Metal content in soil and a wild leafy vegetable, *Bidens pilosa* L. on the Witwatersrand gold fields

By

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DECLARATION

I declare this dissertation is my own, unaided work. It is being submitted for the degree of Master of Science in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

(Signature of Candidate)

Signed at Johannesburg on 31 August 2017.

ABSTRACT

Metal pollution could result in health and economic risks to communities. Plant absorbs both essential and toxic elements from polluted soil and water. Consumption of contaminated vegetables and incidental ingestion of soils have been confirmed to be a pathway of contaminants for humans and livestock. Previous studies have established that some species which are utilized as wild leafy vegetables (WLVs) grow on polluted land in Witwatersrand goldfields, and people harvest these vegetables for consumption. An edible plant that is harvested and consumed is *Bidens pilosa (*black jack). Samples of soil and corresponding *B. pilosa* leaves were collected from a mine contaminated site on the Witwatersrand known as the Varkenslaagte, in Gauteng Province which is being monitored since the implementation of phytoremediation about 14 years ago. Metal concentrations were compared with those in soil and leaf material collected from different sites, viz., (i) the nearby Kraalkop nature reserve (n=2); (ii) a commercial/industrial site in Johannesburg's central business district (n=1) and (iii) a residential site in Johannesburg (n=1). Different soil particle size fractions and leaf material subjected to different treatments were analysed for iron (Fe), zinc (Zn), arsenic (As), and lead (Pb) concentrations. Concentration of As in unfractionated soils were highest in Kraalkop plot 2 (52.6 mg/kg). Johannesburg commercial area had the least As concentration (6.3 mg/kg). Zinc concentrations in soils were highest in Johannesburg commercial area (649.3 mg/kg) and lowest in Varkenslaagte (84.0 mg/kg). Kraalkop plot 1 had the highest Fe concentrations (61000 mg/kg) and Johannesburg residential suburb had the lowest Fe concentrations (31720 mg/kg). Lead concentrations in soils were highest in Johannesburg commercial area (185.9 mg/kg) and lowest in Varkenslaagte (32.6 mg/kg). While the mean concentrations of Pb, As and Zn in soil and *B. pilosa* in Varkenslaagte (n=8) was in some cases lower than concentrations in some of the comparison sites, the metal concentrations in some plots in Varkenslaagte were above the concentrations of the samples obtained from the comparison sites. This indicates that metal concentrations are still high in some areas of the Varkenslaagte despite remediation. There was no significant difference in distribution of metal concentrations between different soil particle size ranges. Different preparatory and cooking methods resulted in different concentrations of Fe and Pb. However, concentrations of As and Zn were not affected by different categories of *B. pilosa* treatment and cooking methods. Concentrations of Zn and Pb exceeded minimum allowable limit levels as stipulated in FAO/WHO, European Union (EU) and South African regulations relating to maximum levels of metals in leafy vegetables. A dietary risk index (DRI) indicated that there is no obvious risk to adult populations from *B. Pilosa* consumption, particularly as this species is not consumed in the same volumes as other, more popular, wild leafy vegetables. However, this preliminary study does not include DRI in children, dietary exposure through consumption of other foods besides *B. pilosa* and does not encompass dermal and inhalation exposure pathways which are important contributing factors to health risk indices.

Key words: Wild leafy vegetables, gold mining, pollution, contaminated land, dietary risk

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ACRONYMS

ADI	Acceptable Daily Intake
AMD	Acid Mine Drainage
ANOVA	Analysis of variance
ARD	Acid Rock Drainage
As	Arsenic
Ca	Calcium
Cd	Cadmium
Co	Cobalt
Cr	Chromium
CRG	Central Rand Group
CRM	Certified reference material
Cu	Copper
DEA	Department of Environmental Affairs
DMI	Daily metal intake
DWAF	Department of Water Affairs and Forestry
EC	Electrical conductivity
EEPP	Ecological Engineering & Phytotechnologies Programme
EU	European Union
FAO	Food and Agricultural Organisation
Fe	Iron
HRA	Health risk assessment
Hg	Mercury
ICP-MS	inductively coupled plasma mass spectrometry

ICP-OES	inductive coupled plasma optical emission spectrometry
IRIS	Integrated risk information system
K	Potassium
Mg	Magnesium
MPRDA	Mineral and Petroleum Resource Development Act
Mn	Manganese
NEMA	National Environmental Management Act
Ni	Nickel
Pb	Lead
ppb	parts per billion
PPE	personal protective equipment
ppm	parts per million
Ra	Radium
RDI	Recommended daily intake
RfD	Reference Dose
SA	South Africa
SD	Standard Deviation
Sr	Strontium
TDI	Total daily intake
TSFs	Tailing Storage Facilities
USEPA	United States Environmental Protection Agency
U	Uranium
USA	United State of America
WHO	World Health Organization

WLV	Wild leafy vegetables
WRG	West Rand Group
XRF	X-Ray Fluorescence
Zn	Zinc

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CHAPTER 1: INTRODUCTION

1.1 General overview

Metal pollution could result in health and economic risks to communities. Plant absorbs both essential and toxic elements from polluted soil and water. Plants are exposed to environmental contaminants through air, water and soil (Arora *et al*., 2008; Abhilash *et al*., 2009). Soil is however the major contaminants reservoir due to its constituents' ability to bind with metals (Dube *et al*., 2001). Contamination of soils causes great concern because soil is the medium from which vegetables extract numerous elements and compounds (Okonkwo *et al*., 2005). Consumption of contaminated vegetables and incidental ingestion of soils have been confirmed to be a pathway of contaminants for humans and livestock.

The Witwatersrand basin is subjected to different sources of metals and metalloids including mining, traffic and industry related sources of metals, which frequently contribute to water, soil and air pollution. Metals are elements with high electrical conductivity and they readily lose electrons in solution to form positive charged cations (Atkins and Jones, 1997). Metalloids are elements with characteristics that are intermediate between metals and non-metals (Crosby, 1998). They conduct electricity (under certain condition), which is a characteristic of metals but they are brittle typical of a non-metal. In most literature, metals and metalloids that are toxic and hazardous are referred to as heavy metals (Duffus, 2002). There are over forty definitions of heavy metals listed in the literature. Most definitions are based on physical and chemical properties such as high density and high relative atomic mass (Duffus, 2002).

According to Lawrence *et al*. (1998), heavy metal is a vague term which refers to any significantly toxic metal in any chemical form with a high relative atomic mass (e.g., lead, cadmium, mercury, arsenic). Heavy metals persist in the environment and can accumulate in plant and animal tissues. Because of the ambiguity in the definition of the term, 'heavy metal' will not be used in this research report. Both metals and metalloids will be referred to as metals in this study. Metals are non-biodegradable and have long half-lives (time it takes for the metal to lose half its physiologic activity) (Asrari, 2014)).

High deposition of metals in soils around mining areas has been reported extensively in literature. Elevated concentrations of Cd, cobalt (Co), Iron (Fe), Nickel (Ni), Zinc (Zn), Uranium (U), Copper (Cu), Manganese (Mn), Radium (Ra) and Pb in the Witwatersrand basin were reported by Marsden (1986). McCarthy (2011) reported the occurrence of high concentrations of Fe and other metals in the Witwatersrand goldfields. Sediments of the Witwatersrand basin have high concentrations of Mn, Fe, Calcium (Ca) and Cu (Tutu *et al*., 2008; Makgae, 2012). Accumulation of metals in naturally enriched soils or from mining and other anthropogenic activities is of great concern due to potential health hazards (Pruvot *et al*., 2006).

One of the major problems associated with high levels of metals in soil is their uptake by edible plants. Uptake can either be through selective metal uptake by the roots in response to concentration gradient or through aerial parts via the plant stomata. Of concern is the ability of some of the metals to bio-accumulate in ecosystems (Censi *et al*., 2006), bioconcentrate in food chain and accumulate in, and affect certain organs and tissues (Akinola *et al*., 2006). This could potentially result in health and livelihood risks especially to communities living in the proximity of the mine (Liu *et al*., 2005; Kachenko and Singh, 2006). Metals may have adverse health effects even in low concentrations (Ikeda *et al*., 2000) because they are non-biodegradable and persistent in nature (Duruibe *et al*., 2007).

Systemic health problems may develop due to excessive metal accumulation (Lacatusu *et al*., 1996). Metals such as lead (Pb) and cadmium (Cd) said to be prevalent in contaminated soils are frequently associated with a number of health effects (Lacatusu *et al*., 1996). Zinc and Pb are often among the metals of concern whenever metal contamination is discussed (Alloway, 1995).

According to Tripathi *et al.* (1997), the main route of exposure for most people is ingestion. Consumption of contaminated vegetables is an important pathway of contaminants into human systems (Sipter *et al*., 2008). Species which are utilized as wild leafy vegetables (WLVs) also grow on Witwatersrand gold mining polluted land (Weiersbye *et al*., 2006; Bubala, 2013) including the Varkenslaagte. The Varkenslaagte is mainly a grassland with hundreds of *Tamarix species* and other species which were planted for the purpose of phytoremediation. There is a large wetland with reedbeds covering a portion of this area. The Varkenslaagte is alongside a gold mine tailings dam and a stream on AngloGold Ashanti's West Wits mine. This site is impacted by windblown dust from nearby tailings dams and it directly receives eroded tailings and acid mine drainage (AMD). Acid mine drainage or Acid Rock Drainage (ARD) is the acidic water which is formed when oxygenated water comes in contact with pyrite mineral (McCarthy, 2011). Pyrite is a minor constituent in mineral deposits and is common in Witwatersrand Basin gold deposits.

People were observed harvesting WLVs in mine-affected areas (Botha & Weiersbye 2010). *Bidens pilosa* (study species) is one of the species reported to be utilised for food in the Witwatersrand goldfields even though it is not as popular as *Amaranthus, Solanum* and *Chenopodium species.* There were inadequate volumes of these species at the study site during the sampling period (March 2016) therefore *B. pilosa* was chosen as the study species.

Wild leafy vegetables are still a crucial component of diet especially for people living in impoverished circumstances. They are rich in protein, vitamins, minerals and essential amino acids and they supplement starch-based staple foods (Afolayan and Jimoh, 2009, Uusiku *et al*., 2010). They are the main source of nutrients in most developing countries, contributing significantly to household food security (Van Rensburg *et al*., 2007; Uusiku *et al*., 2010) especially for the low-income communities where food choice is influenced by price. They remain a low-cost source of nutrients in the diet of many low income households (Grivetti and Ogle, 2000; Lyimo *et al*., 2003) compared to animal derived foods. However, consumption of WLV is not only limited to low income earners. Some people enjoy WLV as a traditional dish alongside meat and starchy foods, usually maize porridge commonly known as pap in South Africa.

Wild leafy vegetables can be hazardous if they are contaminated. However, the potential health hazard is not only determined by elevated levels of metals in raw vegetables. Cooking methods, serving portion, consumption frequency, bioaccessibility (fraction of a substance that is released in the gastrointestinal tract which is available for intestinal absorption) and bioavailability (amount of a substance that can reach systemic circulation) (Oomen *et al*., 2002) are important factors in determining the potential health risk.

Cooking can change the elemental properties of food (Fabbri & Crosby, 2016). Cooking processes resulted in decrease in As levels in boiled string beans and potatoes, although they result in increase in As, Cd and Pb concentrations in rice and olive oil (Perello *et al*., 2008). If fruits and vegetables are not washed properly, fine dust particles on the surface of vegetables may contribute to contaminants load. Most people who consume WLV live with limited access to water resources. It is possible that vegetables may not be adequately washed or will be washed in contaminated water.

Dust particles do not only adhere to vegetables, but also to skin and hands. Risk assessors interested in determining the potential risks from exposure to contaminated soils usually sieve soils to less than 250 µm based on recommendations for Pb contaminated sites

(USEPA, 2000). Recent papers emphasize that particle sizes that adhere to hands are much smaller than 250µm. Several trials by Choate *et al.* (2006) have shown that most particle sizes that adhere to hands are less than 63µm despite the particle size of the bulk soil.

Levels of contamination in soils can be influenced by particle size distribution. Coarse particles have a small surface area for interaction compared to finer particles (<100µm). Small particle sizes have a large surface area to mass ratio and are more reactive (Spalt *et al.* (2009). Studies by Bright *et al.* (2006); Spalt *et al.* (2009); Yu and Li (2011) have shown that there is a difference in metal concentration distribution across different soil particle size range.

People who work on contaminated sites are likely to transfer dust particles that stick to their hands to their food. Some contaminated sites are far from clean water sources, and it is possible that hands will not be adequately washed or in fact not washed at all. Dust may also adhere to food when site workers are eating since contaminated sites may be far from designated eating areas (if any).

The probability of the exposed individual to suffer the adverse effect from exposure to a contaminant is determined during risk assessment exercise. This probability helps the risk assessors to know whether there is any potential for the exposed population or individual to suffer any adverse effects from the exposure. Risk is the probability of harm occurring due to a particular hazard under given exposure conditions (Duffus, 2002). Risk assessment is a procedure used to attempt to estimate risk. It involves identifying and quantifying the risk resulting from exposure to a chemical or physical substance (hazard). It takes account of all possible routes of exposure to a given hazard (Duffus, 2002).

1.2 Problem statement

The negative legacy of mining such as AMD and TSFs resulting in elevated concentrations of metal contaminants in soils and water is a major cause of concern (Oelofse *et al*., 2007). In the Witwatersrand goldfields, mines (abandoned and operational) and TSFs intersperse human settlements (towns, informal settlements etc.). Harvesting of wild leafy vegetables from mining footprints may expose humans utilizing the vegetables to metal pollutants (Kneen *et al*., 2015). This study aims to determine the concentration of metals in rhizosphere soils and *Bidens pilosa* occurring on the Varkenslaagte, a highly contaminated mining site in Gauteng Province, in order to evaluate potential risks to human health through oral ingestion. Metal concentrations in soils and *B. pilosa* leaves were compared with those in samples collected from a nearby nature reserve (Kraalkop), which is known to be less contaminated than the Varkenslaagte (pers. comm. I Weiersbye, 2015), as well as a commercial/industrial and a residential site in Johannesburg.

1.3 Key questions

This research study sought to answer the following questions:

- Elements such as Pb are known to be relatively inert in soils. However, even small quantities may be a health hazard. Do concentrations in the leaves of B. pilosa fall within regulatory guidelines?
- Do different condiments added during food preparation affect elemental contents?
- Does the metal concentration of soils in the study area increase with decreasing soil particle sizes?

1.4 Objectives

1. To determine metal concentrations in the soils and leaves of *B. pilosa* from the Varkenslaagte and compare trends in concentrations with samples collected from Kraalkop nature reserve and two Johannesburg sites.
2. To determine the distribution of metals in different soil particle size fractions
3. To compare metal concentrations in soils and *B. pilosa* with international and national maximum permissible standards for metals in soils and leafy vegetables.
4. To determine if there is any difference in metal concentrations between unwashed, washed, boiled and *B. pilosa* cooked in tomato and onion.
5. To determine the dietary risk index of consuming cooked *B. pilosa* leaves.

1.5 Report structure

This report is made up of 5 chapters. Chapter 1 introduces the study and provides the rationale, key questions, study aim and objectives. Chapter 2 gives an overview of the history of gold mining in South Africa, introduces the study species and its significance as food, and further reviews similar studies and their findings. Chapter 3 describes the study site location, vegetation and climate. Experimental design, data collection methodology and analysis procedures are provided. Chapter 4 presents the findings of the present study by reporting on metal concentrations in different sites, different soil particle sizes, vegetables exposed to different preparation and cooking methods. Discussion on the findings of this study was also covered in in this chapter. Finally, chapter 5 then draws conclusions and recommendations based on study findings.

CHAPTER 2 LITERATURE REVIEW

This chapter gives an overview of mining impacts in South Africa, provides a description of toxicity and benefits of metals within the scope of the present study, describes the benefits of WLV and B. pilosa and dietary risk assessments.

