ELEMENTAL COMPOSITION AND NUTRITIONAL VALUE OF THE EDIBLE FRUITS OF COASTAL RED MILKWOOD (*MIMUSOPS CAFFRA*) AND TRANSVAAL RED MILKWOOD (*MIMUSOPS ZEYHERI*) AND THE IMPACT OF SOIL QUALITY

by

Sihle Vitalis Mngadi

Submitted in fulfilment of the academic requirement for the degree of Master of Science in the School of Chemistry and Physics, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Durban, South Africa

2017

Elemental Composition and Nutritional value of the edible fruits of Coastal red milkwood (*Mimusops caffra*) and Transvaal red milkwood (*Mimusops zeyheri*) and the impact of soil quality

SIHLE VITALIS MNGADI

2017

A thesis submitted to the School of Chemistry and Physics, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Westville, for the Degree of Master of Science.

This thesis has been prepared according to Format 4 as outlined in the guidelines from the College of Agriculture, Engineering and Science which states:

This is a thesis in which chapters are written as a set of discrete research papers, with an overall introduction and final discussion where one (or all) of the chapters have already been published. Typically, these chapters will have been published in internationally recognized, peer- reviewed journals.

As the candidate's supervisor(s), I have approved this thesis for submission.

Supervisor:

Name: Prof SB Jonnalagadda Signature Date:

Co-Supervisor:

Name: Dr Roshila Moodley Signature Date: 11/07/2017

ABSTRACT

Mimusops caffra and *Mimusops zeyheri*, both of the plant family Sapotaceae are indigenous plant species that grow widely in most parts of South Africa and the edible fruits of these species are picked and eaten raw in rural communities across South Africa. This is done to help overcome the challenge of dietary diversity especially in these resource-limited communities where diets are based on starch staples that lack fruit and vegetables. Despite the dependence on these fruit for food and nutrition security, information on their nutritional value is lacking. The aim of this research was therefore to analytically investigate the elemental distribution of essential and toxic elements in the two edible fruits and corresponding growth soil using Inductively Coupled Plasma- Mass Spectrometry (ICP-MS). The nutritional value of the fruits was assessed to evaluate the plants potential as a source of nutrients and the impact of soil quality on elemental uptake was evaluated to determine the plants potential to accumulate toxic metals.

The elemental analysis showed concentration of the elements in *M. caffra* fruit to be (in descending order): K > Na> Ca > Mg > Si > Al > Fe > Zn > Mn > Ni > Cr > Cu > Pb > Mo > Sb > As > Se > V > Cd > Co whilst for *M. zeyheri* fruit it was (in descending order): K > Na> Ca > Mg > Fe > Al > Zn > Mn > Cu > Cr > Sr > Pb > As > Li > Ni ≈ Co > Rb > U > Bi > Ga > Be > Tl > Mo > Ba > Ag > Cd. The concentration of most essential elements in both fruits was found to be within acceptable limits. *M. caffra* fruits were found to be rich in Fe, Si and Cr and *M. zeyheri* fruits were found to be rich in Cr and Mn. Analysis of soil and plant showed that *M. caffra* and *M. zeyheri* do not tend to accumulate toxic elements and would therefore be safe for human consumption. Statistical analyses showed that contamination in soil by the various heavy metals came from various sources however soil contamination did not affect the

concentration of heavy metals in the fruits thereby indicating the plants ability to take up metals to meet metabolic needs and exclude metals, if at elevated levels, for survival.

ABBREVIATIONS

AC	Applied current
B	Exchangeable
BAF	Bioaccumulation factor
BCR	Community Bureau of Reference
CEC	Cation exchange capacity
CGS	Council for Geoscience
COA	Certificate of analysis
CRM	Certified reference material
DC	Direct current
DRI	Dietary reference intake
EDTA	Ethylenediamminetetraacetic acid
FAO	Food and Agriculture Organization
HIV/AIDS	Human Immune Virus Acquired Immune Deficiency Syndrome
I_{geo}	Geoaccumulation index
ICP-MS	Inductively coupled plasma-mass spectrometry
ND	Not determinable
ppm	Parts per million
r	Correlation coefficient
RDA	Recommended dietary allowance
SANB	South African National Biodiversity
SD	Standard deviation
SOM	Soil organic matter
T	Total

ST	Total soil concentration
UKZN	University of KwaZulu-Natal
UL	Tolerable upper intake level
WHO	Word Health Organization

DECLARATION 1 - PLAGIARISM

I, Sihle Mngadi, declare that

- The research reported in this thesis, except where otherwise indicated, is my original research.
- This thesis has not been submitted for any degree or examination at any other university.
- This thesis does not contain other persons’ data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
- This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
    - Their words have been re-written but the general information attributed to them has been referenced
    - Where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.
- This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References sections.

