B.R.S.H., Özyildirim, I. and Tanidir, C. 2008. Case report: Is Pica an eating disorder or an obsessive-compulsive spectrum disorder? *Progress in Neuro-Psychopharmacology & Biological Psychiatry*. 32(8): 2010 – 2011.

Holland, C.V. and Kennedy, M.W. 2002. *World Class Parasites: Volume 2 - The Geohelminths: Ascaris, Trichuris and Hookworm*. First Edition. Kluwer Academic Publishers, Boston.

Hooda, P.S., Henry, C.J.K., Seyoum, T.A., Armstrong, L.D.M. and Fowler, M.B. 2002. The potential impact of geophagia on the bioavailability of iron, zinc and calcium in human nutrition. *Environmental Geochemistry and Health*. 24: 305 – 319.

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

Hooda, P. and Henry, J. 2009. Geophagia and Human Nutrition. In: *Consuming the Inedible: Neglected Dimensions of Food Choice*. Editors: Macclancy, J., Henry, J. and Macbeth, H. Berghahn Books, New York.

Hotez, P.J. 2000. Pediatric geohelminth infections: Trichuriasis, ascariasis and hookworm infections. *Seminars in Paediatric Infectious Diseases*. 11(4): 236 - 244.

Hotez, P.J., Bundy, D.A.P., Beegle, K., Brooker, S., Drake, L., De Silva, N., Montessor, A., Engels, D., Jukes, M., Chitsulo, L., Chow, J., Laxminarayan, R., Michaud, C.m., Bethony, J., Correa-Oliveira, R., Shu-Hua, X., Fenwick, and Savioli, L. 2006. Chapter 24: Helminth Infections: Soil-transmitted Helminth Infections and Schistosomiasis. In: *Disease Control Priorities in Developing Countries*. Second edition. Editors: Jamison, D.T., Breman, J.G., Measham, A.R., Alleyne, G., Claeson, M., Evans, D.B., Jha, P., Mills, A. and Musgrove, P. World Bank Publications, Oxford.

Hunter, B.T. 2003. The Widespread Practice of Consuming Soil. *Consumer's Research Magazine*. [Accessed 31 March 2008].

<http://www.highbeam.com/doc/1G1-112542811.html>

Katz, J.M. 2008. Poor Haitians Resort to Eating Dirt. Associated Press. [Accessed 11 October 2010]. http://news.nationalgeographic.com/news/2008/01/080130-AP-haiti-eatin_2.html

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

Keyton, J. 2006. *Communication research: Asking questions, finding answers*. Second Edition. McGraw-Hill. [Accessed 11 February 2011] http://highered.mcgraw-hill.com/sites/0073049506/student_view0/glossary.html

Kikouama, J.R.O, Konan, K.L., Katty, A., Bonnet, J.P., Baldé, L. and Yagoubi, N. 2009. Physiochemical characterization of edible clays and release of trace elements. *Applied Clay Science*. 43: 135 – 141.

Kleynhans, K.P.N. 1997. *Collecting and preserving nematodes: A manual for a SAFRINET course in practical nematology*. ARC-Plant Protection Research Institute, Pretoria.

Knishinsky, R. 1998. *The Clay Cure*. First edition. Healing Arts Press, Rochester, Vermont.

Kuczynska, E. and Shelton, D.R. 1999. Method for Detection and Enumeration of *Cryptosporidium parvum* Oocysts in Feces, Manures, and Soils. *Applied and Environmental Microbiology*. 65(7): 2820 – 2826.

Lacey, E.P. 1990. Broadening the perspective of pica: literature review. *Public Health Report* . 105 (1): 29 – 35.

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

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

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

Maier, R.M., Pepper, I.L. and Gerba, C.P. 2000. *Environmental Microbiology*. First edition. Academic Press, San Diego, California.

McGreevy, P.D., Hawson, L.A., Habermanna, T.C. and Cattleb S.R. 2001. Geophagia in horses: a short note on 13 cases. *Applied Animal Behaviour Science*. 71: 119 – 125.

Mielke HW. 1999. Lead in the inner cities. *American Scientist*. 87: 62 – 73.

Munsell Color. 2000. *Munsell Soil Color Charts*. Washable edition. New Windsor, New York.

Murray, P.R., Rosenthal, K.S. and Pfaller, M.A. 2009. *Medical Microbiology*. Sixth edition. Mosby Elsevier, Philadelphia.