2.1 Impact of gold mining

Mining has produced a legacy of polluted land. Segmentation of rocks to finer particles during mining creates a large surface area for metals to interact with the environment. This often leads to increased levels of metals such as Pb, As, Cd, Zn, U, Co, chromium (Cr), Mn, Cu, Nickel (Ni) and Vanadium (V) in the environment (Rosner and Van Schalkwyk, 2000) Gold mining in South Africa brought extensive socio-economic development for some. Benefits of the mining boom were, however, accompanied by a host of environmental problems such as AMD. Complex health problems (e.g. silicosis and tuberculosis) became prevalent among mineworkers and communities living in the proximity of mining areas.

2.1.1 Gold mining legacy and legislation in South Africa (SA)

The world’s largest known gold deposit is the Witwatersrand basin (Winde and Stoch, 2010). The 1886 gold discovery in Langlaagte farm by George Harrison provided the onset of development of the City of Johannesburg. The gold mining industry expanded to the East Rand, West Rand, Welkom and Klerksdorp goldfields (Reichardt, 2012). Gold become the biggest part of the South African economy and although waning in importance still forms a cornerstone of the economy of SA (McCarthy, 2011). Land contamination in South Africa due to mining activities dates back over 130 years.

Contamination of soils and water in the Witwatersrand Basin goldfields has occurred due to gold and uranium mining activities (Naicker *et al*., 2003). The Chamber of Mines of

South Africa (2004) estimated that approximately 200 000 tonnes of tailings are produced for every ton of gold produced. Approximately 221 million tonnes of gold mining related waste are produced in South Africa per annum (DWAF, 2001). There are over 6 billion tonnes of tailings in Witwatersrand alone (Chapman, 2011; Pratt, 2011, Winde 2009) and tailings dumps are still prominent near Johannesburg (Monna *et al*., 2006). The gold mining footprint is much greater than just tailing deposits. The Witwatersrand goldfields' soils and water remain heavily impacted even long after abandonment of mining activities (Naicker *et al*., 2003). Impacts stem from continuous discharge of AMD from TSFs, as well as dust from abandoned and existing mines (Oelofse, 2008).

Metal contaminants released into the environment during mining activities may result in serious environmental damage and threaten the health and safety of communities living in the proximity of mining areas. Metals remain in the environment long after mining activities have ceased if effective remediation techniques are not implemented (CSIR, 2009). Before the Minerals Act (No 50 of 1991) and National Environmental Management Act (NEMA) (No 107 of 1998) were put into effect, the "polluter pays principle" did not apply and mining companies were seldom held liable for the impacts from mining activities (Stacey *et al*., 2010). Mining companies had scant or no regard for the environment, as they deplete the ore then liquidate or leave the country, thus abandoning the mines in an unrehabilitated state (Swart, 2003). The government and the communities were to inherit the legacy of adverse land degradation which resulted in environmental, social, economic, and health problems.

There has been improvement in the South African legal framework governing mining practices, mine closure and land rehabilitation. The objective of the Mineral and Petroleum Resources Development Act (MPRDA) (Act 28 of 2002) is to ensure that the state fulfils its responsibilities protecting and benefiting communities from the mineral industry. These responsibilities include the regulation of land rehabilitation and handling of AMD and TSFs for operational and closed mines (Van der Schyff, 2012). AMD is one of the major ecological and socio-economic risks associated with mining activities. Environmental pollution due to AMD affects many countries with current or historic mining activities (Johnson and Hallberg, 2005). The characteristics of AMD are low pH, high electrical conductivity and high sulphide mineral concentrations (Oelofse, 2008; Galvan *et al*., 2009). The pH largely affects the physical or chemical form of an element. It therefore influences bioavailability and toxicity of elements (Ritter *et al*., 2002).

Tailing Storage Facilities in the Witwatersrand basin contain mineral pyrite (Fadiran *et al.*, 2014). When the mineral pyrite comes into contact with oxygenated water, AMD is produced. Flow of AMD into streams can negatively impact on water quality and aquatic organisms (Fadiran *et al*., 2014).

In terms of the current legislative framework, mining companies are liable for the impacts caused to the environment during exploring, mining and processing of minerals. They therefore have a legal obligation to rehabilitate the impacted land. The MPRDA requires that the mining right holder put aside a budget for rehabilitation during its operational phase. A mine closure certificate can only be issued upon adequate environmental rehabilitation and upon producing relevant documents such as an environmental risk report and closure plan (Krause and Snyman, 2014).

Mining companies are to implement measures above minimum requirements to protect communities and the environment from their operations (Bitala *et al*., 2009). Some mining companies have developed sustainability strategies for their operational and post closure phases (e.g. AGA, 2009). In an effort to remediate soil and ground water contamination from mine wastes, some mining companies are implementing diverse methods including phytotechnologies to manage seepage, dust from TSF and elements assimilation by plants.

Phytotechnology is the use of plants, algae, microorganism or non-living biomass to improve the quality of soil, sediments, surface and underground water (Glick, 2003). A study conducted in the western part of the Witwatersrand basin to assess whether *Rhus lancea* and *Tamarix usneoides* can remediate AMD demonstrated that use of these tree species resulted in a 7% increase in pH, an increase in conductivity by 88% and 18% decrease in redox potential. It was noted that *Rhus lancea* and *Tamarix usneoides* species are capable of improving the physical and chemical characteristics of mine soils (Arendze, 2015).

The AGA (2013) sustainability report states that a remediation programme was introduced for the Varkenslaagte clean-up (AGA, 2013). Indigenous trees and Eucalyptus species were planted to control AMD seepage from TSFs. The remediation programme aimed to ensure compliance with statutory requirements for discharge water (AGA, 2013), so that metal concentrations in discharge water would fall within guideline values.

Guideline values are derived from laboratory tests/ experiments and are established based on total metal concentrations in soils. Total metal concentration data are not sufficient to provide accurate risk prediction as metal toxicity is related to metal species, electric conductivity etc. (Chapman *et al*., 2003). Guideline values for metals in soils or food are prepared to provide guidance for general population protection. The guidelines may however not protect all population subsets, for example babies, pregnant women or people with certain disorders may not be protected by some guidelines (Duffus, 2002). Table 1 presents the maximum permissible limit of metals in soils as established by standard regulatory bodies. Variation in permissible limits between countries or organisations may be due to differing strategies for the setting of limits or may result from variations in baseline environments including soil type and characteristics (Chapman *et al*., 2003).

Table 1: Maximum allowable limits of selected metal concentrations in soils (mg/kg)

Element/ Standard	As	Zn	Pb	Fe
*FAO/WHO Guidelines, (2001)	20	300	100	50000
USEPA-IRIS (2005)	75		420	
**South Africa WRC (1997)	2	46.5	6.6	
EU Guidelines (2006)		300	100	

**Limit metal concentrations for agricultural applications
*Chiroma *et al.* (2014)

2.1.2 Soil pH and metal toxicity

pH

Soil pH strongly influences metal distribution and toxicity. pH measures the hydrogen ion (H^+) concentration, with pH ranging from 0 to 14 based on a logarithmic scale. A pH of 7 indicates a neutral solution, while a pH value less than 7 indicates that solution is acidic. The greater the acidity, the lower the pH value. A pH value above 7, on the other hand, indicates that a solution is alkaline; alkalinity increases with increase in pH value. A low pH results in metal dissolution from rocks and sediments (Saria *et al*., 2006) and increases metal mobility (WRC, 2014a). Some metals of concern in soil include As and Pb. Iron and and Zn are essential elements, however, they can cause adverse effects in high concentrations.

Zinc

Concentrations of Zn in unpolluted soils globally is expected to range from 10 mg/kg to 300 mg/kg with 40 mg/kg as the mean (Adriano, 2001). Most organisms bioaccumulate Zn. Both low and high Zn levels have adverse effects in organisms (Eisler, 1993). Low pH promotes Zn uptake by plants whereas high clay content, high cation exchange capacity and high phosphate levels in soils restrict Zn uptake (Bodek *et al*., 1988).

Lead

Lead from a wide range of industrial sources, car exhausts and dust can contaminate soils and plants. Lead does not move readily in soil, and it tends to concentrate on surface layers. Thus shallow-rooted plants are more likely to be contaminated by Pb. The mobile and toxic Pb^{2+} is also the most stable oxidation state under many conditions. In the soil, Pb may react with anions to form less soluble salts (Cao *et al*., 2009). Low bioavailability and toxicity of lead is related to high pH (McBride *et al*., 1997), inversely high bioaccessibility and toxicity is related to low pH. Levels of Pb in unpolluted soils are expected to be below 1 mg/kg (Alloway, 1995).

Arsenic

Arsenic exists in +5, +3, 0 and -3 oxidation states (Mohan and Pitman, 2007) and in a range of chemical forms in sediments (Hasegawa *et al*., 2009). Arsenic is non-essential and is generally toxic to plants. It usually occurs as a component of S-containing ores as a metal arsenide (ATSDR, 2003a). Arsenic is often associated with geological sources; however human activities such as fossil fuel combustion, insecticides and mining have increased As levels in soils and ground water (Bhattacharya *et al*., 2007; Sawyer *et al*., 2003).

Iron

Under typical environmental conditions, Fe can occur in its divalent state as ferrous or Fe^{2+} or in its trivalent state as ferric or Fe^{3+}. It predominantly occurs in the form of less soluble and unavailable Fe^{3+} oxide in soil. The state in which Fe occurs is determined by pH and/ or electrical conductivity (EC) conditions. Availability of other chemicals such as sulphur determines Fe compounds formed (Bodek *et al*., 1988). Soil alkalinity and oxidizing conditions aid Fe^{3+} precipitation. Fe^{2+} compound solution is encouraged in acidic and reducing conditions (Bodek *et al*., 1988).

2.2 Wild leafy vegetables habitats and preparatory methods

Wild leafy vegetables grow in public places such as wetlands, roadsides, veld, riparian zones, backyards, domestic dumps and artisanal mining sites (Botha, 2013; Bubala, 2013). These plants may pose risks to human health when consumed (Turkdogan *et al*., 2003). Despite a decline in consumption in recent years during changing cultural norms, they are still used as food (Van Rensberg *et al*., 2007). Some species bio-accumulates and store metals in the edible parts of the plants (Botha and Weiersbye, 2015). International and South African regulatory bodies have set the guidelines for protection of populations from metals adverse effects. The maximum permissible limits of metals in leafy vegetables are given in Table 2 below.

Table 2: Guideline values for metals in leafy vegetables; (mg/kg)

Metal	Zn	As	Pb	Fe
*FAO/WHO Guidelines (2001)	100	0.1**	0.3	425
EU guidelines (2006)	100	-	0.3	
Department of Health (2004) (SA)	-	-	0.3	

*Chiroma *et al*., (2014)

** 0.1 mg/kg is As guideline for fats and is used extensively in literature as guideline for leafy vegetables. For the purpose of this report, 0.1 mg/kg will be adopted as guideline value for As in leafy vegetables

The consumption pattern in South Africa varies depending on socio-economic factors, rural-urban migration and fresh produce availability (Van Rensberg *et al*., 2007). It is reported that WLVs are consumed by 95% of the population in Kanana, located in the North-West Province of South Africa (Bubala, 2013) less than 100km from the Varkenslaagte where the present study was conducted. Tomato and onion are often used to enhance flavour when cooking WLV dishes in this region (Bubala, 2013).

In most WLV dish preparations, young tender leaves are used. In certain dishes, for example in the preparation of pumpkin leaves (*Cucurbita pepeo* and *C. maxima)* and spider flower (*Cleome gynandra),* young stem, fruits and flowers may be utilised. Dishes can be prepared from a single species or by combining two or more species for a desired taste. Leafy vegetables are either boiled, steamed or fried and at times mixed with condiments such as tomatoes, onions, peanut butter for enhanced flavour (Van Rensberg *et al*., 2007).

2.2.1 Potential benefits of wild leafy vegetables

Wild leafy vegetables are rich in protein, vitamins, minerals and essential amino acids and they supplement starch-based staple foods (Okafor, 1981). Leafy vegetables help remove free radicals and inhibit oxidative degradation of lipids in the body (Gupta and Bains, 2006). They are the main source of energy and protein in most developing countries (Ladeji *et al*., 1995). Wild leafy vegetables also contribute significantly to household food security (Van Rensburg *et al*., 2007) especially the low-income communities where food choice is influenced by price. They remain a low-cost source of nutrients in the diet of many people (Grivetti and Ogle, 2000; Lyimo *et al*., 2003) compared to animal derived foods. Low-income sectors of communities faced with inadequate access to food and limited choices depend on WLVs for ensuring healthy nutrient intake (Dovie *et al*., 2002). However, consumption of WLV is not only limited to low income earners. Some people enjoy WLV as a traditional dish alongside meat and starchy foods, usually maize porridge commonly known as pap in South Africa.

Leafy vegetables contain essential nutrients that can be used as an energy source, for building and repair of body tissues and for contributing to regulatory and protective systems. They maintain the alkaline reserve of the body and buffer acidic substances produced during food digestion (Fayemi, 1999). Leafy vegetables are high in carbohydrates, vitamins and mineral content, low in calories and minute quantities of utilizable energy (Robinson, 1990). Several studies have indicated that WLVs have as high or even higher micronutrients levels in comparison to cultivated leafy vegetables (Odhav *et al*., 2007; Steyn *et al*., 2001; Weinberger and Msuya, 2004). Table 3 gives nutritional values of different leafy vegetables.

Table 3: Nutritional value of wild and cultivated leafy vegetables *(Uusiku et al., 2010)*

Species	Energy kJ (kcal)	Moisture (g)	Protein (g)	Fibre (g)	Fat (g)	Carbohydrates (g)
Solanum spp. *(*Black nightshade*)*	228–241 (55–58)[b,d,g]	83–90[b,d,g]	3–5[b,d,g]	1**g , 2–6§[b,d]	0.6[b]	2z [d] , 9y [b]
Bidens pilosa (Black jack)	163–222 (39–53)[a,b,d]	85–88[a,b,d]	3–5[a,b,d]	3–6§[a,b,d]	0.4–0.6[a,b,d]	2z[d], 8y[a,b]
Chenopodium album *Lamb's quarters*	212–247 (44–59)[b,d]	83–85[b,d]	4–5[b,d]	2§[b,d]	0.8[b]	2z [d] , 8y [b]
Brassica spp. (Various)	100–142 (24–34)[c]	92–94[c]	1–2[c]	2–4§[c]	0.1–0.3[b]	5–6y[c]
Spinacea oleracea (Spinach)	125 (30)[d]	92[d]	3[d]	3§[d]	0.4[d]	1z[d]

'§' stands for dietary fibre, '**' stands for crude fibre, 'y' stands for carbohydrate value by difference, 'z' stands for available carbohydrate.

[a] FAO 1990; [b] Odhav *et al*. (2007); [c] Mosha and Gaga (1999); [d] Kruger *et al*. (1998); [g] Oboh *et al*. (2005)

Mineral content in WLVs can contribute significantly to recommended daily nutrient intake for all age groups (Table 4), demonstrating the WLV importance in combating malnutrition especially in Africa.

Table 4: recommended daily nutrient intakes (RDI) of selected micronutrients for different age groups provided by FAO/WHO (mg/kg). *(Uusiku et al., 2010)*

Age (yrs)	Sex	Fe[a] (mg)	Zn[b] (mg)
1–3		5.8	8.3
4–6		6.3	9.6
7–9		8.9	11.2
10–18	Male	14.6 (10–14 yrs)	17.1
		18.8 (15–18 yrs)	
	Female	32.7 (10–14 yrs)	14.4
		31.0 (15–18 yrs)	
19–65	Male	13.7	14.0
	Female	29.4	9.8

[a] Based on a diet with 10% iron bioavailability, [b] Based on a diet with low zinc bioavailability.

2.2.2 Bidens pilosa

Bidens pilosa is among the WLVs harvested for food in the Witwatersrand Basin goldfields. It is commonly known as 'black jack' in English, '*mokolonyane'* in Sotho, '*umhlabangubo'* in Zulu and '*ucucuza'* in Ndebele. It is well known in many countries and cultures. It has been naturalised to many parts of South Africa (Bartolome *et al*., 2013), originating from South America. *Bidens pilosa* thrives in soils with minimal nutritive value which are moderately dry and preferably situated in full sun. Research by Bubala (2013) shows that a portion of between 107.5g to 122.4g of less popular WLVs is consumed at a frequency ranging from once a week to only being eaten occasionally. *Bidens pilosa* is classified as a less popular vegetable (not in the top eight of vegetable list) species most frequently harvested for food in Kanana based on the research by Bubala (2013).