Signed

……………………………………………………………………………………

DELARATION 2 - PUBLICATIONS

Publication 1

Title: Elemental composition and nutritional value of the edible fruits of Coastal red milkwood (*Mimusops caffra*) and the impact of soil quality on chemical characteristics

Authors*:* Sihle Mngadi, Roshila Moodley and Sreekanth B. Jonnalagadda

Journal: *Journal of Environmental Science and Health, Part B*

Publication 2

Title: Heavy metal distribution and nutritional value of the edible fruits of Transvaal red milkwood (*Mimusops zeyheri*) and impact of soil quality

Authors: Sihle Mngadi, Roshila Moodley and Sreekanth B. Jonnalagadda

Journal: Manuscript prepared for *Environmental Monitoring and Assessment*

In the preparation of the above manuscripts, I performed all the experiments and interpreted the data. The co-authors contributed in verifying the scientific content as well as editing the manuscript.

Signed: ……………………………………………………………………………

ACKNOWLEDGEMENTS

“Do not let this Book of the Law to depart from your mouth, meditate on it day and night and do everything that is written in it and thus you will be successful and prosperous” (Joshua 1(8-12). I would like to thank my supervisors: Prof. S.B Jonnalagadda and Dr Roshila Moodley who provided support and guidance throughout the project.

I would also like to acknowledge the following individuals for the success of my research:

- Dr Colin Southway for assisting with analytical investigation at the UKZN PMB Campus.
- My Mom Monica Dlamini for being supportive financially with the research.
- My Brother Dr Sphiwe Dlamini for encouraging me to complete my studies.
- Mrs Nontobeko Magwaza for always advising me to complete the project.
- My colleagues in the Natural Products Research group for their support.
- My colleagues at Umgeni Water: Mr N. Khumalo, Ms H. Dlamini and Mr S. Khuboni for continual advice towards the completion of the project.
- Dr N. Mahlangeni and Ms B. Shelembe for assisting me in the laboratory.
- Council for Geoscience for financial assistance.
- Dr L.J Jordaan for assisting with correcting/reviewing all my manuscripts.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE – GENERAL INTRODUCTION

1.1 INTRODUCTION

Accessibility and availability to nutritionally balanced food is one of the critical concerns of the United Nations charter that is expressed in the constitutions of member states signatory to the charter (Food and Agriculture Organisation (FAO), 1996). South Africa, like other developing countries, faces the problem of malnutrition, poverty and a bad healthcare system, which should not be the case especially in a country where there is a high rate of people who are suffering from HIV/AIDS (Goggin et al., 2009). Recent research has shown that approximately 14 million people live with food insecurity and 1.5 million children suffer from malnutrition (WHO, 2005). This is because most people from rural communities are not eating a balanced diet as they cannot afford commercially available fruits and vegetables due to economic and physical inaccessibility (Moodley et al., 2007). In light of this, there should be more focus on promoting indigenous locally grown fruit and vegetable as these are easily accessible and of high nutritional value.

The accumulation of heavy metals in soil is a serious problem since most people rely on consuming locally grown foods such as Amadumbe and sweet potato which can accumulate these heavy metals (Mlambo et al., 2015). Plants absorb these heavy metals from the soil and they are thus introduced into the food chain and, if these are at elevated concentrations, they can cause serious problems (Street et al., 2008; Liu et al., 2013). It is therefore critical to determine the potential toxicities of these metals in food to ensure that they comply with standards set by health organisations such as the World Health Organisation (WHO, 2005).

For the human body to function properly, it requires a balance of all of the essential elements. These essential elements are required for different metabolic activities in the body however, their concentrations need to be within a specific range for optimal functioning of the body (Mahlangeni et al., 2016). Macronutrients (S, N, P, K, Na, Mg, Fe and Ca) are essential nutrients required by the body at higher levels and these play a major role in the human body for different metabolic activities. Micronutrients (Zn, Co, F, Mn, B and Si) are required at lower levels but these nutrients are also vital for proper functioning of the body (Rengel et al., 1999).

Most plants are exposed to metals based on different factors such as fertilisers, nature of soil, presence of industries in the area and pesticide treatment (Chunilall et al., 2004). Plants also depend on many factors for nutrition such as the ability of the soil to provide nutrients, circulation of nutrients in soil and the movement of nutrients through the plant (Chunilall et al., 2004). The movement of these elements occur via different processes such as wet deposition, gas deposition and dust fall. Soil parameters such as its cation exchange capacity (CEC), organic matter content and pH are the three critical factors that affect the uptake of elements by most plants (Greger, 2011).

Mimusops caffra (Coastal red milk wood) and *Mimusops zeyheri* (Transvaal red milk wood) are two indigenous plant species that produce berry-like, sweet pulpy edible fruits which are rich in vitamin C and these are normally picked by people in most rural areas in South Africa. They are normally eaten raw and used for making different products such as alcoholic beverages, jelly, juice preservatives and jam (Pooley, 1993). Despite the well-documented ethno-botanical literature in South Africa, very little information on the chemical and nutritional quality of wild edible fruits is available including the fruits of *M. caffra* and *M. zeyheri*.