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

Neher, D.A. 2001. Role of Nematodes in Soil Health and Their Use as Indicators. *Journal of Nematology*. 33(4): 161 – 168.

New World Encyclopedia contributors. 2008. Nematode. New World Encyclopedia. [Accessed 18 October 2010].

<<http://www.newworldencyclopedia.org/entry/Nematode?oldid=830222>>

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

Ngozi, P.O. 2008. Pica practices of pregnant women in Nairobi, Kenya. *East African Medical Journal*. 85(2): 72 – 79.

Ozumba, U.C. and Ozumba, N. 2002. Patterns of helminth infection in the human gut at the University of Nigeria Teaching Hospital, Enugu, Nigeria. *Journal of Health Science*. 48(3): 263 – 268.

Peters, W and Gilles, H.M. 1989. *A Colour Atlas of Tropical Medicine and Parasitology*. Third edition. English Language Book Society, Wolfe Publishing, London.

Poinar, G.O., Jr. 1983. *The Natural History of Nematodes*. First edition. Prentice-Hall, Inc., Englewood Cliffs.

Prasad, A.S., Halsted, J.A. and Nadimi, M. 1961. Syndrome of iron deficiency anemia, hepatosplenomegaly, hypogonadism, dwarfism and geophagia. *The American Journal of Medicine*. 31(4): 532 – 546.

Reid, R.M. 1992. Cultural and medical perspectives on geophagia. *Medical Anthropology: Cross-Cultural Studies in Health and Illness*. 13(4): 337 – 351.

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

Reinbacher, W.R. 2003. *Healing earth: The third leg of medicine - A history of minerals in medicine*. First books library, Bloomington.

Saathoff, E., Olsen, A., Kvalsvig, J.D. and Geissler, P.W. 2002. Geophagy and its association with geohelminth infection in rural schoolchildren from northern KwaZulu-Natal, South Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 96(5): 485 - 90.

Salyers, A.A. and Whitt, D.D. 2005. *Revenge of the microbes – How bacterial resistance is undermining the antibiotic miracle*. First edition. ASM Press, Washington, DC.

Sandor, F. and Estrada, J. 2008. Soil Testing: Perennial Crop Support Series, Jalalabad Afghanistan. Roots of Peace. 2008-001-AFG.

Schistosomiasis Research Group. 2009. Nematoda. University of Cambridge.

[Accessed 18 October 2010].

http://www.path.cam.ac.uk/~schisto/helminth_taxonomy/taxonomy_nematoda.html

Shoff, W.H., Shepherd, S.M., Greenberg, M.E. 2008. Ascariasis. eMedicine.

[Accessed 12 May 2010] <http://emedicine.medscape.com/article/996482-overview>

Smith, B., Rawlins, B. G., Cordeiro, M. J. A. R, Hutchins, M. G., Tiberindwa, J. V., Sserunjogi, L. and Tomkins, A.M.. 2000. The bioaccessibility of essential and potentially toxic trace elements in tropical soils from Mukono District, Uganda. *Journal of the Geological Society*. 157(4): 885 – 891.

Srivastava, M.L. 2007. *Soil Science*. First edition. Shree Publishers & Distributors, New Delhi.

Strydom, H.A., King, N.D., Fuggle, R.F. and Rabie, M.A. 2009. *Environmental management in South Africa*. Second edition. Juta and Company Ltd., Cape Town.

Trivedi, T.H., Daga, G.L. and Yeolekar, M.E. 2005. Geophagia Leading to Hypokalemic Quadripareisis in A Postpartum Patient. *Journal of Association of Physicians of India*. 53: 205 – 207.

Van Bruggen, A.H.C. and Semenov, A.M. 2000. In search of biological indicators for soil health and disease suppression. *Applied Soil Ecology*. 15: 13 – 24.

Vermeer, D.E. and Frate, D.A. 1979. Geophagia in rural Mississippi: environmental and cultural contexts and nutritional implications. *The American Journal of Clinical Nutrition*. 32: 2129 – 2135.

Viglierchio, D.R. 1991. *The World of Nematodes*. First edition. University of California, Davis, California.

Von Humboldt, A. and Bonpland, A. 1853. *Personal narrative of travels to the equinoctial regions of America, during the years 1799-1804: Volume 3*. H.G. Bohn, London. [Accessed 13 October 2010]
http://books.google.co.za/books?hl=en&lr=&id=h88XAAAAYAAJ&oi=fnd&pg=PA1&dq=von+humboldt+1853&ots=y05Yhs_PL8&sig=G8av6Pz3sQ81jsQTUeCfWWRvXis#v=onepage&q=von%20humboldt%201853&f=false

Von Garnier, C., Stünitz, H., Decker, M., Battegay, E. and Zeller, A. 2008. Pica and refractory iron deficiency anaemia: a case report. *Journal of Medical Case Reports*. 2(324).