Bidens pilosa (Figure 1) is used as a medicinal plant and food source for humans and has no recorded adverse effects on humans (Karis and Ryding, 1994). Its shoots are used to prepare a vegetable dish especially in times of limited food supply in most African rural areas (Vorster and Jansen Van Rensburg, 2005). It is a source of energy, protein fat, carbohydrate, fibre, some vitamins, calcium (Ca), phosphorus and carotene. Pharmacological studies have revealed that tannins, essential oils and ascorbic acids are present in *B. pilosa*, and the plant is used in the treatment of gastrointestinal disease (Vorster and Jansen Van Rensburg, 2005).

Figure 1: *B. pilosa* at flowering stage - picture taken at Varkenslaagte, March, 2016

2.3 Potentially harmful effects of metals in plants and animals

Metals in their ionic forms are highly reactive. Xenobiotics or non-essential toxic elements such as Pb, Cd, Mercury (Hg), U and As can be harmful to plants and animals even at very low concentrations (Bowen, 1979). They are frequently associated with many chronic illnesses in humans. They can easily interface biological systems in different ways. For instance, Hg and Cd can easily bind to S in proteins resulting in dysfunction in bio-molecules (Kasprzak, 2002). Normal functioning of many enzymes in the body can be inhibited by preferential binding of thiol groups, for example, interaction between Pb and enzymes involved in the synthesis of haem (Moore *et al*., 1987).

Some metals can induce oxidative modification of proteins and DNA resulting in carcinogenicity (Kasprzak, 2002). Non-essential metals can mimic essential elements by binding to sites meant for essential elements. Manganese can mimic Fe, while Cd, Cu and Ni can mimic Zn (Bridges and Zalpus, 2005).

Zinc

Zinc is a plant micronutrient and is significant in many physiological processes such as enzymatic reactions, metabolic processes and oxidation-reduction reactions. Zinc toxicity in plants results in growth inhibition. In humans, Zn is important in cellular functions and is involved in improving the immune system (Hafeez *et al*., 2013). Deficiency in Zn affects immune and gastrointestinal function (Welch, 1993). It is important for bone formation, normal growth, DNA synthesis, brain development, as well as wound healing and is a component of different enzymes (Adelekan and Abegunde, 2011). Approximately 6.8% of deaths in children between 0-5years in 2004 in Africa were due to deficiency in Zn (Roth *et al*., 2008). Acute Zn toxicity results in nausea, vomiting, epigastric pain, abdominal cramps and bloody diarrhoea (Fosmire, 1990). In some cases, excessive Zn ingestion results in difficulty in writing, lethargy, slight staggering of gait and light-headedness (Fosmire, 1990). There is no recorded information in Zn toxicity effects in humans. According to Kabata-Pedias and Pedias (1992), the normal range of Zn concentrations in mature leaves is 27-100 mg/kg. If Zn concentrations are between 10-20 mg/kg, then the leaves are said to be deficient in Zn whereas concentrations between 100-400 mg/kg are considered toxic. It has been noted that elevated Zn can result in impaired Cu uptake (FAO/WHO, 2011). Zinc and Fe distribution in food supply is similar; hence some dietary components which affect Zn distribution also affect Fe (Hotz and Brown, 2004).

Iron

Iron is a plant micronutrient essential for plant growth, and is important in many enzymes and enzyme activities (Welch, 2002). Excess Fe can produce stunted growth of roots and tops, dark green foliage, or dark brown to purple leaves on some plants (Kabata-Pedias and Pedias, 1992). In humans, Fe is involved in a wide variety of metabolic processes such as oxygen transport, electron transport and DNA synthesis. Excessive Fe leads to free radical formation resulting in tissue damage. Iron deficiency is common in pregnant women, women of child bearing age, and in children (FAO/WHO, 2011). No acute effects of Fe toxicity have been reported in humans. Normal Fe concentration range in mature leafy tissue is between 100 mg/kg to 500 mg/kg. Concentrations less than 50 mg/kg and above 500 mg/kg are considered deficiency and toxic respectively (Kabata-Pedias and Pedias, 1992). Excess intake of Fe may result in Fe deposition in tissues such as the liver, heart, and pancreas. People with impaired Fe regulation ability suffer from liver cirrhosis, diabetes, adrenal insufficiency or heart failure (FAO/WHO, 2011).

Lead

Lead is not an essential element for plants; it is however absorbed by plants and accumulates in roots while a small fraction reaches the leaves (Sharma and Dubey, 2005) and is toxic to plants (). Stunted growth, chlorosis and blackening of the root system can result due to Pb. It impacts on mineral nutrition and water balance, and inhibits photosynthesis (Sharma & Dubey, 2005). High accumulation of Pb can cause colic, headaches, anaemia, brain and central nervous system damage (Rehman *et al*., 2013), renal failure, convulsions, coma, and even death. Subtle effects on metabolism and intellectual deficiency occur at low exposures (USEPA-IRIS, 2005). Health impacts from lead such as effects on enzymes and neurobehavioral development in children may occur at very low Pb blood levels (USEPA-IRIS, 2005). In addition, Pb bioaccumulates primarily in the skeleton and the body burden of Pb varies depending on age, nutrition, health status, gestation and lactation. Hence risk values cannot truly give an indication of the potential risk (USEPA-IRIS, 2005).

Arsenic

Arsenic is non-essential and generally toxic to plants. Upon translocation from roots to shoots, it can severely inhibit plant growth by slowing expansion and biomass accumulation causing the plant reproductive potential to be compromised (Garg and Singla, 2011). At higher concentrations As interferes with critical metabolic processes leading to plant death (Finnegan & Chen, 2012). High As concentrations originating from food such as rice, vegetables (via soils) and drinking water has resulted in human health problems (Ng *et al*., 2001).

In humans, As metabolism can produce free radicals resulting in receptor oxidative damage carcinogenesis and mutagenesis (Ng *et al*., 2001). It is associated with lung, kidney, bladder, and skin disorders (ATSDR, 2003a). Hair, nails and skin are the main long-term storage sites of As; hence it is important to test these keratin-rich tissues to estimate total As burdens (McCally, 2002). Arsenic also interferes with haem synthesis pathways (Ng *et al*., 2005). Arsenic is regarded as the most hazardous metal (ATSDR, 2003a).

2.4 Exposure of plants and human to contaminants

Ability of different plant species to absorb metals can be determined by transfer factors/ bioconcentration factors (BCF) (Rattan *et al*., 2005). Transfer of metals from soil to plants is of concern because of potential risk to human and livestock health. Bioconcentration factor is an index to evaluate the potential of a metal to be transferred from soil to plants. It is determined by the equation below:

$$\mathrm{BCF} = \frac{Metal\ concentration\ in\ leaves}{Metal\ concentration\ in\ corresponding\ soils}$$ (Cui *et al.*, 2005)

Food chain exposure is an important pathway for higher order organisms such as humans who consume plants and animals that might have accumulated metals. Deleterious effects such as impaired reproduction, reduction in metabolism, increase in sensitivity to chemicals and or physical stresses exist due to exposure (Duffus, 2002). Frequent exposure to contaminated soils, food or water even if metal concentrations are low may pose risk because metals bio-concentrate in in living organisms. Risk can be reduced if exposure is reduced.

Bioaccumulation of contaminants in tissues inaccessible to mechanisms that eliminate toxins is of particular concern. Toxins may be slowly released from such tissues into the body resulting in protracted exposure even though contamination levels outside the body have been reduced (McCally, 2002). Humans can potentially be exposed to higher concentrations of contaminants compared to the concentrations found in soils where contaminants originated (biomagnification) (Sharma *et al.*, 2009).

Factors such as age, sex, genetics, lifestyle, level of health and diet of an individual also determine the magnitude of toxicity. Metals absorption in human digestive system depends on the metal chemical form, age and nutritional status of a person (McCally, 2002). Metals are excreted primarily through the digestive tract and kidneys but tend to accumulate in the kidneys, bones or liver for a long period (McCally, 2002). The persistent and accumulative behaviour of metals results in their ability to concentrate throughout the food chain causing toxicity in the human body (Sharma et al, 2009).

High absorption of metals into tissues and organs can cause essential nutrient depletion in the body of humans and other organisms, resulting in health risks (Arora *et al*. 2008). The effects may be severe and occur rapidly due to high concentrations (acute effects) or may occur over a long period of time and involve lower exposure (chronic effects).

For essential elements such as Zn and Fe, there is an optimum dose range required for good health. Carcinogens such as Pb have a low exposure threshold. Reference Dose (RfD) is an estimate of the safe daily intake of a substance or chemical without a health risk over a lifetime (US-EPA-IRIS, 2006). Relative risks from metals in food can be quantified using a Dietary Risk Index based on mean concentrations of metals in food, average serving portion of food and RfDs.

2.5 Dietary exposure assessment

Toxicity is the capacity of a chemical to produce injury to a living organism. Exposure to a hazardous substance or mixture including through use, production or disposal can cause adverse effect to organisms or environment (IUPAC, 1993): safe handling and usage of such substances must be practiced. Injury is caused by the chemical itself or compounds formed when it is transformed in the body of an organism. Exposure is the process where a hazard reaches a target cell, tissue, organ or population. Level of exposure is determined taking into account factors such concentration, frequency and duration of exposure (IUPAC, 1993).

To conduct a health risk assessment (HRA), four steps are required as follows: (i) hazard identification, (ii) exposure assessment, (iii) dose response assessment and (iv) risk characterization (USEPA, 2001). Thus an HRA provides a comprehensive overview and understanding of health risk from hazardous substances.

The present study can be seen to be honing on one component of an HRA in that it investigates specifically the risk of ingestion exposure to metals through consumption of *B. pilosa* by adult populations. Dermal and inhalation exposure is beyond the scope of the study.

Risk assessment is calculated by estimating daily metal intake (DMI) (in this case through ingestion of *B. pilosa)*. Daily Metal Intake is determined according to the method of Khan *et al*. (2008):

$$DMI = \frac{Cmetal * Wfood * 0.085}{Bw}$$

C_{metal} = mean metal concentration in the leafy vegetables (mg/kg)

W_{food} = average mass of leafy vegetable consumed per day per person (kg/person/day) = 0.1075kg (based on average consumption of least popular WLV (107.5g) as determined by Bubala (2013). No report was made on *B. pilosa* consumption in the area; however, labourers on site confirmed they use it for food, albeit infrequently. In this research, a mass of 0.1075g per serving of *B. pilosa* will be assumed. The DMI thus provides an overestimation of risk.

B_w= average body weight of an adult. A body weight of 70kgs is assumed (DEA, 2010)

0.085=conversion factor of fresh weight to dry weight to match the metal content which is expressed in terms of dry mass. The results obtainable after calculations are then equated to the RfD set out by USEPA-IRIS as a safe limit (Table 5). The RfD for Pb was obtained from DEA, 2010. IRIS has not set the RfD limits therefore DEA value has been adopted as a secondary source of reference.

Table 5: Oral reference dose (RfD) for metals in food (mg/kg.d) (USEPA IRIS, 2006)

Metal	As	Pb	Zn
RfD	3.00×10^{-4}	$*3.6 \times 10^{-3}$	0.3

*- (DEA 2010) Data sourced from World Health Organisation

Due to occurrence of changes in certain blood enzymes and in children's neurobehavioral development at very low lead levels, USEPA IRIS considered it inappropriate to develop an RfD for lead. A DRI for Pb was calculated in this report as an estimate of risk but must be treated with caution.

The potential risks of dietary exposure pathway can be assessed by dietary risk index (DRI).

DRI= $\frac{\mathrm{DMI}}{\mathrm{RfD}}$ (USEPA, 2002)

If DRI is < 1, there is no obvious risk and is assumed that population exposed is safe (USEPA, 2004).

Arsenic is carcinogenic. The risk of an exposed individual to develop cancer over a lifetime due to exposure to a carcinogen is obtained by multiplying the DMI by a slope factor. The carcinogenicity of arsenic needs to be included in a health risk assessment. . However, this aspect is beyond the scope of the present study. Apart from the small sample size, the cumulative effects of different exposure routes would need to be considered.

CHAPTER 3: MATERIALS AND METHODS

This chapter describes the location and physico-chemical parameters of the primary study site (Varkenslaagte). The locations and descriptions of the comparison sites (Kraalkop and Johannesburg) are then provided. Data collection methods, sample preparation and analysis are also covered in this chapter.

The Witwatersrand basin comprises a north-east to south-west trending feature 350km x160km underlying southern Gauteng, North-West Province and northern Free State Province (AngloGold Ashanti, 2012). The gold and uranium bearing basin is one of the greatest metallogenic provinces in the world (AngloGold Ashanti, 2012). The sedimentary strata are known as the Witwatersrand Supergroup which is made up of the West Rand Group (WRG) and the Central Rand Group (CRG). The lowermost sedimentary strata of the WRG mostly comprise shales and quartzites, and the quartzites and conglomerates of the CRG overlie the WRG. These sedimentary layers are an accumulation of riverine deposits and have high gold and uranium concentrations associated with certain conglomerates layers (McCarthy and Rubisge, 2005).

3.1 Study sites

The study was conducted from the Varkenslaagte with comparison samples collected from the Kraalkop and Johannesburg.

3.1.1Varkenslaagte

This study was conducted in South Africa (SA), on the West Wits mine property of AngloGold Ashanti. The West Wits Mine area has some of the world’s deepest mines and tailing/slime dams derived from decades of mining activities. The area is situated at the border of Gauteng Province and North-West Province (Figure 2a), approximately 75km west of Johannesburg and 7km South of Carletonville. Fochville and Potchefstroom are to the south and west of the study site situated at 12km and 50km away respectively (AGA, 2013) (Figure 2b). The West Wits operation is situated within the Merafong City Local Municipality, which is within the West Rand District Municipality. The Merafong City Local Municipality comprises Carletonville, Fochville, Wedela and the western portion of Gatsrand (AngloGold Ashanti, 2012).

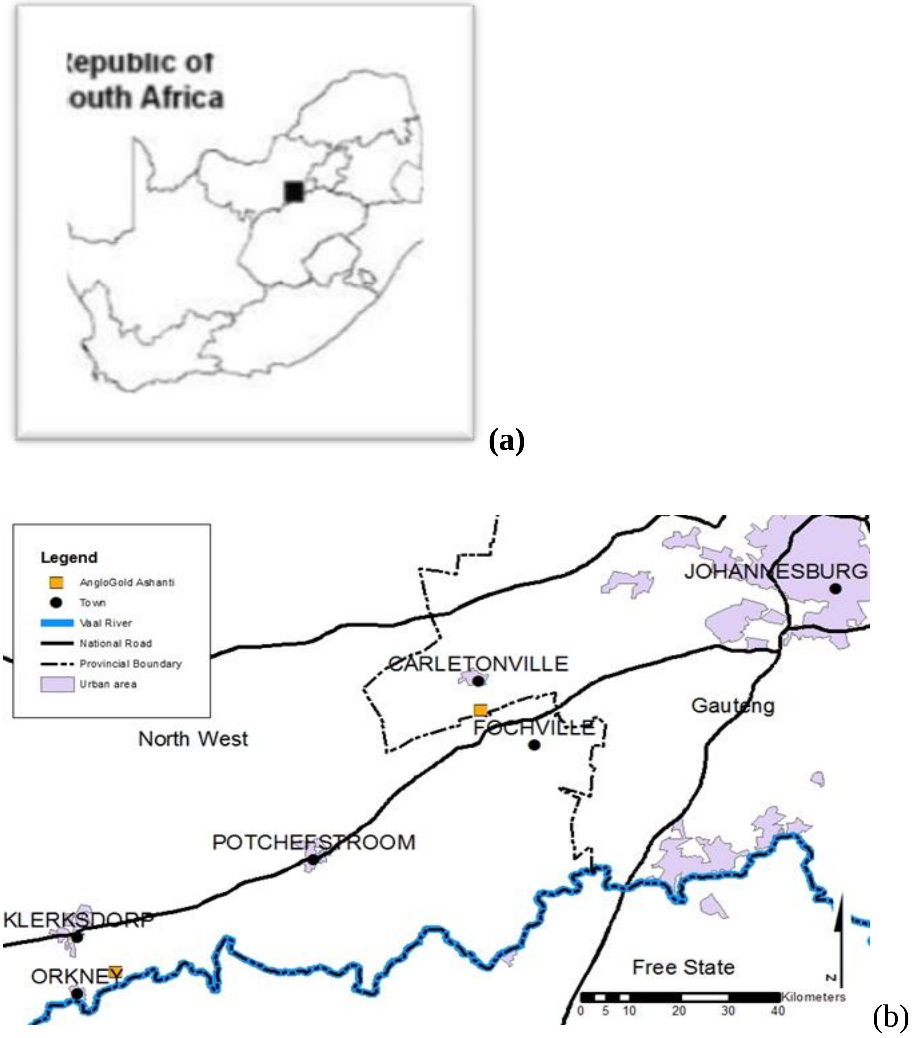

Figure 2: (a) Location of the study site within SA. (b) Location of Anglo Gold Ashanti's West Wits operations near Carletonville (map by D. Furniss)

Topography

The area is characterised by undulating plains interspersed by rock peaks (AGA, 2009). A prominent rock ridge known as the Gatsrand extends from the eastern extent of the

West Wits operations to the western extremity forming a watershed. The mine infrastructures including headgear and the TSFs have become a characteristic part of the landscape since the commencement of mining operations (AngloGold Ashanti, 2012).