1.2 PROBLEM STATEMENT

There are more than 50 000 edible plants in the world but, of these, only a few including rice, corn and wheat are consumed in significant amounts. In South Africa, traditional diets in most rural communities consist mostly of starchy and legume staples to obtain nourishment therefore malnutrition is common in these communities (Shackleton et al., 2005). In some low income communities, people tend to consume wild fruits to introduce dietary diversity to their meals as commercially available fruits are not accessible or economically affordable (Ewell & Matura, 1991). Despite this fact, there is limited information on the chemical and nutritional quality of wild fruits. This knowledge can contribute to the understanding of the nutritional value of fruits grown in the wild and their potential can be exploited to improve food insecurity in these communities. Also, awareness on the consumption of wild fruits may be created and promoted.

1.3 AIMS AND OBJECTIVES

The aim of the study was to analytically investigate two plant species from the *Mimusops* genus (*Mimusops caffra* (Coastal red milkwood) and *Mimusops zeyheri* (Transvaal red milkwood)) which produce edible fruits. The nutritional value of these wild fruits was investigated to evaluate their potential as a food source and the impact of soil quality on elemental uptake was determined to assess for metal toxicities.

The objective of this study was:

- To collect *M. caffra* samples from various regions along the east coast of KwaZulu-Natal, South Africa.
- To collect *M. zeyheri* samples from various regions in Gauteng and North West Province, South Africa.

- To determine the concentration of elements in *M. caffra* and *M. zeyheri* fruits and concentration of elements (total and exchangeable) in the growth soil from the different sampling sites.
- To evaluate the nutritional value of the fruits of both species by comparing to dietary reference intakes (DRIs) for essential nutrients.
- To compare elemental concentrations in fruits to concentrations in soil to evaluate the impact of soil quality on uptake by the plant and to assess for metal toxicities for the two species.
- To determine the proximate chemical composition (moisture, ash, protein and carbohydrate) in the fruits.
- To evaluate the impact of different soil parameters (pH, soil organic matter and cation exchange capacity) on elemental uptake by the two species.
- To statistically analyse the data to reveal relationships in soil or between soil and plant.

1.4 REFERENCES

1. Chunilall, V, Kindness, A, Jonnalagadda, SB. 2004. Heavy metal uptake by spinach leaves grown on contaminated soils with lead, mercury, cadmium, and nickel. Journal of Environmental Science and Health, 39 (3): 473 - 481.
2. Ewell, PT, Matuura J. 1991**.** Tropical root crops in a developing economy. Proceedings, 9th Symposium of the International Society for Tropical Root Crops: Accra, Ghana. pp 20–26.
3. FAO, 1996. Rome Declaration on World Food Security and World Food Summit Plan of Action. World Food Summit, 13 – 17 November 1996, Rome, Italy.
4. Goggin, K, Pinkston, M, Gqaleni, N, Puoane, T, Wilson, D, Berkley-Patton, J, Martinez, DA. 2009. The role of South African traditional health practitioners in HIV/AIDS prevention and treatment. In: HIV/AIDS global frontiers in prevention/ intervention. Taylor & Francis: New York.
5. Greger, M. 1999. Metal availability and bioconcentration in plants: Heavy Metal Stress in Plants from Molecules to Ecosystems. J. Eds. Springer Verlag: Heidelberg, Germany. 1-27.
6. Liu, X, Song, Q, Tang, Y, Li, W, Xu, J, Wu, J, Wang, F, Brookes, PC. 2013. Human health risk assessment of heavy metals in soil-vegetable system: A multi-medium analysis. Science of the Total Environment, 463-464: 530-540.
7. Mahlangeni, N, Moodley, R, Jonnalagadda, SB. 2016**.** Heavy metal distribution in *Laportea peduncularis* and growth soil from the eastern parts of KwaZulu-Natal, South Africa. Environmental Monitoring and Assessment. 188(2), 76.
8. Mlambo, L, Moodley, R, Koorbanally N. 2015. Elemental composition of the fruits of Baboon grape (*Rhoicissus digitata*) and impact of soil quality on chemical characteristics. Journal of Food and Nutrition Research, 325-368.

9. Moodley, R, Kindness, A, Jonnalagadda, SB. 2007. Chemical composition of Macadamia nuts (*Macadamia integrifolia*) and impact of soil quality. Journal of Environmental Science and Health Part A, 42: 2097-2104.
10. Pooley, E. 1993. The Complete Field Guide to Trees of Natal, Zululand and Transkei; Natal Flora Publications Trust: Durban, South Africa; 311-322.
11. Rengel, Z, Batten, GD, Crowley, DE. 1999. Agronomic approaches for improving the micronutrient density in edible portions of field crops. Field Crops Res, 60, 27-40.
12. Shackleton, SE, Shackleton, CM, Netshiluvhi, TR, Geach, BS, Ballance, A, Fairbanks, D.H.K. 2005. Use patterns and value of Savanna resources in three rural villages in South Africa. Economic Botany, 56(2): 130-146.
13. Street, RA, Stirk, WA, van Staden, J. 2008. South African traditional medicinal plant trade-Challenges in regulating quality, safety and efficacy. Journal of Ethnopharmacology, 119: 705-710.
14. World Health Organisation. 2005. National policy on traditional medicine and regulation of herbal medicine: Report of a WHO global survey, Geneva.