Watson, C.M. and Hickey, P.W. 2008. Paediatric Hookworm Infection. *eMedicine*. [Accessed 17 May 2010]. <http://emedicine.medscape.com/article/998401-overview>

WHO, Geneva. 2008. Soil-transmitted helminthiasis. *Weekly Epidemiological Record*. 83: 237 - 252.

Wilson, M.J. 2003. Clay mineralogical and related characteristics of geophagic materials. *Journal of Chemical Ecology*. 29(7): 1525 – 1547.

Winn, W., Allen, S., Janda, W., Koneman, E., Procop, G., Schreckenberger, P and Woods, G. 2006. *Koneman's Color Atlas and Textbook of Diagnostic Microbiology*. Sixth edition. Lippincott Williams and Wilkins, Philadelphia.

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

Yeates, G.W. 1998. Soil nematode assemblages: Regulators of ecosystem productivity. *Phytoparasitica*. 26(2): 97 -100.

Yeates, G.W. and Bongers, T. 1999. Nematode diversity in agroecosystems. *Agriculture, Ecosystems and Environment*. 74: 113 – 135.

Young, S.L., Goodman, D., Farag, T.H., Ali, S.M., Khatib, M.R., Khalfan, S.S., Tielsch, J.M. and Stoltzfus, R.J. 2007. Geophagia is not associated with *Trichuris* or hookworm transmission in Zanzibar, Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 101(8): 766 - 772.

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

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

Appendices

Appendix A: Recipes

Appendix B: Questionnaire: Human geophagia

Appendix C: Data collection sheets

Appendix A

Recipes

Ammonium bicarbonate (AMBIC) saturated solution:

11.9 grams ammonium bicarbonate (B&M Scientific) per 100 ml distilled water (dH₂O), pH adjusted to 8.6 at 22°C (Vario pH hand-held pH meter).

Zinc sulphate flotation solution:

161.5 grams zinc sulphate (B&M Scientific) per 100 ml dH₂O adjusted until the specific gravity (SG) reaches 1.4 (1 ml of solution weighed using an APX-402 measuring scale).

Lugol's iodine:

1 gram iodine and 2 grams potassium iodide per 100 ml deionised water.

Saturated sugar flotation solution:

450 grams of table sugar (Spar/PicknPay) per liter of water.

Appendix B

Questionnaire: Human Geophagia

QUESTIONNAIRE RELATED TO HUMAN GEOPHAGIA: VENDORS**INTRODUCTION**

The University of Limpopo, Limpopo Province and Central University of Technology, Bloemfontein, Free State Province, South Africa in collaboration with the Universities of Swaziland and Botswana are carrying out a study to characterize habits related to human and enzootic geophagia in South Africa, Botswana, and Swaziland. It is also designed to physico-chemically, microbiologically, mineralogically and ecologically characterize the soils that are preferred by geophagic individuals and animals in these three countries. This exercise is mainly for academic purposes. However, information provided may be generally used to improve methods of harvesting geophagic soils that will guarantee the health of geophagic individuals. Strict confidentiality of the information will be guaranteed at all times. Respondents are therefore requested cooperate with the interviewees in to facilitate this study.

COPYRIGHT@2007 BY G. EKOSSE et al. All rights reserved. No part of this document may be reproduced, stored in a retrieval system or transmitted in any form or by any means, electronic, mechanical, photocopying, recording, or otherwise, without the written permission of the copyright owner.

For Office use only

Number 1-4

Date of Interview: _____ (dd/mm/yy)

 5-10

Name of interviewee (optional): _____

 11-13Country:

| | | |
|-----|----------|-----------|
| RSA | Botswana | Swaziland |
|-----|----------|-----------|

 14Region:

| | | |
|------------|--|--|
| Free State | | |
| Limpopo | | |
| North West | | |
| Gauteng | | |

 15

District: _____

 16-17**A. DEMOGRAPHIC INFORMATION****1. Geographic Information**1. Location:

| | | |
|-------|----------|-------|
| Rural | Suburban | Urban |
|-------|----------|-------|