Climate

The rainfall in the study area occurs in the summer months (October to April) with an average annual precipitation of approximately 650mm-1200mm (Herbert, 2008) and inters-annual variation between 25-30% (Schulze, 1996). Heavy thunderstorms resulting in erosion and runoff occur between November and February and deliver above 60% of the area's rainfall. The dry season occurs between May and September with average precipitation at around 50mm. Evaporation is estimated at between 2000- 2250mm per day (Schulze, 1997). The mean monthly statistics indicate January as the warmest month with maximum temperatures of 25^{o}C-27.5^{o}C. July records the lowest temperatures of 0-2^{o}C and regular frost occurs in winter (Schulze, 1997). The highest wind speed has been recorded in September, with the lowest in February and the average wind speed measured in Potchefstroom being 4.7m/s. The prevailing wind direction is northerly (Herbert, 2008).

Soil, land use and land cover

The northern portion of the 3785.8ha West Wits lease land falls in Gauteng while the southern area falls in North-West province (AGA, 2013). Infrastructure such as mining plants, tailing dams, shafts and related operations, excavations and residential areas occupy 1460.4ha (AGA, 2013). The northern part is made up mainly of gently sloping midslopes and footslopes, largely transformed by a history of agriculture (croplands) and mining activities. The only part in the northern portion that is almost natural is a small area in the northwest corner which supports grassland (AGA, 2013). The central and southern portions consist mainly of slightly steep to steep rocky crest midslopes and footslopes. Approximately 80% of the central portion (plateau) is still in its natural state (grassland) although in some areas it is overgrazed. About 60% of the southern area has been transformed by a history of croplands and mining activities, with only a third is still in its natural grassland state (AGA, 2013).

Vegetation

The vegetation on the Varkenslaagte comprises mainly grassland and reedbeds in wetlands and falls between the Savanna and Grassland Biomes (Mucina and Rutherford, 2006). The two TSFs that contribute to the contamination of Varkenslaagte are Old North Complex TSF and the Savuka TSF, which are located within AGA West Wits mine land (AGA, 2013). In an effort to reduce AMD contamination on the Varkenslaagte and in order to satisfy a legal obligation, West Wits mine land has been undergoing AMD and metals remediation through a combination of woodlands allowed to grow on the site for fourteen years and reedbeds for four years (AGA, 2013). For purposes of comparison, samples were obtained from several places in the Witwatersrand goldfields including the Kraalkop Nature Reserve near the West Wits Mine and under the N14 freeway bridge outside Kraalkop nature reserve.

3.1.2 Kraalkop

Kraalkop Nature Reserve is owned by AngloGold Ashanti and falls within the lease area for West Wits Mine. It is located within the vicinity of the West Wits Mine on a 700ha Rocky Highveld Grassland. It is located adjacent to N14 towards Potchefstroom between Carletonville and Fochville. Kraalkop catchment does not receive AMD but does receive dust from the tailings dam. Kraalkop is known through prior research to be less contaminated (I. Weiersbye, Personal Communications). Figure 3 below shows the location of the Varkenslaagte and Kraalkop as shown on the google earth image.

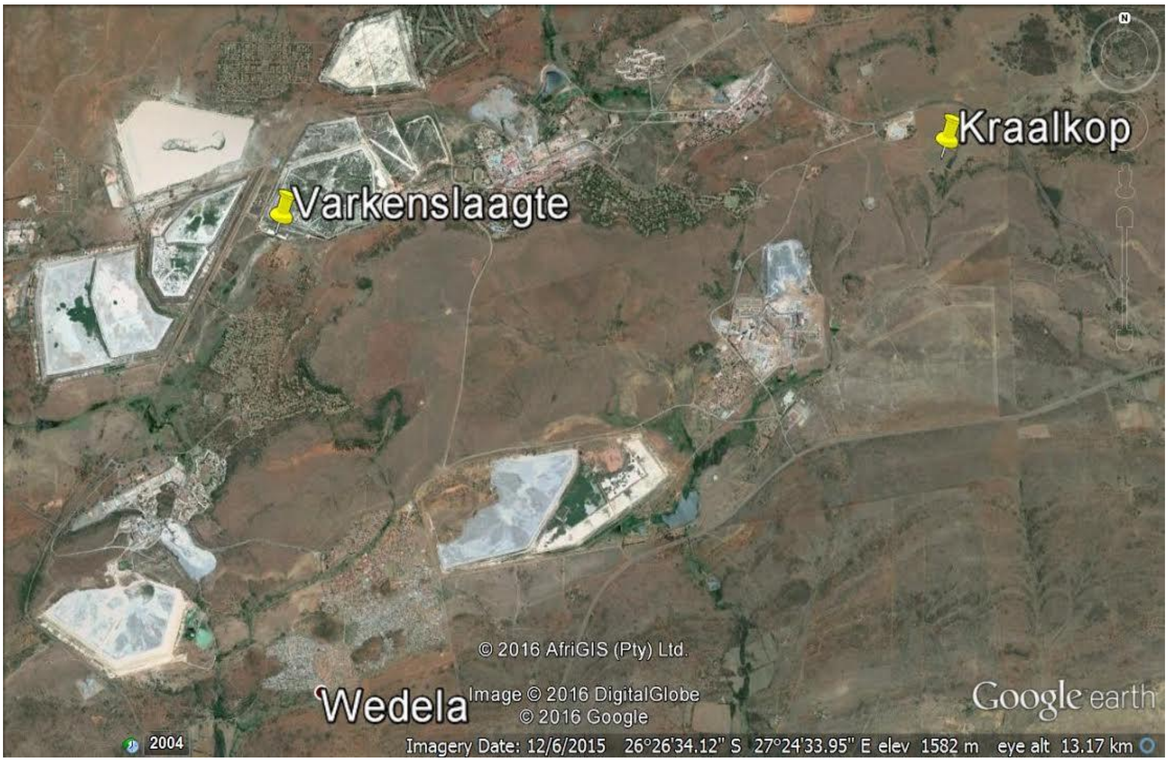

Figure 3: The Varkenslagte and Kraalkop and shown on google earth

3.1.3 Johannesburg

Additional comparison samples were collected from two sites in Johannesburg. Johannesburg commonly known as "*Egoli*" which means a place of gold (AGA, 2009) is located at the heart of Gauteng and has the deepest mines in the world surrounding it from over a century of mining activities. One plot in Johannesburg residential area located at Brixton and another plot in Johannesburg commercial/ industrial area (University of Witwatersrand). The Johannesburg central business district is characterized by high traffic volumes.

3.2 Experimental design and protocol

The schematic diagram in figure 4 below summarises the experimental set up of the present study.

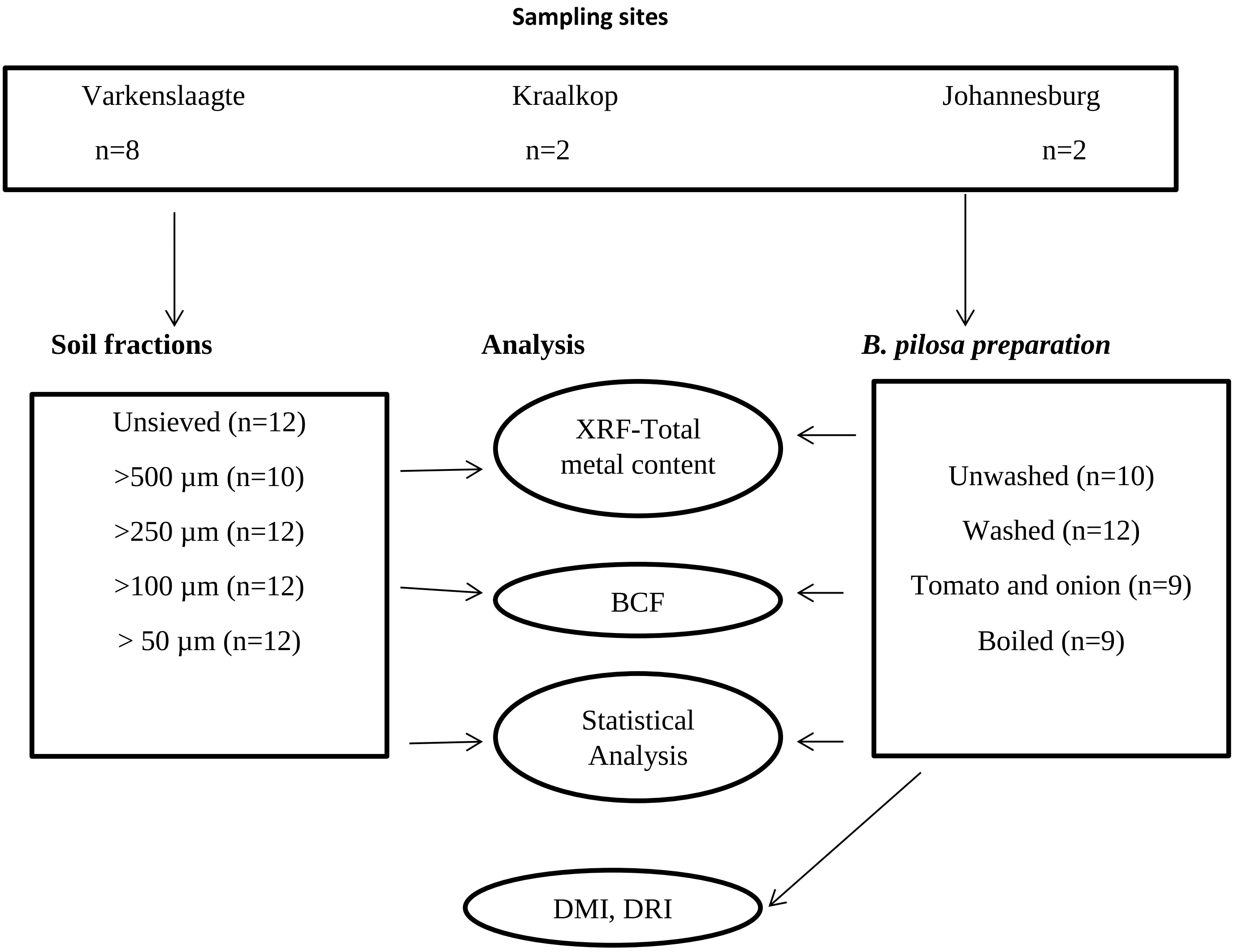

Figure 4: Schematic diagram showing experimental setup

Soils samples from different sites were sieved into different particle sizes as shown on the schematic diagram above. *Bidens pilosa* leaves were exposed to different cooking methods vis. unwashed, washed, cooked in tomato and onion and boiled. Both soil and vegetable samples were analysed using XRF (X-Ray Fluorescence) and results were statistically interpreted. DMI and DRI where calculated from cooked vegetable samples. Cooked samples (boiled and tomato and onion) only were used because *B. pilosa* is mostly consumed as cooked vegetable in the study area.

3.3 Data collection

Fieldwork was conducted during a single site visit from 7 to 18 March 2016.

3.3.1 Sampling

Samples were obtained from eight plots at Varkenslaagte as shown on figure 5 below.

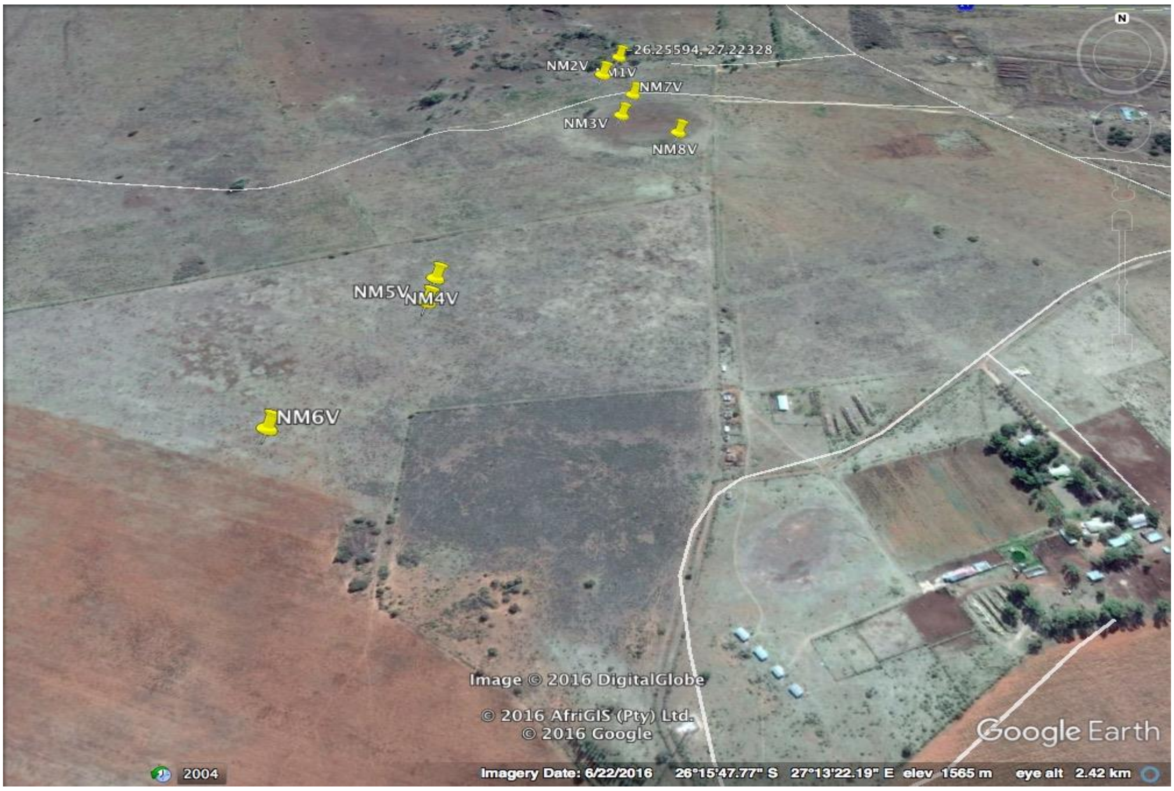

Figure 5: Google earth image showing the location of 8 plots at the Varkenslaagte

There was only one plot where *B. pilosa* was growing inside Kraalkop nature reserve. After a long search and enquiring with people around Kraalkop, a second sample was obtained about 100m outside Kraalkop reserve, under N14 Freeway Bridge. This plot is referred to as Kraalkop plot 2 in the report and the former which is located inside the reserve is referred to as Kraalkop plot 1. Figure 6 shows the location of the two plots at Kraalkop where comparison samples were obtained.