CHAPTER TWO - LITERATURE REVIEW

2.1 THE PLANT FAMILY SAPOTACEAE

Sapotaceae, is a family of flowering plants belonging to the order Ericales, which consists of 800 species of evergreen trees and shrubs and about 65 genera (Table 1). Most of the species in this family produce edible fruits, of which, most are of economic use (SANB, 2016). Some of the fruits are used for traditional and western cosmetics and medication. Most fruits are used for making different products such as jelly and alcoholic beverages and they are also eaten raw by people.

Table 1 : Diversity and distribution of Sapotaceae species (SANB, 2016; Pooley, 2003).

Genus	No. of species	Distribution
Mimusops balata	1	America and some parts of Africa
Mimusops elengi	20	Coastal tropical Africa to Southern Africa
Amuphospermum anitilogum	1	Queensland New South Wales and Papau New Guinea
Aubreginia tainsil	1	Tropical part of Africa (Ghana)
Enylerophytum magalismontanum	600	Subtropical part of KwaZulu-Natal, South Africa
Nimeyera whitei	24	Poorer soils in Queensland
Sideroxylon acunae	81	North and South America, Africa & Asia
Pouteria austria	3	Rainforest in Australia
Synespalum brevipes	23	African tropics, subtropical low lands of Angola and Zimbabwe
Synespalum dilcifum	20	West Africa

2.2 PHYTOCHEMISTRY OF SAPOTACAEAE SPECIES

Species from the Sapotaceae family are known to consist of a wide range of chemical constituents such as saponins, flavonoids and polyphenolic compounds. The biological activities of some traditional medicinal plants belonging to the family Sapotaceae have been investigated (Moustafa et al., 2016). *Manilkara zapota* fruits were reported to contain phenolic compounds such as myricetin-3-O-α-L-rhaminopyranoside, apigenin-7-O-α-L-rhamnopyranoside and caffeic acid. The leaves of this plant were found to contain quercetin, (+)-catechin, (-)-epicatechin, (+)-gallocatechin, gallic acid, dihydromyricetin, methylchlorogenate, methyl-4-O-galloylchlorogenate and 4-ogalloylchlorogenic acid, and these compounds are known to have anti-inflammatory activity (Moustafa et al., 2016). The leaves of *Pouteria* species were found to contain myricetin-3-O-α-L-arabinopyranoside, myricetin-3-O-β-D-galactopyranoside and myricetin-3-O-α-L-rhaminopyranoside (Moustafa et al., 2016).

2.3 THE GENUS *MIMUSOPS*

Mimusops is a genus containing 57 species that belong to the plant family Sapotaceae that is native to tropical and subtropical regions of Asia, Africa, Australia, and various oceanic islands. *Mimusops elengi* is commonly found in most parts of India and it has been used for centuries for medicinal purposes (Baby and Raj, 2010). One of the popular species of *Mimusops* found in the lower regions of East Africa to South Africa is *Mimusops obavata*. In South Africa, it is widely found in the region of KwaZulu-Natal to Eastern Cape, Gauteng, and North West and extends up to Limpopo (Boon et al., 2010). This plant has whitish flowers which turn into oval, narrow tip edible fruits which turn shiny yellow to red when ripe. The fruits act as a source of food since they are enjoyed by people because of its sweetness (Leister, 2005). In addition, it has been used in traditional medicine. Table 2 highlights some of the medicinal uses of *Mimusops* species.

Table 2: *Mimusops* species commonly used for traditional purposes.

Plant species	Part used	Therapeutic uses
Mimusops balata	Leaf	Decoction as astringent used to treat diarrhoea and ulcers (Schlickmann and da Silva, 2015).
Mimusops obavata	Root/bark/fruits	Used to treat gonorrhoea, stomach pains, fruit is eaten (Leister, 2005).
Mimusops elengi	Seed/bark/leaves/ stem/flowers/ seed	Ensure safe delivery during pregnancy, cure cancer related diseases and toothache, used to fix loose teeth, seed is used as inhibitory activity against HIV protease, fruits are used for gonorrhoea and flowers are used as perfume (Gami et al., 2012).
Mimusops zeyheri	Leaves	Inflammation, bleeding gums, tuberculosis, sexually transmitted diseases (Okatchi et al., 2012).

2.4 PHYTOCHEMISTRY OF *MIMUSOPS* SPECIES

Biological studies on *Mimusops* species have shown some species to possess antifungal, gastroprotective and antinociceptive properties (Gami et al., 2012). The active compounds that were isolated from the fruits of *M. balata* were taxafolin, urosilic acid, spinasterol and taraxerone (Schlickmann, 2015). The leaves of *M. elengi*, extracted using ethanol, yielded β-carotene, hentriacontane, β-sitosterol and quercetin (Manjeshwar et al., 2011). The bark of *M. elengi* contained saponins and tannins. The flowers of *M. elengi* contained D-mannitol, β-sitoterol, β-sitosterol-D-glucoside and lupeol (Gami et al., 2012). *M. hexandra* and *M. manilkara* fruits were found to be rich in triterpenoids (Gami et al., 2012).