 18

2. Specify town or area: _____

 19-20**2. Personal and Demographic Information**3. Gender:

| | |
|------|--------|
| Male | Female |
|------|--------|

 21

4. Age: _____ (years)

 22-235. Ethnic Group:

| | |
|--------------------------|------------------------------|
| <input type="checkbox"/> | Afrikaans |
| <input type="checkbox"/> | English |
| <input type="checkbox"/> | Sesotho |
| <input type="checkbox"/> | Setswana |
| <input type="checkbox"/> | siSwati |
| <input type="checkbox"/> | isiXhosa |
| <input type="checkbox"/> | isiZulu |
| <input type="checkbox"/> | Sepedi |
| <input type="checkbox"/> | Other, please specify: _____ |

 24 25-26

| | | | | |
|--|-----|--|--|-------|
| 6. Marital Status: | | Married | | 27 |
| | | Divorced | | |
| | | Single | | |
| | | Living together | | |
| | | Cohabiting | | |
| 7. In addition to your shop, do you have ANOTHER source of income? | Yes | No | | 28 |
| 7.1 If YES , please specify other income source: | | | | |
| | | Wage employment | | 29 |
| | | Non wage employment | | |
| | | Other, please specify: _____ | | 30-31 |
| 8. Highest educational level reached: | | | | |
| | | No schooling | | 32 |
| | | Primary | | |
| | | Secondary | | |
| | | Tertiary | | |
| | | Literacy program | | |
| B. SOCIO-ECONOMIC AND CULTURAL ASPECTS | | | | |
| 1. HABITS | | | | |
| 9. Are you presently in the habit of eating soil/clay? | Yes | No | | 33 |
| 9.1 If YES , how often do you eat soil/clay? | | Once a month | | 34 |
| | | Once a week | | |
| | | Once a day | | |
| | | More than once a day | | |
| 9.2 If YES , for how long have you been eating soil/clay? _____ (years) | | | | 35-36 |
| 10. What is/are your reason(s) for eating soil/clay? | | | | |
| | | Standard practice (cultural, traditional, spiritual) | | 37 |
| | | Craving | | 38 |
| | | Medicinal value | | 39 |
| | | Supplement diet | | 40 |
| | | Ritualistic | | 41 |
| | | When hungry | | 42 |
| | | When pregnant | | 43 |
| | | To attract customers | | 44 |
| | | Encourage customers | | 45 |
| | | Taste on behalf of customers | | 46 |
| | | Don't know | | 47 |
| | | Other, please specify: _____ | | 48 |
| | | | | 49-50 |

| | | | | | | | | | | | | | | | | | | | | | | | | | | |
|--|---|-------|---|---|---|---|---|----------------------|---|---|---|---|---|--------|---|---|---|---|---|---------|---|---|---|---|---|----------------------|
| 11. How do other people (non customers and other passersby) perceive the SALE of soil/clay? | <input type="checkbox"/> Positive <input type="checkbox"/> Negative <input type="checkbox"/> Indifferent <input type="checkbox"/> Do not know | 51 | | | | | | | | | | | | | | | | | | | | | | | | |
| 12. How do other people (non customers and other passersby) perceive the CONSUMPTION of soil/clay? | <input type="checkbox"/> Positive <input type="checkbox"/> Negative <input type="checkbox"/> Indifferent <input type="checkbox"/> Do not know | 52 | | | | | | | | | | | | | | | | | | | | | | | | |
| 2. DEMOGRAPHIC, SOCIO-ECONOMIC and CULTURAL CHARACTERISTICS of CUSTOMERS | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 13. What is the age group of the majority of your customers for the consumed soil/clay you sell? | <input type="checkbox"/> Children under 20 <input type="checkbox"/> Adults 21 - 30 <input type="checkbox"/> Adults 31 – 40 <input type="checkbox"/> Adults 41 – 50 <input type="checkbox"/> Adults 51 - 60 <input type="checkbox"/> Elderly 60+ | 53 | | | | | | | | | | | | | | | | | | | | | | | | |
| 14. What is the gender of the majority of your customers that purchase soil/clay? | <input type="checkbox"/> Females <input type="checkbox"/> Males <input type="checkbox"/> Both | 54 | | | | | | | | | | | | | | | | | | | | | | | | |
| 15. Where do the majority of your customers that purchase soil/clay come from? | <input type="checkbox"/> Urban <input type="checkbox"/> Rural <input type="checkbox"/> Suburban | 55 | | | | | | | | | | | | | | | | | | | | | | | | |
| 16. What is your estimation of the economic status of the majority of your customers that purchase soil/clay? | <input type="checkbox"/> High income group <input type="checkbox"/> Middle income group <input type="checkbox"/> Low income group <input type="checkbox"/> All income groups | 56 | | | | | | | | | | | | | | | | | | | | | | | | |
| 17. What is the quantity of soil/clay that is usually bought by majority of your customers? | <table border="1"> <tr> <td>Daily</td> <td>1</td> <td>2</td> <td>3</td> <td>4</td> <td>5</td> </tr> <tr> <td>More than once a day</td> <td>1</td> <td>2</td> <td>3</td> <td>4</td> <td>5</td> </tr> <tr> <td>Weekly</td> <td>1</td> <td>2</td> <td>3</td> <td>4</td> <td>5</td> </tr> <tr> <td>Monthly</td> <td>1</td> <td>2</td> <td>3</td> <td>4</td> <td>5</td> </tr> </table> | 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 | 57 58 59 60 |
| 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 | | | | | | | | | | | | | | | | | | | | | |
| 18. What is the cost of the unit quantity? _____ (R/Pula) | | 61-62 | | | | | | | | | | | | | | | | | | | | | | | | |