Figure 6: Google earth imagery showing the sampling plots in Kraalkop

Two more samples for comparison purposes were obtained in Johannesburg: one from a residential garden in Brixton, about 10km west of Johannesburg central business district (Figure 7) and the second sample was obtained from a flowerbed adjacent to University of Witwatersrand Sontonga avenue (off Yale road) entrance (Figure 8).

Figure 7: Google earth image showing the sampling site in Johannesburg residential area

Figure 8: Google earth image showing the sampling site within Johannesburg commercial area.

Sample locations were marked with a Garmin model portable Global Positioning System (GPS) to an accuracy of 5meters and the plot name and GPS location is given on table 6 below:

Table 6: GPS coordinates of sampling sites location

PLOT NAME	GPS COORDINATES
KNM 1	S26.25.324/E027.27.088
KNM 2	S26.25.049/E027.27.368
NM1V	S26.25.597/E027.22.329
NM2V	S26.25.651/E027.22.298
NM3V	S26.25.772/E027.22.322
NM4V	S26.26.155/E027.22.088
NM5V	S26.26.188/E027.22.093
NM6V	S26.26.395/E027.21.940
NM7V	S26.25.704/E027.22.340
NM8V	S26.25.812/E027.22.411
JHB01	S26.11.2399/E27.59.3611
JHB02	S26.11.3375/E28.014366

The number of plants uprooted per plot depended on the size of the *B. pilosa* available. *Bidens pilosa* leaves from each plot were bulked together to form one composite sample and put in polythene bags. A wet mass of between 1kg to 1.5kg leaves was obtained per plot. Only leaves were required for analysis because they constitute the edible parts of *B. pilosa*.

An unwashed portion of approximately 100g was removed and placed in separate polythene zip lock bags. The rest of the leaves were washed twice in tap water and once in deionized water to remove dust and any foreign substance or object on the surface of the leaves. Samples were placed on drying trays before storing in labelled, clean zip lock bags. Nitrile gloves were worn at all times during sample handling to avoid contamination Corresponding soil samples were obtained to a depth of 15 cm (0-15cm) from the same locations where *B. pilosa* samples were uprooted. This depth of soil sample was sufficient because *B. pilosa* is a shallow rooted plant. A shovel and garden fork were used to dig the soil samples and were washed before and after sampling to avoid cross-contamination of samples. Soil samples obtained from each plot were thoroughly mixed to give one composite sample for each plot. Approximately 3kg of soil was collected from each plot. Figure 9 below shows the researcher collecting *B. pilosa* samples opposite a TSF in the Varkenslaagte.

Figure 9: Sampling by the researcher opposite AGA TSF

The samples were stored in polythene bags (Figure 10) and either refrigerated or stored in cooler boxes at temperatures between -4^{o}C to 8^{o}C before taking them to the EEPP cold room at Wits University. This was done so that the leaves will not decompose.

Figure 10: Plucking of B. pilosa leaves into a polythene bags by the researcher and assistant during sampling

3.3.2 Soils

A portable pH meter (WTW multiparameter pH/Cond 340i and ORP, German), calibrated at pH 4.0 and pH 7.0 at room temperature, was used to determine soil pH in 1:2 ratio of soil: water suspension. Approximately 200g of soil material from each plot was air dried in an oven at room temperature until all moisture was removed. Ten grams (10g) of air-dried soil was removed before sieving. The remaining soil samples were then sieved in an Retsch type AS 200, Germany electrical shaker into the following particle sizes: >2mm, >500µm, >250µm and >100µm and >50µm. Greater than 2mm particle size was mostly stones and leaves, it was not analysed further. For >500µm, >250µm and >100µm and >50µm, approximately 5g of each sample was placed in a plastic cup then covered with a thin formvar foil and analysed using XRF.

3.3.3 Bidens pilosa

Bidens pilosa washed leaves from each plot were weighed and separated into three portions as follows:

- +/_200g was boiled in distilled water
- +/-200g was cooked in tomato and onion puree prepared in the laboratory
- +/-300g was left uncooked.

Tomato and onion were prepared as follows:

Approximately one kilogram of tomatoes (4 large tomatoes) and 250g of onion (1 large onion) was cooked in glassware, mashed using plastic spoons and supernatant was filtered and stored in a fridge at +/- 4^{o}C. Figure 11 below shows sample preparation and weighing in the lab.

Figure 11: Sample preparation and weighing in EEPP laboratory, Witwatersrand University

Cooking was done in controlled conditions in the laboratory using test tubes and a Spectroquant TR320 at temperature of 95^{o}C-97^{o}C. Three millilitres of either tomato and onion puree or distilled water was used to cook approximately 70g of leaves in a test tube. The samples were then freeze-dried in a Labconco vacuum freeze drier (USA) at -50^{o} C for a period of 3-5 days. After freeze drying, the leaves were ground using a ceramic coffee grinder. The ground samples were then placed in clean-labelled specimen bags and stored in the cold room before analysis.

3.3.4 Total Metal Content Analysis

Analytical work was conducted by the Ecological Engineering Laboratory in the Department of Plant, Animal and Environmental Sciences (APES) at the University of the Witwatersrand. Major and trace elements in the samples were analysed using a semi-quantitative Spectroscout Portable Energy-Dispersive (ED-XRF) Analyser (SPECTRO) Geo + (1) with twin tubes for light to heavy elements detection. For quality assurance purposes (accuracy and precision), all soil and plants samples were measured together with certified reference materials (CRM). Certified reference materials were inserted at a frequency of two for every 10 samples. There are different standards for soil/sediments and plant material. The CRM used for soils were stream sediments NCS DC 73315a and GSD 12-1 from China National Analysis Centre for Iron and Steel. Plant samples were analysed alongside the spinach leaves (1570a) supplied by National Institute of Standards and Technology, USA and orchard leaves (1577) CRM supplied by LECO Corporation.

Metal selection was based on metals reported to have elevated concentrations in the Witwatersrand goldfields by prior studies and good CRM agreements for both plants and soil samples. The selected metals on this criterion were Fe, Zn, As and Pb.

3.3.5 Statistical procedure

In order to determine metal concentration distribution among variables, the experimental units were grouped according to the treatments they had been subjected to. Statistical analysis was performed using the Statistical Packages for Social Sciences version 23. Descriptive statistics (mean, median, standard error, standard deviation, minimum and maximum) were calculated for each dependent variable. Descriptive statistics was reported as mean ±standard deviation (SD) and range. The permissible limit guidelines were used as criteria to compare mean concentrations of selected element for all the sites and different treatments.

According to Galpin and Krommenhoek (2014), the one way ANOVA parametric test and Kruskal Wallis non parametric tests are tests conducted on three groups or more. When any of the parametric test assumption is not met, the Kruskal Wallis test becomes more appropriate (Galpin and Krommenhoek, 2014) provided its assumptions are met.

To determine whether hypothesis testing was to be done using parametric or non-parametric test, normality test was carried out followed by homogeneity of variance. Where assumptions of ANOVA were violated, Kruskal Wallis was used. However, where ANOVA and Kruskal Wallis gave the same results, the ANOVA results were adopted because it is a robust test (Leedy and Ormrod, 2013). Any test resulting in statistical difference triggered a post hoc pairwise comparison to determine where the differences lie.

ANOVA and Kruskal Wallis were conducted to determine differences in metal concentrations in soil particle fractions and different treatment and cooking methods in B. pilosa leaves.

3.3.6 Dietary exposure assessments calculations

The RfDs are often more recent than the PTWIs hence were used in the calculations.   The advantage of using the more recent USEPA RfDs is that they were often derived using update toxicological dose-response methodology, for example Bench Mark Dose Modelling (BMD modelling) which has several advantages over using only a NOAEL or LOAEL as POD (point of departure). In practice, mostly used are the RfD rather than the PTWI if both are available (M. Fourie, Personal Communications) .

CHAPTER 4: RESULTS AND DISCUSSION

Chapter 4 presents the findings of the study. Total metal concentrations in soil fractions from different sites, B. pilosa leaves from different sites and leaves subjected to different treatment were presented. Bioconcentration factors, DMI, and DRI was also calculated. The presented results are also discussed in this chapter.

4.1 Soils

Soil pH, electrical conductivity, CRM results and total metal content of soil fraction are reported in this section.

4.1.1 pH and electrical conductivity

The pH and conductivity values of soils are presented in Table 7 below. The pH values ranged from 4.13 to 7.59. Varkenslaagte and Kraalkop pH range was 4.13-6.12 and Johannesburg pH were close to neutral (6.45 and 7.59). Conductivity ranged from 99.3 µS/cm to 243.63 µS/cm.

Table 7: pH and conductivity in soils collected from the study sites in March 2016

Sample	GPS	pH	Conductivity µS/cm
KNM 1	S26.25.324/E027.27.088	4.87	104.4
KNM 2	S26.25.049/E027.27.368	5.53	175.3
NM1V	S26.25.597/E027.22.329	5.21	158.1
NM2V	S26.25.651/E027.22.298	5.26	177.6
NM3V	S26.25.772/E027.22.322	4.13	243.6
NM4V	S26.26.155/E027.22.088	5.69	208.8
NM5V	S26.26.188/E027.22.093	5.83	178.3
NM6V	S26.26.395/E027.21.940	6.12	197.8
NM7V	S26.25.704/E027.22.340	5.02	210.4
NM8V	S26.25.812/E027.22.411	4.83	99.3
JHB01	S26.11.2399/E27.59.3611	6.45	126.4
JHB02	S26.11.3375/E28.014366	7.59	85.2

4.1.2 Certified reference material (CRM) percentage agreements

Certified reference materials agreements of the elements listed in Table 8 were above 91% with standard deviation of less than 2%.

Table 8: Certified reference material analysis for total elements in soils; (mg/kg; mean ± standard deviation (SD); n=7)

CRM	Element	Certified value	Measured value	% agreement
GSD12	Fe	34132.33	34772.86±689.4	98
NCS733159	Fe	36860.12	37392.86±413.55	98
GSD12	Zn	498	548.37±4.91	91
NCS733159	Zn	263	287.8±3.62	91
GSD12	As	115	111.67±2.38	97
NCS733159	As	74	68±0.90	92
GSD12	Pb	285	313.49±1.49	91
NCS733159	Pb	102	102.07±1.46	99

4.1.3 Mean metal concentrations (mg/kg) in soils collected from different locations

The results are presented in table 9 below.

Table 9: Mean metal concentrations (mean± SD) of soils before sieving (mg/kg; Varkenslaagte; n=8; Kraalkop; Johannesburg minimum and maximum values

Site	Fe	Zn	As	Pb
Varkenslaagte	39026±11335.3	[ç] 84±33.6	[ç]18.5±11	[ç] 32.6±12
Varkenslaagte Minimum	18650	35.5	[ç]7.8	[ç] 19.0
Varkenslaagte Maximum	54310	[ç] 140.6	[ç]43.5	[ç] 53.45
Kraalkop (average)	[a]61000±2050.6	[ç] 93.2±49.6	[cç]35.1±24.8	[ç] 96.3±96.9
Kraalkop plot 1	[a]62450	[ç] 58.1	[ç]17.5	[ç] 27.8
Kraalkop plot 2	[a]59550	[ç] 128.1	[cç]52.6	[b ç] 164.8
Johannesburg (average)	33805±2948.6	[b, ç] 476.1± 244.9	[ç]7.2±1.2	[b ç] 112.7±103.6
Jhb residential	31720	[b, ç] 302.9	[ç]8	[ç] 39.4
Jhb Commercial	35890	[b, ç] 649.3	[ç]6.3	[b ç] 185.9

[a] exceeds maximum allowable iron limits (table 2)

[b] exceeds FAO/WHO and EU maximum allowable limits

[c] exceeds FAO/WHO maximum allowable limits

[d] exceeds FAO/WHO and EU maximum allowable limits

[ç] exceeds SA limits

Generally, Pb, As and Zn concentrations exceeded the maximum acceptable metal concentrations in soil for agricultural application in SA shown in table 1. The soils in Varkenslaagte and the comparison sites are therefore not commendable to be used for agricultural purposes.

Lead concentration at Johannesburg commercial area and Kraalkop plot 2 exceeded the maximum allowable limits set by FAO/WHO, SA and EU. Studies by Monna *et al.* (2006) investigating the origin of atmospheric Pb in Johannesburg characterised isotopic composition of potential sources such as mine dumps, coal, gasoline and lichen samples. The study made the following findings (i) Pb gasoline was the major contributor to Pb pollution,

(ii) concentrations of Pb were high in lichens from urban open space and traffic areas, (iii) 80-90% of Pb concentrations in urban open spaces or traffic areas emanate from automotive traffic, (iv) tailings near Johannesburg townships contributed less than 15% of total lead, (v) lichens sampled close to goldfields did not show any impacts of contamination from mine dumps.

These results are in line with the findings of Coetzee *et al*. (2004) who reported high Pb concentrations in children living in Johannesburg. Exhaust gases has been an important contributor to Pb contamination in Johannesburg (Formenti *et al*., 1998). Kraalkop plot 2 had the second highest Pb concentration at 164.8 mg/kg. High Pb contamination might be emanating from exhaust gases. Kraalkop plot 1 and Varkenslaagte had lower Pb concentrations. This may be because they are located away from heavy traffic

Zinc concentrations in soils were highest in Johannesburg soils and exceeded the FAO/WHO, SA and EU maximum allowable limits. Anthropogenic activities which emit Zn to the atmosphere include urban waste stream, industry, corrosion, tire wear, hot-deep galvanised steel products and agriculture (Pacyna and Pacyna 2001). Anthropogenic sources may be the reason for high Zn concentrations in Johannesburg sites because of high industrial and traffic emissions in Johannesburg compared to Kraalkop and Varkenslaagte.

Concentrations of As were highest in Kraalkop plot 2 exceeding the FAO/WHO maximum acceptable standards and SA maximum permissible limits. Arsenic concentrations in Kraalkop plot 1, Varkenslaagte and the Johannesburg exceeded the SA maximum acceptable limit of soils for agricultural applications shown in table 1. Human activities such as mining, waste disposal and manufacture of As-bearing chemicals increases As levels in the environment (Duker *et al*., 2005).

Identification of a specific benchmark for Fe is not easy as Fe bioavailability and toxicity to plants depends on specific pH, EC and soil-water conditions (Kabata-Pendias and Pendias, 1992). Plants absorb Fe in its ferrous state (Fe^{2+}), although the Fe form occurring in well drained soils is $Fe^{3+.}$ While Fe is abundant in soil and rocks some Fe^{3+} compounds are highly insoluble resulting in its unavailability to plants (Kabata-Pendias and Pendias, 1992).

Prior studies in the West Wits mine reported elevated metal concentrations (Makgae, 2012). Generally, the present study results show lower metal concentrations in Varkenslaagte soils compared to Kraalkop plot 2 and Johannesburg commercial site. However, some plots in the Varkenslaagte have high Pb, As and Zn concentrations.

4.1.4 Mean metal concentrations in varying soil particle sizes

Mean concentrations of metals in different fractions, SD and range are presented in table 10 below.

Table 10: Metal concentrations across different soil particle size range (mean± SD; mg/kg; n=10; n=12)

Treatment	Fe	Range	Zn	Range	As	Range	Pb	Range
Greater than 50µm	39871±16615	64730	127.7±166.6	593.2	15.6±14.4	54.2	59.9±84.6	278.9
Greater than 100µm	37599±15387	51750	101.5±86.3	303.6	14.1±12.4	44.2	51.8±65.8	224.0
Greater than 250µm	40006±18402	59190	89.8±58.1	212.6	14.0±11.8	44.1	60.2±78.0	219.8
Greater than 500µm	49473±18975	62580	85.1±54.2	146.0	18.0±16.8	57.5	91.2±147.9	450.9
Before sieving	41818±12930	43800	150.8±171.7	613.8	19.4±14.3	46.3	56.6±56	166.9

The average metal concentrations of Fe, Zn, As and Pb were the same across different particle size range as presented by the ANOVA p values in table 11. Fe ($p=0.805$); Zn ($p=0.607$); As ($p=0.334$); Pb ($p=0.316$).