2.5 ANALYTICAL STUDIES ON *MIMUSOPS* SPECIES

The uptake, accumulation and concentration of heavy metals in edible plants need to be studied since, if at elevated levels that are not phytotoxic, they can cause serious health effects if ingested. Eliton et al (2011) reported that *M. Zeyheri* fruits contained 91.1%, 83.3%, 9.3% and 2.8% of dry matter, organic matter, protein and ash, respectively. The leaves of *M. Zeyheri* were found to contain N (6.33%), P (0.33%), K (1.25%), Ca (0.39%), Mg (0.06%), Zn (0.0029%), Cu (0.0014%), Fe (0.0409%), Al (0.007407%) and Mn (0.005185%) (Okatchi et al., 2012). Findings from a study conducted in Botswana on the root of *M. Zeyheri* showed the plant to contain Cr (0.73 mg kg^{-1}), As (0.73 mg kg^{-1}) and Pb (0.20 mg kg^{-1}) (Okatchi et al., 2012). All of the elements were within permissible limits therefore the plant was regarded safe for medical use.

Research conducted in Bangladesh on the fruits of *M. elengi* showed them to be rich in Ca and K (Mannan et al., 2016). The heavy metals Cd, Cr, Cu and Pb were found to be at safe levels for consumption since their concentrations were within recommended limits for edible plants set by FOA/WHO (Mannan et al., 2016). On the other hand, studies conducted in Pakistan on *M. elengi* bark showed that the plant may not be safe for human consumption or medicinal purposes as Hg levels were found to exceed maximum permissible limits (Table 3) (Akram et al., 2015).

In South Africa, not much research has been conducted on the chemical composition and nutritional value of *Mimusops* species. Therefore, this research focuses on the two indigenous plants that are consumed as food and used for medicinal purposes, *M. caffra* (Coastal red milkwood) and *M. zeyheri* (Transvaal red milkwood).

Table 3: Heavy metals content of *M. elengi* bark compared to permissible limits.

Metal	**Concentration (Akram, 2015) mg kg $^{-1}$**	**Permissible limits (WHO, 2005) mg kg $^{-1}$**
Na	2.89	51430
Fe	0.82	20
Mg	2.00	2000
Ca	5.08	614
Cu	0.08	10
Zn	0.20	50
Ni	0.00	1.5
Mn	0.13	200
Cd	0.00	0.3
Hg	0.49	0.1

2.6 *MIMUSOPS CAFFRA*

Mimusops caffra, commonly known as Coastal red milkwood (English), umThunzi (Zulu), umHlophe (Xhosa) and Moepel (Afrikaans), belongs to the plant family Sapotaceae.

Figure 1: Fruits of Coastal red milkwood (*Mimusops caffra*).

M. caffra is an indigenous shrub or small to medium-sized evergreen tree that grows in large, uniform groups in the dune forests of the KwaZulu-Natal coast, Southern Africa and Mozambique (Botha et al., 2003). It is commonly known as umThunzi which means shade tree (Pooley, 1993). The tree normally grows 4 – 10 m height and occasionally reaches a height of 25 m. The bark is dark grey in colour and contains milky latex. The leaves are hardy and leathery, with a blue-green upper colour and have whitish hairs below (Palmer et al., 1972). The wood of this tree is strong and it is commonly used for construction purposes and boat building. The bark is used in traditional medicine to treat wounds and sores (Pooley, 1993). The plant species produces berry-like fruit (Figure 1) which are eaten raw by most communities in South Africa and are used for jellies and alcoholic beverages (Pooley, 2003). Research conducted by Simelane et al. (2014) showed that the fruits of *M. caffra* contain the pentacyclic triterpenoid, ursolic acid. Biological studies on the leaves and bark of *M. caffra* showed the extracts to possess good antimalarial activity relative to chloroquine (Simelane et al., 2014).

2.7 *MIMUSOPS ZEYHERI*

M. zeyheri, also known as Transvaal red milkwood or Mmupudu, is widely distributed in most parts of Northern South Africa (Gauteng, Limpopo, North West) and other parts of Africa. The tree grows to a height of 15 m and can withstand different soil and climatic conditions such as draught and frosty conditions (Mashela and Mollel, 2001). The leaves of this plant are glossy and dark green in colour. Normally, the leaves flush in winter, the flowers blossom between April and September during the harvest of fruits. The fruits are oval with pointed tips ripening yellow or orange from April to September (Figure 2) (Mashela and Mollel, 2001). This plant has gained its popularity amongst people through its tasty, sweet fruit that is rich in vitamin C (Mashela and Mollel, 2001). The fruits are used to make jams, jellies, fermented juices and different beverages (Eliton et al., 2011). Ripened fruits are also sold in street and open markets.

Figure 2: Fruits of Transvaal red milkwood (*Mimusops zeyheri*) (Eliton et al., 2011).