| C. THE IMPORTANCE/SIZE OF BUSINESS | | | | | | | | | | | | |
|------------------------------------|---|---|------------------------------|---|---|---|--|--|--|-----|-------|-------|
| 19. | For how long have you been selling soil/clay? _____ years | | | | | | | | | | | 63-64 |
| 20. | On average, how much soil/clay do you sell? | | | | | | | | | | | |
| | Daily | 1 | 2 | 3 | 4 | 5 | | | | | 65 | |
| | Weekly | 1 | 2 | 3 | 4 | 5 | | | | | 66 | |
| | Monthly | 1 | 2 | 3 | 4 | 5 | | | | | 67 | |
| 21. | How much do you get from the sale of soil/clay in a day? _____ R/Pula | | | | | | | | | | | 68-69 |
| 22. | Do you sell other things beside the soil/clay at your shop? | | | | | | | | | Yes | No | 70 |
| 22.1 | If YES , can you list these other items sold together? | | | | | | | | | | | |
| | | | | | | | | | | | 71-74 | |
| | | | | | | | | | | | 75-78 | |
| | | | | | | | | | | | 79-82 | |
| 23. | Which of these other items are used as traditional medicine? | | | | | | | | | | | |
| | | | | | | | | | | | 1-4 | |
| | | | | | | | | | | | 5-8 | |
| | | | | | | | | | | | 9-12 | |
| 24. | What importance does soil/clay selling play in your business? | | | | | | | | | | | 13 |
| | | | Very important | | | | | | | | | |
| | | | Important | | | | | | | | | |
| | | | Not important | | | | | | | | | |
| D. INDIGENOUS KNOWLEDGE | | | | | | | | | | | | |
| 25. | What kind of soil/clay do you sell? | | | | | | | | | | | 14 |
| | | | Soil | | | | | | | | | 15 |
| | | | Clay | | | | | | | | | 16 |
| | | | Soil from termite mounds | | | | | | | | | 17 |
| | | | Other, please specify: _____ | | | | | | | | | 18-19 |
| 26. | How do you usually obtain the soil/clay sold? | | | | | | | | | | | |
| | | | Buy from supplier(s) | | | | | | | | | 20 |
| | | | Buy from other vendors | | | | | | | | | 21 |
| | | | From a termite mound | | | | | | | | | 22 |
| | | | From the wild | | | | | | | | | 23 |
| | | | Other, please specify: _____ | | | | | | | | | 24 |
| | | | | | | | | | | | | 25-26 |