Table 11: Statistical tests and p-values for different soil particle sizes showing that variances are homogeneous $\alpha=0.05$; ($p>0.05$; H_0: $v_1=v_2=v_3$ and H_I: $v_1 \neq v_2 \neq v_3$ and that average metal concentration in different particle sizes were not different ($p>0.005$; H_0: $\mu_1 = \mu_2 = \mu_{3 \text{ and}}$ H_1: $\mu_{1\neq}$ $\mu_{2\neq}$ μ_3)

Variable	Levene test	ANOVA
Fe	p=0.844	p=0.527
Zn	p=0.181	p=0.659
As	p=0.975	p=0.854
Pb	p=0.128	p=0.866

There was no difference in metal distribution across different particle size range. These results differ to the findings of similar studies that have shown a difference in elemental concentrations across different particle sizes (Sheppard and Evenden, 1994; Layton and Beamer, 2009). Studies conducted by the Geological Survey of Namibia (2006b) showed slightly elevated concentrations of As, Cu, Pb, Cd and Zn in finer particle sizes compared to larger particle sizes. The discrepancy in the results may be attributable to the use of a non-digestive analysis technique, the XRF in this study. In digestive techniques such as the ICP-MS/OES which was used in other studies, the process is more effective for small particle size than larger particle size because small particle sizes have relatively large surface areas therefore analysis may be more efficient in small particle sizes compared to large particle sizes. The small sample size might also be reason for no differences being determined across particle sizes in the present study.

4. 2 *Bidens pilosa*

Results obtained during analysis of *B. pilosa* leaves are presented in this section.

4.2.1 Certified Reference Materials

CRM agreements were between 72-85%, with a SD of less than 15%. CRM calculations are given in Table 12 below.

Table 12: Certified reference material percentage agreements for orchard and spinach leaves (mg/kg; mean SD; n=3)

CRM	Element	Certified value	Measured value	% agreement
Orchard 1577	Fe	300	409.25±28.90	74
Orchard 1577	Zn	25	34.03±3.17	74
Spinach 1570	Zn	82.3	98.4±1.19	84
Orchard 1577	As	10	14.68±3.46	72
Orchard 1577	Pb	45	34.13±4.46	76

The CRM agreements in plants were lower than the CRM agreements in soils. This might be because of

- Different matrices in which these CRMs exist. The XRF has shown to be effective on soil matrix compared to plant matrix.
- Concentrations of elements in the matrix may affect performance efficiency of the XRF. The metal concentrations in plant CRMs are very low compared to sediments CRMs (for example As in orchard leaves is 10 mg/kg and in GSD12 sediments it is 115 mg/kg; Iron concentrations in orchard leaves versus Fe concentration in NCS733159 sediments is 300 mg/kg and 36860 mg/kg respectively)
- Different analysis programmes and sample holders are used for soils and plants. The analysis programme for soils is optimised for high Silicon (Si) soil matrices, and the analysis programme for plants is optimised for low Si dicot plant matrices.

These differences may be the reason for lower percentage agreements in *B. pilosa* leaves compared to soils.

4.2.2 Mean concentrations of metals in B. pilosa leaves harvested from different sites in the Witwatersrand Basin goldfields

The table below (table 13) gives the mean concentrations of metals in *B. pilosa* from different sites.

Table 13: Concentration of metals in *B. pilosa* washed leaves (mg/kg; mean SD; Johannesburg and Kraalkop minimum and maximum values; Varkenslaagte: n=8)

Location	Fe	Zn	As	Pb
Varkenslaagte	[e]438.9±106.7	79.9±42.3	[g]0.23±0.19	[h]0.57±0.65
Varkenslaagte minimum	284.7	26.4	0.01	0.12
Varkenslaagte maximum	615.8	[f]157.6	[g]0.6	[h]2.1
Kraalkop average	[e]570.4±55.5	[f]149.5±99.3	[g]0.17±0.00	[h]1.55±0.35
Kraalkop plot 1	[e]609.6	79.2	[g]0.17	[h]1.3
Kraalkop plot 2	[e]531.1	[f]219.7	[g]0.17	[h]1.8
Johannesburg average	329.2±99.4	[f]164.0±108.8	[g]0.43±0.38	[h]0.87±0.75
Jhb residential	258.9	87.1	[g]0.7	[h]0.333
Jhb commercial	399.5	[f]240.9	[g]0.17	[h]1.4

[e] exceeds maximum allowable limits for Fe

[f] exceeds FAO/WHO and EU maximum allowable limits

[g] exceeds FAO/WHO maximum allowable limits

[h] exceeds FAO/WHO, EU and SA maximum allowable limit.

Mean concentration of Zn in *B. pilosa* from Varkenslaagte is below FAO/WHO and EU standards (Table 2). However, the maximum concentration of Zn in B. pilosa from

Varkenslaagte exceeds the guideline values. Samples from Johannesburg commercial and Kraalkop plot 2 had elevated Zn concentrations compared to average and maximum Varkenslaagte concentrations and FAO/WHO and EU standards. *Bidens pilosa* samples across the three sites had higher Pb concentrations than 0.3 mg/kg FAO/WHO, EU and SA guidelines given in Table 2. The highest Pb concentration was found in Kraalkop (1.55 mg/kg) which was more than five times above standard; almost three times the average concentration of *B. pilosa* from Varkenslaagte but lower than maximum concentration in Varkenslaagte (2.1 mg/kg). Johannesburg residential concentrations exceeded the guidelines by 0.03 mg/kg. Although there were limited sample sizes for Johannesburg and Kraalkop, this study basically reflects high metal levels in Johannesburg commercial site and Kraalkop plot 2 compared to the Varkenslaagte average. However, the maximum As and Pb concentrations of *B. pilosa* from Varkenslaagte are higher than that of Johannesburg and Kraalkop samples.

4.2.3 Mean concentrations of metals in B. pilosa leaves subjected to different preparation and cooking methods

Mean metal concentrations of *B. pilosa* across different preparation and cooking methods are given in table 14 below.

Table 14: Metal concentrations in B. pilosa leaves exposed to different preparation and cooking methods (mean SD; mg/kg; unwashed: n=10; washed: n=12; tomato and onion: n=9; boiled: n=9)

Treatment	Fe	Range	Zn	Range	As	Range	Pb	Range
Unwashed	[w]611.5±274.2	812.6	[x]113.8±64.1	202.3	[y]0.31 ±0.3	0.833	[z]0.9±0.9	2.733
Washed	[w]442.5±117.2	356.9	[x]105.5±67.5	214.5	[y]0.26±0.2	0.633	[z]0.8±0.7	1.967
Tomato &0nion	357.2±99.0	281.4	68.0±26.3	94.4	[y]0.27 ±0.4	1.133	[z]2.6±1.0	3.367
Boiled	427.7±100.0	287.3	86.9±39.0	123.7	[y]0.30±0.2	0.633	[z]1.7±1.7	3.967

[W] exceeds the Maximum allowable limit of Fe

[X] exceeds FAO/WHO and EU guideline standards

[Y] exceeds FAO/WHO guideline standards

[Z] exceeds FAO/WHO, EU and South African standards.

There was no difference in concentrations of Fe, As, Zn and Pb between washed and unwashed treatments. The reason why there was no difference between concentrations in unwashed and washed vegetables could be due to sampling being undertaken while it was raining; therefore, dust levels in the atmosphere would have been minimal and the rains might also have washed off the dust that would otherwise have accumulated on the surface of the leaves.

Concentrations of Zn in washed and unwashed samples slightly exceeded the FAO/WHO and EU standards (Table 2) (unwashed 113.8 mg/kg; washed (105.5 mg/kg).

Cooking in the present study resulted in increases in Pb concentrations. Lead concentrations were: tomato and onion (2.6 mg/kg) > boiled (1.7 mg/kg) > unwashed (0.9 mg/kg) > washed (0.8 mg/kg). Boiling reduced the concentrations of Zn and Fe by over 18% and 0.5% respectively. According to Uusiku *et al*. (2010), Fe and Zn total concentrations are slightly affected or not affected at all by cooking (in water). In this study, there was no difference between concentrations of Zn and Fe in boiled and raw washed leaves. Cooking in tomato and onion reduced Zn concentrations by over 30% but significantly increased the concentration of Pb in *B. pilosa* cooked in tomato and onion. It is known that metal availability and mobility increase as pH decrease. Because tomato is slightly acidic, this might be the reason for the increase in Pb concentration in *B. pilosa* cooked in tomato and onion. However, the reasons for the decrease in Zn concentrations are unknown as there are no prior researches on effect of cooking in tomato and onion mixture. This is currently an unexplained association which can be investigated in future research as the characteristics of the cooked mixture of tomato and onion have not yet been explored.

There was no significant difference in the concentrations of Fe and Zn in washed and cooked *B. pilosa* (boiled in distilled water or cooked in tomato and onion). A difference was noticed between unwashed raw *B. pilosa* and *B. pilosa* cooked in tomato and onion.

Concentrations of As in *B. pilosa* from different sites were above the FAO/WHO standard of 0.1 mg/kg (Table 2).

An ANOVA test showed that the concentration of Fe and Pb is not the same across categories of treatment (9<n<12) (Fe (p=0.013) and Pb (p= 0.003) respectively). Distribution of As and Zn concentrations is the same across categories of treatment As (p=0.973) and Zn (0.268) (Table 15).

Table 15: Statistical test and p-values (different preparation and cooking methods)

Variable	Levene test	ANOVA	Kruskal Wallis
Fe	p=0.005	p=0.013	p=0.042
Zn	p=0.119	p=0.268	
As	p=0.651	p=0.973	
Pb	p=0.002	p=0.003	p=0.009

There was no difference in As and concentration across the categories of treatment. From these results, it can be concluded that boiling or cooking *B. pilosa* in tomato and onion has no effect on As concentrations. Arsenic concentrations across different treatments exceeded the FAO/WHO limit of 0.1 mg/kg.

Iron concentrations at Kraalkop and Varkenslaagte exceeded the maximum permissible limit of 425 mg/kg (Table 2). It is not yet established whether there are any health effects associated with high Fe concentrations in the blood. Iron concentrations at both Johannesburg sites were below the maximum permissible limit of Fe concentration in leafy vegetables.

A Tukey pairwise comparison used to determine where the difference in Fe and Pb lies has shown that there is a difference in Fe concentrations between unwashed and tomato and onion (p=0.01). There is a difference in Pb concentrations between (i) unwashed and tomato and onion (p=0.011) and (ii) between washed and tomato and onion (p=0.005). Multiple test comparison results are given in table 16 below:

Table 16: Tukey multiple test comparisons to show where there is a difference between treatments

Dependent variable	Treatment (i)	Treatment (j)	p-value
Fe	Unwashed	Washed	0.098
		tomato & onion	0.01
		Boiled	0.092
	Washed	tomato & onion	0.65
		Boiled	0.997
Pb	Unwashed	Washed	0.998
		tomato & onion	0.011
		Boiled	0.326
	Washed	tomato & onion	0.005
		Boiled	0.22

4.3 Bioconcentration factor between unfractioned soil concentrations and concentration in *B. pilosa* washed leaves

Bioconcentration factors in Table 17 below were calculated by dividing the mean metal concentration of washed *B. pilosa* leaves by mean metal concentration of unsieved soils. Unwashed and cooked leaves were not used because other factors (such as dust on the surface and cooking effects) may otherwise have resulted in an inaccurate value not representative of the true BCF. Concentrations of unfractioned soil were used in BCF calculations.

Table 17: Ratio of metal concentrations in *B.pilosa* leaves to corresponding soil concentrations

		Fe	Zn	As	Pb
Average	BCF	0.011	0.700	0.013	0.014
Varkenslaagte	BCF	0.011	0.951	0.012	0.017
Kraalkop		0.009	1.604	0.005	0.016
Jhb Residential		0.008	0.288	0.088	0.008
Jhb Commercial		0.011	0.371	0.026	0.008

High BCF indicates stronger metal accumulation by the plant. Mean BCF decrease as follows: Zn (0.700) > Pb (0.014) > As (0.013 > Fe (0.011). These findings are in line with the findings of Sun *et al*., (2009).

Concentrations of As from uncontaminated soils ranges from 0.1 mg/kg to 40 mg/kg with an average of 6 mg/kg. The BCF of As in these soils is 0.037 (Kabata-Pedias and Pedias, 1992).

Lead is accumulated in plant roots, with only a small amount translocated to other parts of the plant (Adriano, 2001). This accounts for low Pb concentrations in *B. pilosa* (0.780 mg/kg) compared to mean concentrations in soils (56.58 mg/kg) (BCF=0.014). The concentration range of Pb in uncontaminated soils is <1 to 300 mg/kg with an average of 19 mg/kg. The mean BCF in uncontaminated soils is 0.0016 whereas the BCF in contaminated soils is in between 0.01 and 1 (Kabata –Pedias and Pedias. 1992). These findings are supported by the findings of the present study where mean BCF of Pb is 0.014.

Zinc is an airborne element. Above-ground plants accumulate high Zn levels (Kabata-Pendias, 1993) and uptake of Zn from the ground is also promoted by low pH (Bodek *et al*., 1988). The pH ranges in soils were between 4 and 7. The pH as an important factor in controlling chemical processes, including elemental uptake, it may have contributed to high BCF. The BCF of Zn in contaminated soils is between 1 and 10 (Kabata-Pedias and Pedias, 1992). The present study BCF is 0.7 which is less than the range of Zn BCF in contaminated soils.

According to Kabata-Pendias and Pendias (1992), iron unavailability in plants is due to factors governing Fe solubility. Increase in redox potential and/ or pH result in a decrease in iron availability to plants (Kabata-Pendias and Pendias 1992). Plants can moderate Fe uptake by acquiring sufficient Fe despite the oxidation state (Kabata-Pendias and Pendias 1992). Concentrations of Fe in an area can vary depending on soil type and presence of other sources.

4.4 Potential dietary hazards through *Bidens pilosa* consumption

Concentrations of cooked *B. pilosa* (boiled and tomato and onion) were used to calculate DMI. According to Bubala (2013), leafy vegetables are cooked before consumption and onion and tomato are used regularly in the dish preparations (Table 18).

Table 18: Daily metal intake (mg/kg.d) and dietary risk index for Zn, As and Pb

		*Zn	As	Pb
Tomato and onion	Metal concentration	68	0.27	2.6
	DMI	8.88E-03	3.52E-05	3.39E-04
	DRI	2.96E-02	1.17E-02	9.42E-02
Boiled	Metal concentration	86.9	0.3	1.7
	DMI	1.13E-02	3.92E-05	2.22E-04
	DRI	3.78E-02	1.31E-02	6.17E-02

*The Zn RfD used to calculate the DRI was obtained from a secondary source of reference, DEA.

Generally, Zn and Pb concentrations in *B. pilosa* at all the sites and in different treatments exceeded the WHO, EU and SA guidelines. However, the DRI of As, Zn and Pb calculated from cooked vegetables (boiled and tomato and onion) were < 1, and the Hazard Index (HI) for As, Zn and Pb (summation of the DRIs of metals under investigation) was < 1 for both boiled and *B. pilosa* cooked in tomato and onion. This indicates no obvious potential risk from consumption of washed and cooked *B. pilosa* from the study site. These results are similar to the findings of Kamunda *et al*. (2016), where health risk assessment for Witwatersrand Basin goldfields was conducted. In Kamunda *et al*. (2016), samples were collected from TSFs and villages around the mine and were analysed using ICPMS. The results revealed a DRI which was less than one and it was concluded that there was no obvious risk for the exposed population. However, when dermal and inhalation exposure pathways were included, a value of 2.13 was obtained, meaning that there was a potential risk to the exposed populations.

Daily metal intake and consequently DRI in the present study and other studies (e.g. Cui *et al*., 2005; Khan *et al*., 2008; Arora *et al*., 2008; Mahmood and Malik, 2014; Khan *et al*., 2015; Kamunda *et al*., 2016) were calculated from the total metal content in vegetables. But it is only a fraction of the metal that is released in the gastrointestinal tract which is available for intestinal absorption (Benito and Miller, 1998). The ingested metal may be absorbed by intestinal epithelium or excreted by liver after being bio transformed, a process referred to as the first pass- effect. The fraction of the contaminants that reach the systematic circulation after ingestion, bioaccessibility, absorption and first pass effect (bioavailable fraction) (Oomen *et al*., 2002) is the one that has to be considered in calculation of risk index values. Therefore, the DMI and DRI in the present study, and other studies on this topic may be overrated. It is important to understand the route of the metals within the body of an organism so that state regulators and mining companies can ascertain the impacts of mining and potential risks to human health in the short, medium and long term land use planning, including mine closure planning. In future risk assessments DRI may be calculated based on the amount likely to reach the circulatory system, not simply the amount ingested.