2.8 SOIL ANALYSIS

Water, nutrients and air are stored in soil and these play a crucial role in the growth and survival of both plants and animals (Mengel et al., 2001). It is crucial for many human activities including plantation of food and it is a source of raw materials such as gold and diamonds that play a major role in a countries economy (Gerrard, 2000). There are different types of soils such as sand, silt and clay. Sandy soils have large particle sizes (0.0625- 2 mm) therefore it cannot hold water which results in nutrients being carried away by runoff (Baker 1982). The particle size for silt is between 0.004 and 0.0625 mm while for clay it is <0.004 mm. Silt has more water holding capacity compared to sandy soil (Webb et al., 1993). Clay with smallest particle size, is chemically active, has good water holding capacity and binds with plant nutrients. Plants normally start their life at horizon A; at this horizon there is a variety of rich nutrients compared to other horizons (Figure 3) (Webb et al., 1993).

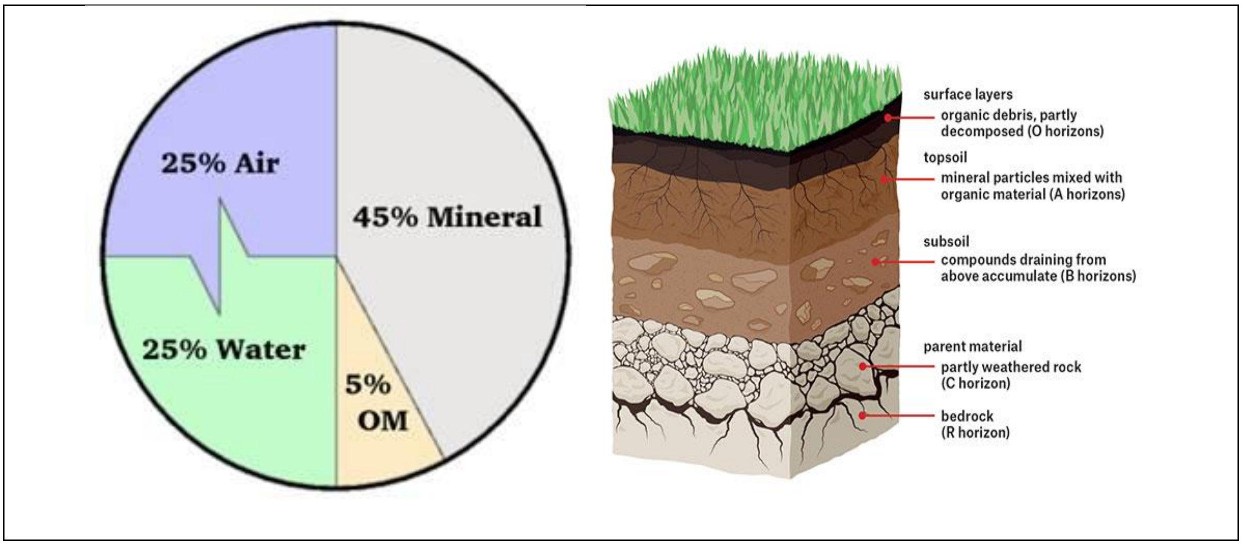


Figure 3: Soil composition and profile
(www.pmfias.com%25252Fsoil-profile-soil-horizon-soil-types-sandy-clayey-loamy%25252)

The O horizon is the surface layer that contains large amounts of organic material derived from plant residues that are not fully decomposed. This layer normally occurs in forest areas. The A horizon is where most biological activities occurs (Webb et al., 1993). This is where organisms such as fungi, worms and anthropoids are found, often in close association with plant roots. The B horizon, also called illuvated horizon, is normally referred to as the subsoil (layer below the A horizon) (Webb et al., 1993). The C Horizon is a layer of unconsolidated parent material found below the B Horizon. This layer contains large amounts of un-weathered rock and usually accumulated soluble compounds that come from the B Horizon (Webb et al., 1993).

Availability and mobility of nutrients for uptake by the plant is affected by different factors such as soil pH, soil organic matter (SOM), temperature, cation exchange capacity (CEC), root type and energy supply for different parts of the plant (Chunilall, 2004). The three most common factors that affect the mobility of nutrients are cation exchange capacity (CEC), soil organic matter (SOM) and soil pH since these are capable of overcoming any pollution in the environment.

2.8.1 Cation exchange capacity (CEC)

Soil contains organic matter that contains negative charges on the surface where adsorption and desorption occur by exchange. The measure of these negative charges on the soil surface that retain positive charged ions like Ca and Mg is referred to as the CEC (Chapman, 1965). If the soil pH increases the exchange capacity of these cations also increases.

In this study, CEC is determined using the Chapman method. In this method, the cations are replaced by ammonium acetate ($CH_3COO^- NH_4^+$) and excess ammonium ions are removed with ethanol (Horneck et al., 1989).