| | | | |
|--|---------------------------------|------------------------------------|-----------------------------------|
| 27. What is the color (s) of the soil/clay bought from the suppliers? | | | |
| <input type="checkbox"/> | Reddish | | 27 |
| <input type="checkbox"/> | Blackish | | 28 |
| <input type="checkbox"/> | Khaki | | 29 |
| <input type="checkbox"/> | Brownish | | 30 |
| <input type="checkbox"/> | Yellowish | | 31 |
| <input type="checkbox"/> | Whitish | | 32 |
| <input type="checkbox"/> | Other, please specify: _____ | | 33 |
| | | <input type="text"/> | <input type="text"/> 34-35 |
| 28. Do customers prefer soil/clay with a particular color? | | <input type="button" value="Yes"/> | <input type="button" value="No"/> |
| | | | 36 |
| 28.1 If YES , what is the color most preferred? | | | |
| <input type="checkbox"/> | Reddish | | 37 |
| <input type="checkbox"/> | Blackish | | 38 |
| <input type="checkbox"/> | Khaki | | 39 |
| <input type="checkbox"/> | Brownish | | 40 |
| <input type="checkbox"/> | Yellowish | | 41 |
| <input type="checkbox"/> | Whitish | | 42 |
| <input type="checkbox"/> | Other, please specify: _____ | | 43 |
| | | <input type="text"/> | <input type="text"/> 44-45 |
| 28.2 Why do they prefer the indicated color? | | | |
| <input type="checkbox"/> | Taste | | 46 |
| <input type="checkbox"/> | Traditional belief | | 47 |
| <input type="checkbox"/> | Easily accessible | | 48 |
| <input type="checkbox"/> | Medicinal value | | 49 |
| <input type="checkbox"/> | Other, please specify: _____ | | 50 |
| | | <input type="text"/> | <input type="text"/> 51-52 |
| 29. For whom are your customers buying the soil/clay for? | | | |
| <input type="checkbox"/> | For their own consumption | | 53 |
| <input type="checkbox"/> | For other members of the family | | 54 |
| <input type="checkbox"/> | For reselling | | 55 |
| <input type="checkbox"/> | Don't know | | 56 |
| <input type="checkbox"/> | Other, please specify: _____ | | 57 |
| | | <input type="text"/> | <input type="text"/> 58-59 |
| 30. Do you give advice to your customers regarding the use of soil/clay? | | <input type="button" value="Yes"/> | <input type="button" value="No"/> |
| | | | 60 |
| 31. How do you administer treatment using the soil/clay? | | | |
| <input type="checkbox"/> | Swallowing | | 61 |
| <input type="checkbox"/> | Drinking | | 62 |
| <input type="checkbox"/> | Smearing | | 63 |
| <input type="checkbox"/> | Enema | | 64 |
| <input type="checkbox"/> | Smoking | | 65 |
| <input type="checkbox"/> | Sniffing | | 66 |
| <input type="checkbox"/> | Other, please specify: _____ | | 67 |
| | | <input type="text"/> | <input type="text"/> 68-69 |
| 32. What are the traditional names of the soil/clay used for treatment? | | | |
| <input type="text"/> | | | 70-71 |
| <input type="text"/> | | | 72-73 |

| | | | |
|---|--------|---------|-------|
| 33. What is the duration of the treatment? _____ days | | | 74-75 |
| 34. Where do you store the soil/clay? _____ | | | 76-77 |
| 35. For how long do you usually store the soil/clay? _____ days | | | 78-79 |
| 36. How are the customers expected to consume the soil/clay? | | | |
| <input type="checkbox"/> Wet | | 1 | |
| <input type="checkbox"/> Dry | | 2 | |
| <input type="checkbox"/> With other food | | 3 | |
| <input type="checkbox"/> With other medicines | | 4 | |
| <input type="checkbox"/> Other, please specify: _____ | | 5 | 6-7 |
| 37. Do you process the soil/clay in a special way before selling it to the customers? | | | |
| <input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Sometimes | | 8 | |
| 38. By whom is soil/clay processing done? | | | |
| <input type="checkbox"/> Vendor | | 9 | |
| <input type="checkbox"/> Customer | | 10 | |
| <input type="checkbox"/> Both | | 11 | |
| <input type="checkbox"/> Other, please specify: _____ | | 12 | 13-14 |
| 39. What method is used for processing? | | | |
| | Vendor | Cusomer | |
| Grinding | | | 15-16 |
| Pounding | | | 17-18 |
| Sieving | | | 19-20 |
| Slurring | | | 21-22 |
| Adding of substances | | | 23-24 |
| Other, please specify: _____ | | | 25-28 |
| 40. Is there any heat treatment of the soil/clay before selling it to the customers? | | | |
| <input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Sometimes <input type="checkbox"/> Doesn't matter | | 29 | |
| 40.1 If YES , which of the following heat treatment is used? | | | |
| <input type="checkbox"/> Baking | | 30 | |
| <input type="checkbox"/> Burning | | 31 | |
| <input type="checkbox"/> Boiling | | 32 | |
| <input type="checkbox"/> Sun exposure | | 33 | |
| <input type="checkbox"/> Other, please specify: _____ | | 34 | 35-36 |