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

Metal concentrations were analysed in rhizosphere soils and raw and cooked leaves of B. pilosa on the Varkenslaagte, and compared with samples collected at Kraalkop nature reserve and two sites in Johannesburg. The results were (i) compared with regulatory standards, (ii) food safety standards for amount likely to be consumed and (iii) conclusions drawn as to whether there are potential health risks from consuming *B. pilosa* from the study area. The findings of this study are that (i) mean concentrations of metals in the study site are generally lower in comparison to metal concentrations on soils and vegetables from Johannesburg and Kraalkop plot two located about 100m outside Kraalkop nature reserve; (ii) the DRI for consumption of *B. pilosa* is less than one which means there is no obvious hazard from consuming *B. pilosa* from the study areas at low volumes. However, only a four elements were analysed. Furthermore, it is always advisable to harvest from safe sites rather than those known to be contaminated.

This study had several limitations and these are:

(i) The project was conducted during the 2015-2016 drought and lack of rains might have affected the metal concentrations and uptake in plants as stated in Tutu *et al*. (2008) who found that pollution load and mobility are affected by seasons.

(ii) The XRF results adequately covered the objectives of the present study however the plant material CRM agreement was lower (between 70-85%) which lowers the confidence in the results. Soil CRM was above 90%.

(iii) This study did not address the cumulative effect of eating other WLVs substituted for *B. pilosa* which might be accumulating higher metal concentrations and being consumed in larger quantities and regularly. A risk assessment was beyond the scope of this research report, however, other studies can build upon the findings of this study.

(iv) The inhalation and dermal exposure pathways were not considered during calculations of the risk index; the cumulative effect of all the exposure pathways might be high.

(v) The DRI was calculated from the total concentrations in vegetables, whereas the concentrations that reach the systemic circulatory system is only a fraction of the total metal concentrations.

Recommendations

- Members of the community may consume products that may be contaminated by metals. The government, learning institutions, researchers and the regulators should conduct and publish more research on risk assessments on mining and post mining areas as well as highly industrialised and traffic congested areas.
- In addition to being aware of the consumption of potentially contaminated food from high metal risk areas, the consumers of WLVs must to be made aware of ways to improve the safety of foods, including washing prior to cooking and to avoid harvesting from hazardous sites in, for example, mining areas.
- Future studies in the goldfields should be conducted over a longer period of time (for example three to four years), with larger sample sizes, over different seasons to be able to see the trends in metal concentrations.
- Dietary risk index calculation needs to take to account the route of a hazard from the time it is introduced into its target organism to estimate the concentration likely to reach the systemic circulation and thus use the value for DRI calculations.

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APPENDICES

APPENDIX 1: Raw data soils

Element	Site	treatment (um)	Plot	Fe	Zn	As	Pb
NM 1V greater 50um	1	50	1	33640	57.2	22.2	30.05
NM 2V greater 50um	1	50	2	37980	45.3	11.1	19.6
NM 3V greater 50um	1	50	3	11550	27.5	4.2	13.7
NM 4V greater 50um	1	50	4	47190	75.6	15.2	25.9
NM 5V greater 50um	1	50	5	48070	86.7	11.3	36.5
NM 6V greater 50um	1	50	6	26670	55.9	7.2	18.2
NM7V greater 50um	1	50	7	35040	59.2	16.6	25.6
NM 8 V Greater 50 um	1	50	8	41450	63.8	14.8	22.9
JHB1 greater 50um	3	50	9	28920	254.3	5.8	37.3
JHB2 greater 50um	3	50	10	32510	620.7	7.8	170.8
KNM1 greater 50um	2	50	11	76280	50.2	12.8	26.2
KNM2 greater 50um	2	50	12	59150	135.8	58.4	292.6
NM1V greater 100um	1	100	1	35970	78.1	23.5	37.95
NM 2V greater 100um	1	100	2	30420	35.4	7.7	15.3
NM 3V greater 100um	1	100	3	11720	26.1	3.8	13.1

NM 4V greater 100um	1	100	4	53910	94.6	17.7	30.9
NM 5V greater 100um	1	100	5	52840	109.1	11	41.5
NM 6V greater 100um	1	100	6	26250	56.5	7.1	17.1
NM7V greater 100um	1	100	7	40490	68.6	18.7	30.1
NM 8V greater 100um	1	100	8	42340	65	14.8	23.5
JHB1 greater 100um	3	100	9	22410	204.6	4.7	26
JHB2 greaer 100um	3	100	10	23130	329.7	4.6	127
KNM1 greater 100um	2	100	11	63470	38.9	8.1	21.7
KNM2 greater 100um	2	100	12	48240	111.1	48	237.1
NM 1V greater 250um	1	200	1	41930	94.3	24.05	38.95
NM 2V greater 250um	1	250	2	33680	37.8	9.2	15.3
NM 3V greater 250um	1	250	3	15600	32.9	5.6	15.5
NM 4V greater 250um	1	250	4	52930	74	14.6	28.8
NM 5V greater 250um	1	250	5	58870	133.4	11.4	53.2
NM 6V greater 250um	1	250	6	28420	60.7	7.9	19.4
NM7V greater 250um	1	250	7	40310	65.9	17.2	28
NM 8V greater 250um	1	250	8	47840	72.1	17.4	26.6
JHB1 greater 250um	3	250	9	13630	96.5	1.9	16.1
JHB2 greater 250um	3	250	10	20040	245.5	5.2	215.8

KNM1 greater 250um	2	250	11	72820	44.1	8.1	29.8
KNM2 greater 250um	2	250	12	54000	120.5	46	235.1
NM 1V greater 500um	1	500	1	54065	105.75	26.2	43.7
NM 2V greater 500 um	1	500	2	43010	39.5	9.9	22.9
NM 3V greater 500um	1	500	3	13730	34.6	5.3	15.4
NM 4V greater 500 um	1	500	4	51720	70	15.6	26.2
NM 5V greater 500um	1	500	5	58280	178.5	9.6	237.7
NM 6V greater 500um	1	500	6	28630	61.6	8.4	18.9
NM7V greater 500um	1	500	7	44070	58	15.9	23.6
NM 8V greater 500um	1	500	8	50280	82	16.4	27.5
KNM1 greater 500um	2	500	11	76310	40.5	9.4	29.3
KNM2 greater 500um	2	500	12	74630	180.6	62.8	466.3
KNM1 Before sieving	2	1000	11	62450	58.1	17.5	27.8
NM1V Before sieving	1	1000	1	38385	120.7	43.5	53.45
KNM2 Before sieving	2	1000	12	59550	128.3	52.6	164.8
NM2V Before sieving	1	1000	2	40130	66.3	13.2	25.2
NM3V Before sieving	1	1000	3	18650	35.5	7.8	19
NM4V Before sieving	1	1000	4	49750	77.6	18.2	34.1
NM5V Before sieving	1	1000	5	54310	140.6	15.8	44.3

NM6V Before sieving	1	1000	6	29640	61.4	10.3	20.5
NM7V Before sieving	1	1000	7	36170	76.8	17.7	27.3
NM8V Before sieving	1	1000	8	45170	92.6	21.3	37.2
JHB1 Before sieving	3	1000	9	31720	302.9	8	39.4
JHB2 Before sieving	3	1000	10	35890	649.3	6.3	185.9

APPENDIX 2: Descriptive statistics by site

	Site		Fe	Zn	As	Pb
	Varkenslaagte	Mean	39025.625	83.938	18.475	32.631
		N	8	8	8	8
		Std. Deviation	11335.27	33.5803	11.0238	12.0073
		Range	35660	105.1	35.7	34.5
		Minimum	18650	35.5	7.8	19
		Maximum	54310	140.6	43.5	53.5
	Kraalkop	Mean	61000	93.2	35.05	96.3
		N	2	2	2	2
		Std. Deviation	2050.6097	49.6389	24.8194	96.8736
		Range	2900	70.2	35.1	137
		Minimum	59550	58.1	17.5	27.8
		Maximum	62450	128.3	52.6	164.8
	Johannesburg	Mean	33805	476.1	7.15	112.65
		N	2	2	2	2

		Std. Deviation	2948.6353	244.9418	1.2021	103.5911
		Range	4170	346.4	1.7	146.5
		Minimum	31720	302.9	6.3	39.4
		Maximum	35890	649.3	8	185.9
	Total	Mean	41817.917	150.842	19.35	56.579
		N	12	12	12	12
		Std. Deviation	12929.921	171.7277	14.3492	56.5334
		Range	43800	613.8	46.3	166.9
		Minimum	18650	35.5	6.3	19
		Maximum	62450	649.3	52.6	185.9

APPENDIX 3: Descriptive statistics -particle sizes

						95% Confidence Interval for Mean			
		N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
Fe	greater 50	12	39870.83	16615.364	4796.443	29313.93	50427.73	11550	76280
	greater 100	12	37599.17	15387.371	4441.951	27822.50	47375.84	11720	63470
	greater 250	12	40005.83	18401.773	5312.134	28313.90	51697.76	13630	72820
	greater 500	10	49472.50	18974.851	6000.375	35898.71	63046.29	13730	76310
	before sieving	12	41817.92	12929.920	3732.547	33602.64	50033.20	18650	62450
	Total	58	41487.07	16396.191	2152.925	37175.91	45798.23	11550	76310
Zn	greater 50	12	127.683	166.5900	48.0904	21.837	233.530	27.5	620.7
	greater 100	12	101.475	86.2674	24.9033	46.663	156.287	26.1	329.7
	greater 250	12	89.808	58.1238	16.7789	52.878	126.738	32.9	245.5
	greater 500	10	85.105	54.1739	17.1313	46.351	123.859	34.6	180.6
	before sieving	12	150.842	171.7277	49.5735	41.731	259.952	35.5	649.3
	Total	58	111.875	119.2546	15.6589	80.519	143.231	26.1	649.3
As	greater 50	12	15.617	14.4007	4.1571	6.467	24.766	4.2	58.4

	greater 100	12	14.142	12.4039	3.5807	6.261	22.023	3.8	48.0
	greater 250	12	14.046	11.8451	3.4194	6.520	21.572	1.9	46.0
	greater 500	10	17.950	16.8344	5.3235	5.907	29.993	5.3	62.8
	before sieving	12	19.350	14.3492	4.1423	10.233	28.467	6.3	52.6
	Total	58	16.161	13.6322	1.7900	12.577	19.746	1.9	62.8
Pb	greater 50	12	59.9458	84.60175	24.42242	6.1924	113.6992	13.70	292.60
	greater 100	12	51.7708	65.78429	18.99029	9.9735	93.5682	13.10	237.10
	greater 250	12	60.2125	78.04509	22.52968	10.6250	109.8000	15.30	235.10
	greater 500	10	91.1500	147.85141	46.75472	-14.6165	196.9165	15.40	466.30
	before sieving	12	56.5792	56.53337	16.31978	20.6596	92.4988	19.00	185.90
	Total	58	62.9931	87.39611	11.47567	40.0135	85.9727	13.10	466.30

APPENDIX 4: Normality test soils

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	treatment (um)	Statistic	df	Sig.	Statistic	df	Sig.
Fe	greater50	.144	12	.200*	.956	12	.721
	greater100	.103	12	.200*	.979	12	.981
	greater250	.111	12	.200*	.967	12	.874
	greater500	.167	10	.200*	.954	10	.713
	before sieving	.135	12	.200*	.973	12	.943
Zn	greater50	.347	12	.000	.581	12	.000
	greater100	.289	12	.007	.768	12	.004
	greater250	.204	12	.179	.819	12	.016
	greater500	.223	10	.173	.815	10	.022
	before sieving	.357	12	.000	.625	12	.000
As	greater50	.306	12	.003	.663	12	.000
	greater100	.202	12	.189	.774	12	.005
	greater250	.222	12	.106	.808	12	.012
	greater500	.337	10	.002	.670	10	.000
	before sieving	.282	12	.009	.781	12	.006
Pb	greater50	.439	12	.000	.561	12	.000
	greater100	.395	12	.000	.599	12	.000
	greater250	.369	12	.000	.591	12	.000
	greater500	.426	10	.000	.573	10	.000
	before sieving	.355	12	.000	.639	12	.000

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

APPENDIX 5: Test of homogeneity of variance

	Levene Statistic	df1	df2	Sig.
Fe	.349	4	53	.844
Zn	1.626	4	53	.181
As	.119	4	53	.975
Pb	1.875	4	53	.128

APPENDIX 6: Anova test, soils

		Sum of Squares	df	Mean Square	F	Sig.
Fe	Between Groups	878049341.300	4	219512335.300	.805	.527
	Within Groups	14445550410.000	53	272557554.900		
	Total	15323599750.000	57			
Zn	Between Groups	35527.157	4	8881.789	.607	.659
	Within Groups	775107.120	53	14624.663		
	Total	810634.276	57			
As	Between Groups	260.217	4	65.054	.334	.854
	Within Groups	10332.448	53	194.952		
	Total	10592.665	57			
Pb	Between Groups	10137.255	4	2534.314	.316	.866
	Within Groups	425233.333	53	8023.270		
	Total	435370.587	57			

APPENDIX 7: Kruskal-Wallis Test Soils

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of K is the same across categories of treatment (um).	Independent-Samples Kruskal-Wallis Test	.663	Retain the null hypothesis.
2	The distribution of Ca is the same across categories of treatment (um).	Independent-Samples Kruskal-Wallis Test	.779	Retain the null hypothesis.
3	The distribution of Fe is the same across categories of treatment (um).	Independent-Samples Kruskal-Wallis Test	.501	Retain the null hypothesis.
4	The distribution of Zn is the same across categories of treatment (um).	Independent-Samples Kruskal-Wallis Test	.723	Retain the null hypothesis.
5	The distribution of As is the same across categories of treatment (um).	Independent-Samples Kruskal-Wallis Test	.613	Retain the null hypothesis.
6	The distribution of Br is the same across categories of treatment (um).	Independent-Samples Kruskal-Wallis Test	.520	Retain the null hypothesis.
7	The distribution of Rb is the same across categories of treatment (um).	Independent-Samples Kruskal-Wallis Test	.829	Retain the null hypothesis.
8	The distribution of Sr is the same across categories of treatment (um).	Independent-Samples Kruskal-Wallis Test	.679	Retain the null hypothesis.
9	The distribution of Pb is the same across categories of treatment (um).	Independent-Samples Kruskal-Wallis Test	.820	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

APPENDIX 8: Raw Data *B. Pilosa*

Sample	Plot	Site	Treatment	Fe	Zn	As	Pb
NM 1V unwashed	1	1	1	579.4	173.3	0.8	1.6
NM 1V washed	1	1	2	576.5	157.6	0.0667	2.1
NM 2V unwashed	2	1	1	676.2	71.9	0.1667	1
NM 2V washed	2	1	2	615.8	83	0.1	0.167
NM 2V TO	2	1	3	499.4	68.1	0.1667	3
NM 2V boiled	2	1	4	552.5	66.8	0.1667	1.4
NM 3V unwashed	3	1	1	388.5	74	0.4	0.333
NM 3V washed	3	1	2	387.8	71.5	0.1667	0.333
NM 3V T&O	3	1	3	299.8	58.3	0.1667	2.5
NM 3V boiled	3	1	4	432.6	69.2	0.1667	1
NM 4V unwashed	4	1	1	557.2	78.8	0.0667	0.333
NM 4V washed	4	1	2	419	78.9	0.3	0.333
NM 4V T&O	4	1	3	354.4	57.4	1.2	0.333
NM 4V boiled	4	1	4	289.6	75.1	0.3	0.333
NM 5V unwashed	5	1	1	1073	117.2	0.9	0.333
NM 5V washed	5	1	2	407	114.7	0.6	0.333
N 5V T&O	5	1	3	379.9	85.2	0.1667	2.9
NM 5V boiled	5	1	4	426.4	68.6	0.3	0.333
NM 6V unwashed	6	1	1	260.4	95.6	0.1667	0.333
NM 6V washed	6	1	2	284.7	31.9	0.0667	0.1333
NM 6V T&O	6	1	3	253.6	31.5	0.1667	3
NM 6V boiled	6	1	4	509.3	104.8	0.8	0.333
NM7V unwashed	7	1	1	401.3	93.2	0.1667	0.0667
NM 7V washed	7	1	2	411.8	26.4	0.4	0.333
NM 7V T&O	7	1	3	218.7	73.7	0.1667	2