Soil CEC is calculated as follows:

$$CEC\ (meq/100g) = [(B - S)) \times M] \times 100 / [Mass\ of\ sample]\ in\ grams$$

Where: B = Titration of blank; S = Titration of sample; M = Molarity of standard alkali solution

2.8.2 Soil organic matter (SOM)

The amount of plant and animal residue in soil is generally described as SOM. SOM plays a major role in understanding the physical properties of soil and also contributes to the ability of how metals bind to soil. Both nutrients and water in the soil are stored by the organic matter. SOM can be determined using two techniques namely dry combustion and wet technique (Chunilall et al., 2004). In the two methods, organic carbon is oxidised and the CO_2 formed is then measured as SOM. Dry combustion involves the ashing of the sample in a furnace at high temperature (>600°C) that totally burns the samples and leaves the dissolved form that is volatile (Chunilall et al., 2004). In the wet digestion method, the organic matter is decomposed by introducing a strong oxidising agent like potassium dichromate ($K_2Cr_2O_7$). This method is called the Walkley–Black Method and it is also applied in this research (Walkley & Black, 1934). The Wackley-Black Method is the oxidation of carbon by potassium dichromate producing CO_2; the excess dichromate ion is titrated using a back titration technique where it reacts with ferrous ion as shown in the equation below: (Walkey & Black, 1934).

a. Dichromate ion reacts with carbon in the soil as follows:

$$2Cr_2O_7^{2-} + 3C^o + 16H^+ \rightarrow 4Cr^{3+} + 3CO_2 + 8H_2O$$

b. Ferrous ion reacts with dichromate as follows:

$$6Fe^{2+} + Cr_2O_7^{2-} + 14H^+ \rightarrow 2Cr^{3+} + 6Fe^{3+} + 7H_2O$$

2.8.3 Soil pH

Soil pH is the most important soil property that determines the plants chemical and biological process (Chunilal et al., 2004). The growth of plants depends on soil pH since it affects the amount of essential nutrients and the level of toxic elements available to the plant (Figure 4). McCauley et al. (2003) reported that metals are more soluble and more exchangeable for plant uptake at low soil pH. Soil pH also affects the solubility of pollutants and how these adsorb to colloids. The mechanism for how pollutants contaminate the environment (food chain, ground and surface water) is determined by soil pH (Brady and Weil, 2002). A mixture of soil slurry and water (1:1 ratio) is used for determining soil pH; a glass electrode is immersed into the mixture and the electrical potential is measured. There are many factors that affect the measurement of soil pH. These include amount of carbon dioxide in the air, ratio of soil and water and the amount of salt and electrolyte (Skoog et al., 2004).

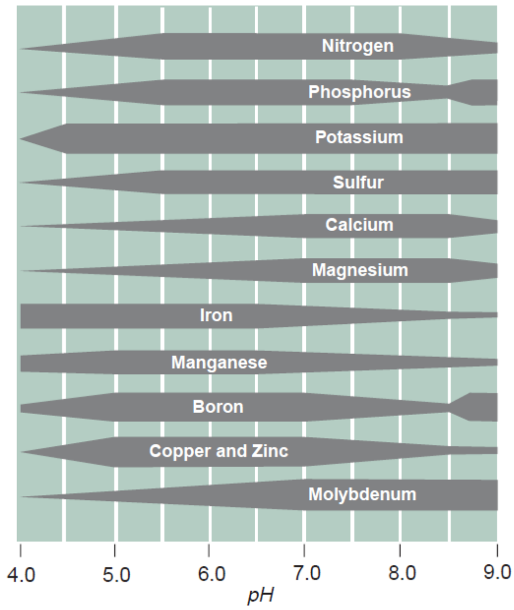


Figure 4: The effect of soil pH on the availability of different nutrients.

http://www.cogs.asn.au/organic-principles/soil-basics/).

2.8.4 Soil-plant relationships

Metals can be found in soil in different forms; available form, in solution, adhered to organic and inorganic colloids or tightly bound to the crystal lattice of clay. There are different extracting methods available to assess mobility and availability of metals in soil. A variety of extractants are available to simultaneously extract exchangeable nutrients from the soil. These include acetic acid, ammonium acetate and ethylenediaminetetraacetic acid (EDTA). Acetic acid plays a major role by releasing bioavailable nutrients that are tightly bound to the soil and further dissolves them (Chen et al., 2004). Acidic ammonium acetate however releases not only the exchangeable metals but also those in the carbonate 'pool' therefore it is more effective in releasing the available metals from the soil, it prevents re-adsorption of these released metals (Ure, 1996).

EDTA is a powerful chelating agent and is used extensively in soil science to determine the bioavailability of elements due to its non-selective nature. EDTA is a chelating agent that has the ability to extract strongly bound ions because of its multidentate nature and is known to complex with different metals (Beckett 1989; Podlesakova et al., 2001; Stover et al., 1976; Njukeng et al., 2013). Simplicity, efficiency, type of nutrient to extract and cost determines the type of extractant to be used for extracting nutrients in soil (Njukeng et al., 2013).