NM 7V boiled	7	1	4	265.2	78.4	0.1667	3.7
NM 8V unwashed	8	1	1	409.4	83.6	0.1667	1.5
NM 8V washed	8	1	2	408.4	74.9	0.1667	0.8
NM 8V T&O	8	1	3	309.5	52.6	0.0667	1.9
NM 8V boiled	8	1	4	388.5	71.8	0.1667	4
JHB1 washed	9	3	2	258.9	87.1	0.7	0.333
JHB2 washed	10	3	2	399.5	240.9	0.1667	1.4
NM 1K unwashed	11	2	1	1040	75.9	0.0667	0.333
NM 1K washed	11	2	2	609.6	79.2	0.1667	1.3
NM 1K T&O	11	2	3	500.1	59.7	0.1667	3.7
NM 1K boiled	11	2	4	525.9	62	0.4	0.333
NM 2K unwashed	12	2	1	729.7	274.2	0.1667	2.8
NM 2K washed	12	2	2	531.1	219.7	0.1667	1.8
NM 2K T&O	12	2	3	399.1	125.9	0.1667	3.7
NM 2K boiled	12	2	4	458.9	185.7	0.1667	4.3

APPENDIX 9: Descriptive statistics by site

Treatment	Site		Fe	Zn	As	Pb
Washed	Varkenslaagte	Mean	438.875	79.863	.233350	.566538
		N	8	8	8	8
		Std. Deviation	106.7002	42.2928	.1885500	.6515362
		Range	331.1	131.2	.5333	1.9667
		Minimum	284.7	26.4	.0667	.1333
		Maximum	615.8	157.6	.6000	2.1000
	Kraalkop	Mean	570.350	149.450	.166700	1.550000
		N	2	2	2	2
		Std. Deviation	55.5079	99.3485	.0000000	.3535534
		Range	78.5	140.5	.0000	.5000
		Minimum	531.1	79.2	.1667	1.3000
		Maximum	609.6	219.7	.1667	1.8000

JHB	Mean	329.200	164.000	.433350	.866500
	N	2	2	2	2
	Std. Deviation	99.4192	108.7530	.3771000	.7544829
	Range	140.6	153.8	.5333	1.0670
	Minimum	258.9	87.1	.1667	.3330
	Maximum	399.5	240.9	.7000	1.4000
Total	Mean	442.508	105.483	.255575	.780442
	N	12	12	12	12
	Std. Deviation	117.2138	67.5439	.2075877	.6896002
	Range	356.9	214.5	.6333	1.9667
	Minimum	258.9	26.4	.0667	.1333
	Maximum	615.8	240.9	.7000	2.1000

APPENDIX 10: Descriptive Statistics B. Pilosa

						95% Confidence Interval for Mean			
		N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
Fe	Unwashed	10	611.510	274.2180	86.7153	415.346	807.674	260.4	1073.0
	Washed	12	442.508	117.2138	33.8367	368.034	516.982	258.9	615.8
	Tomato and onion	9	357.167	98.9983	32.9994	281.070	433.263	218.7	500.1
	Boiled	9	427.656	99.9315	33.3105	350.841	504.470	265.2	552.5
	Total	40	462.215	184.2379	29.1306	403.293	521.137	218.7	1073.0
Zn	Unwashed	10	113.770	64.0734	20.2618	67.935	159.605	71.9	274.2
	Washed	12	105.483	67.5439	19.4983	62.568	148.399	26.4	240.9
	Tomato and onion	9	68.044	26.2809	8.7603	47.843	88.246	31.5	125.9
	Boiled	9	86.933	39.0409	13.0136	56.924	116.943	62.0	185.7
	Total	40	94.958	54.7298	8.6535	77.454	112.461	26.4	274.2
As	Unwashed	10	.306690	.3013405	.0952922	.091124	.522256	.0667	.9000
	Washed	12	.255575	.2075877	.0599254	.123680	.387470	.0667	.7000
	Tomato and onion	9	.270400	.3501653	.1167218	.001239	.539561	.0667	1.2000
	Boiled	9	.292611	.2086723	.0695574	.132211	.453011	.1667	.8000

	Total	40	.280023	.2600343	.0411150	.196859	.363186	.0667	1.2000
Pb	Unwashed	10	.863170	.8665557	.2740290	.243273	1.483067	.0667	2.8000
	Washed	12	.780442	.6896002	.1990704	.342291	1.218593	.1333	2.1000
	Tomato and onion	9	2.559222	1.0496621	.3498874	1.752381	3.366064	.3330	3.7000
	Boiled	9	1.748000	1.7350800	.5783600	.414299	3.081701	.3330	4.3000
	Total	40	1.419050	1.2963986	.2049786	1.004442	1.833658	.0667	4.3000

APPENDIX 11: Normality test

	Treatment	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	Df	Sig.	Statistic	df	Sig.
Fe	Unwashed	.169	10	.200*	.910	10	.278
	Washed	.246	12	.043	.905	12	.184
	Tomato and Onion	.147	9	.200*	.942	9	.601
	Boiled	.162	9	.200*	.931	9	.495
Zn	Unwashed	.312	10	.007	.693	10	.001
	Washed	.274	12	.013	.856	12	.044
	Tomato and Onion	.193	9	.200*	.895	9	.222
	Boiled	.364	9	.001	.630	9	.000
As	Unwashed	.379	10	.000	.724	10	.002
	Washed	.332	12	.001	.799	12	.009
	Tomato and Onion	.505	9	.000	.454	9	.000
	Boiled	.282	9	.037	.682	9	.001
Pb	Unwashed	.330	10	.003	.794	10	.012
	Washed	.325	12	.001	.817	12	.015
	Tomato and Onion	.183	9	.200*	.897	9	.234
	Boiled	.246	9	.123	.765	9	.008

APPENDIX 12: Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
Fe	4.998	3	36	.005
Zn	2.085	3	36	.119
As	.550	3	36	.651
Pb	5.786	3	36	.002

APPENDIX 13: Anova Test

		Sum of Squares	Df	Mean Square	F	Sig.
Fe	Between Groups	337615.771	3	112538.590	4.108	.013
	Within Groups	986185.040	36	27394.029		
	Total	1323800.811	39			
Zn	Between Groups	11966.918	3	3988.973	1.370	.268
	Within Groups	104851.720	36	2912.548		
	Total	116818.638	39			
As	Between Groups	.017	3	.006	.076	.973
	Within Groups	2.621	36	.073		

	Total	2.637	39			
Pb	Between Groups	20.658	3	6.886	5.523	.003
	Within Groups	44.888	36	1.247		
	Total	65.545	39			

APPENDIX 14: Multiple Comparisons

Multiple Comparisons							
Tukey HSD							
						95% Confidence Interval	
Dependent Variable	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Fe	Unwashed	Washed	169.0017	70.8678	.098	-21.861	359.865
		Tomato and onion	254.3433*	76.0472	.010	49.531	459.156
		Boiled	183.8544	76.0472	.092	-20.958	388.667
	Washed	Unwashed	-169.0017	70.8678	.098	-359.865	21.861
		Tomato and onion	85.3417	72.9837	.650	-111.220	281.903
		Boiled	14.8528	72.9837	.997	-181.709	211.414
	Tomato and	Unwashed	-254.3433*	76.0472	.010	-459.156	-49.531

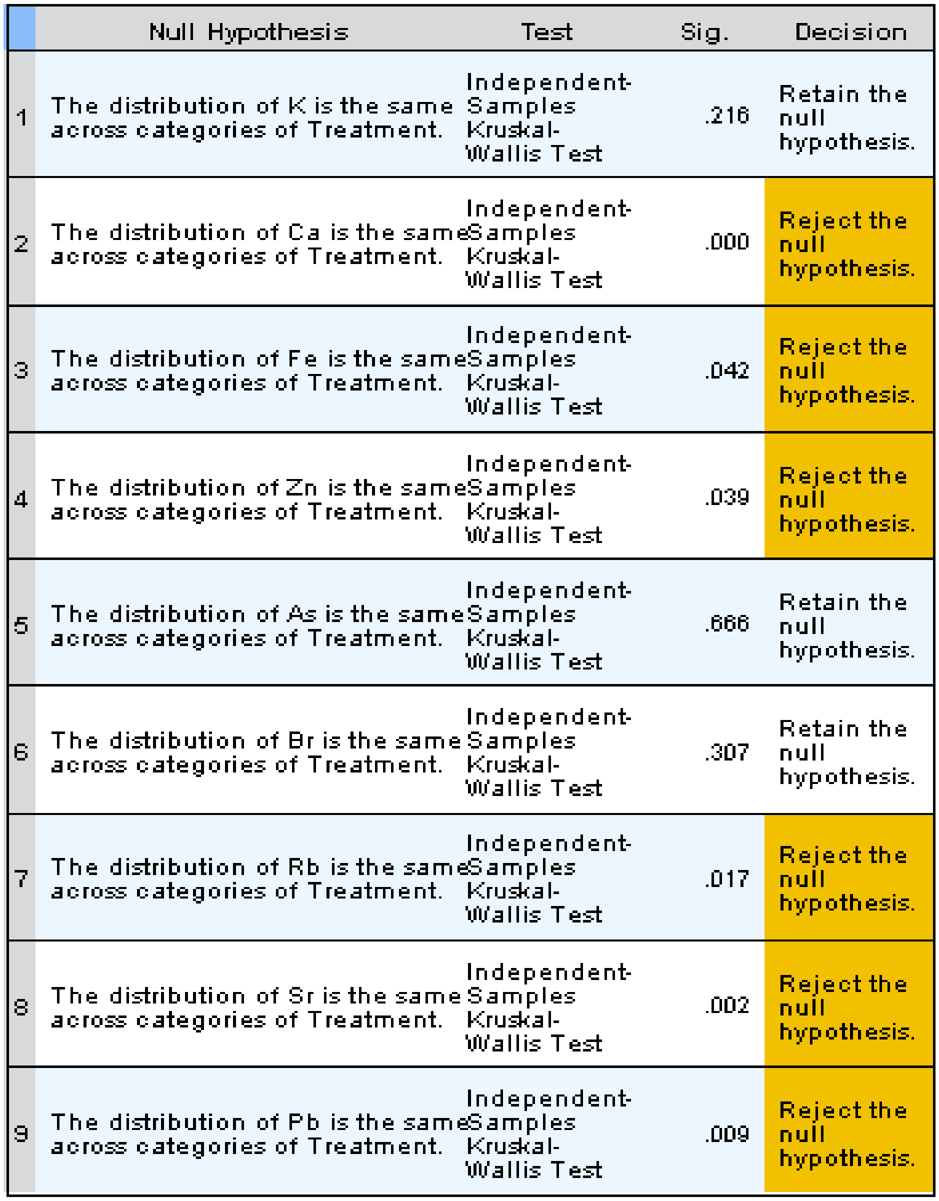
	onion	Washed	-85.3417	72.9837	.650	-281.903	111.220
		Boiled	-70.4889	78.0228	.803	-280.622	139.644
	Boiled	Unwashed	-183.8544	76.0472	.092	-388.667	20.958
		Washed	-14.8528	72.9837	.997	-211.414	181.709
		Tomato and onion	70.4889	78.0228	.803	-139.644	280.622
Zn	Unwashed	Washed	8.2867	23.1077	.984	-53.948	70.521
		Tomato and onion	45.7256	24.7966	.270	-21.057	112.508
		Boiled	26.8367	24.7966	.702	-39.946	93.620
	Washed	Unwashed	-8.2867	23.1077	.984	-70.521	53.948
		Tomato and onion	37.4389	23.7977	.406	-26.654	101.531
		Boiled	18.5500	23.7977	.863	-45.543	82.643
	Tomato and onion	Unwashed	-45.7256	24.7966	.270	-112.508	21.057
		Washed	-37.4389	23.7977	.406	-101.531	26.654
		Boiled	-18.8889	25.4408	.879	-87.407	49.629
	Boiled	Unwashed	-26.8367	24.7966	.702	-93.620	39.946
		Washed	-18.5500	23.7977	.863	-82.643	45.543
		Tomato and onion	18.8889	25.4408	.879	-49.629	87.407
As	Unwashed	Washed	.0511150	.1155223	.971	-.260013	.362243

		Tomato and onion	.0362900	.1239655	.991	-.297577	.370157
		Boiled	.0140789	.1239655	.999	-.319788	.347946
	Washed	Unwashed	-.0511150	.1155223	.971	-.362243	.260013
		Tomato and onion	-.0148250	.1189715	.999	-.335242	.305592
		Boiled	-.0370361	.1189715	.989	-.357453	.283381
	Tomato and onion	Unwashed	-.0362900	.1239655	.991	-.370157	.297577
		Washed	.0148250	.1189715	.999	-.305592	.335242
		Boiled	-.0222111	.1271859	.998	-.364752	.320329
	Boiled	Unwashed	-.0140789	.1239655	.999	-.347946	.319788
		Washed	.0370361	.1189715	.989	-.283381	.357453
		Tomato and onion	.0222111	.1271859	.998	-.320329	.364752
Pb	Unwashed	Washed	.0827283	.4781156	.998	-1.204945	1.370402
		Tomato and onion	-1.6960522*	.5130595	.011	-3.077838	-.314267
		Boiled	-.8848300	.5130595	.326	-2.266616	.496956
	Washed	Unwashed	-.0827283	.4781156	.998	-1.370402	1.204945
		Tomato and onion	-1.7787806*	.4923908	.005	-3.104901	-.452660

		Boiled	-.9675583	.4923908	.220	-2.293679	.358562
	Tomato and onion	Unwashed	1.6960522*	.5130595	.011	.314267	3.077838
		Washed	1.7787806*	.4923908	.005	.452660	3.104901
		Boiled	.8112222	.5263879	.424	-.606460	2.228904
	Boiled	Unwashed	.8848300	.5130595	.326	-.496956	2.266616
		Washed	.9675583	.4923908	.220	-.358562	2.293679
		Tomato and onion	-.8112222	.5263879	.424	-2.228904	.606460
*. The mean difference is significant at the 0.05 level.							

APPENDIX 15: Kruskal Wallis test

Hypothesis Test Summary



	Null Hypothesis	Test	Sig.	Decision
1	The distribution of K is the same across categories of Treatment.	Independent-Samples Kruskal-Wallis Test	.216	Retain the null hypothesis.
2	The distribution of Ca is the same across categories of Treatment.	Independent-Samples Kruskal-Wallis Test	.000	Reject the null hypothesis.
3	The distribution of Fe is the same across categories of Treatment.	Independent-Samples Kruskal-Wallis Test	.042	Reject the null hypothesis.
4	The distribution of Zn is the same across categories of Treatment.	Independent-Samples Kruskal-Wallis Test	.039	Reject the null hypothesis.
5	The distribution of As is the same across categories of Treatment.	Independent-Samples Kruskal-Wallis Test	.666	Retain the null hypothesis.
6	The distribution of Br is the same across categories of Treatment.	Independent-Samples Kruskal-Wallis Test	.307	Retain the null hypothesis.
7	The distribution of Rb is the same across categories of Treatment.	Independent-Samples Kruskal-Wallis Test	.017	Reject the null hypothesis.
8	The distribution of Sr is the same across categories of Treatment.	Independent-Samples Kruskal-Wallis Test	.002	Reject the null hypothesis.
9	The distribution of Pb is the same across categories of Treatment.	Independent-Samples Kruskal-Wallis Test	.009	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.