Soil stores nutrients, both organic and inorganic. The movement of nutrients in soil depends on many factors such as nutrient mobility, nutrient absorption, nutrient concentration and soil structure. There are three mechanisms by which the plant draws nutrients from the soil; root extension, mass flow and diffusion (Mengel et al., 2001). Root extension is when the roots grow out of the soil. Mass-flow occurs when soil solution containing dissolved nutrients moves into the root surface since the roots store water. The amount of nutrients transported to the root depends on the concentration of the nutrients and also the amount of water. Diffusion is described by the movement of nutrients from a region of a high concentration (soil) to a region of a low concentration (root surface) (Brady and Weil, 2002).

Plants have the ability to control appropriate concentration levels of essential and non-essential metals (Anderson et al., 2001). The amount of metal present in the plant is crucial; if the metal is found at high levels then it may have a negative impact on the plant (Wei et al., 2009). Plants respond in different ways when exposed to high concentrations of metals in soil. These are excluders, indicators and hyper-accumulators (Naicker et al., 2016). Excluders are plants that are not sensitive to heavy metals. These plants maintain a constant concentration of heavy metals in plant tissue. Hyper-accumulator plants have the ability to tolerate and accumulate heavy metals (Callan et al., 2006). These plants are normally found in contaminated sites. Naicker et al. (2016) reported that the plant *Berkheyi coddi* is a hyper-accumulator of Ni (>3 % Ni m/m).

Plants require essential nutrients (both micro and macronutrients) for growth and survival. Humans also require these nutrients for growth and survival since they are used for different processes in the human body (Rengel, Z et al., 1999).

2.8.5 Heavy metals

The amount of heavy metals in soil is increasing significantly due to human activities such as agricultural and food waste, industrial waste, mining activities, municipal waste, atmospheric deposits etc. which later affect the environment (Zhang et al., 2011). These heavy metals have a negative impact on humans, plants and other microorganisms in the environment. Previously, contamination of soil was not a threat (Wood, 1983). However, recently this has become a concern worldwide due to different health issues associated with high concentrations of heavy metals.

A study conducted in Nanjing (China) showed heavy metal contamination by Pb from burning leaded gasoline (Chen, 2007). Agricultural activities such as application of fertilisers and pesticides results in heavy metal contamination of soil. High Cd uptake by plants is due to Cd from fertilisers (Zhang et al., 2011). High concentrations of heavy metals in the soil affects plants if present at levels above threshold levels. Zhang et al. (2011) reported that citrus fruits growing in soil containing more than 50 mg kg^{-1} Cu will not grow properly.

Humans are more affected by the metal contamination in soil. Heavy metals affect the environment and human health through pollution of water, food and air. Recent research conducted in most cities in China showed that most children were found to have high Pb content in their bloodstream exceeding their normal value (100 g L^{-1}). Lead enters the human body and thus causes diseases related to cancer (Chong et al., 2001). Arsenic is also one of the heavy metals known to cause health problems. In 1951, an As factory produced As which contaminated soils, water and also the plants growing in the surrounding area. Arsenic in the soil reached extremely high levels (92.7 mg kg^{-1}) and caused all plant life to die. Furthermore, the high As content caused the deaths of more than 400 workers in the factory due to cancer-related conditions (Chen, 2007).

2.8.6 Determination of soil contamination

Soil contamination can be assessed using various methods, one of which is by determining the geoaccumulation index (I_{geo}) (Yaqin, 2008). I_{geo} is classified based on the degree of pollution as indicated by Table 4.

Table 4: Different classes to assess the pollution in soils (Stoffers et al., 1986).

I_{geo} value	I_{geo} class	Soil quality
0	0	Unpolluted
0-1	1	Unpolluted to moderately polluted
1-2	2	Moderately polluted
2-3	3	Moderately polluted to strongly polluted
3-4	4	Strongly polluted
4-5	5	From strongly to extremely polluted
>5	6	Extremely polluted

2.9 NUTRITION STATUS IN SOUTH AFRICA

South Africa is affected by malnutrition and food insecurity and these are a threat to the country especially due to the high rate of people infected by HIV (Mngadi et al., 2015). Provision of stable and healthy food is also a challenge. Recent research conducted in most parts of rural South African communities showed that they rely on locally grown fruits and vegetables to overcome the issue of malnutrition and food insecurity (WHO, 2005). In 2001, approximately 54% of deaths in children living in most developing countries were related to malnutrition (WHO, 2005)

The major nutrients that humans eat daily are carbohydrates, proteins and fats. These are required in high amounts (Table 5). Carbohydrates provide energy and they are the body's main source of fuel (Hu et al., 2001). They play a major role in ensuring proper functioning of tissues and cells in the human body. Simple carbohydrates are obtained from fruits and vegetables and complex carbohydrates are obtained from cereals, starchy vegetables and legumes (Hu et al., 2001). Proteins are essential for physiological processes and perform different functions in every system in the human body. Fats or lipids provide energy for muscles and other bodily processes. They also play a role in digestion and absorption of food and nutrients (Table 5, 6 and 7) (Hu et al., 2001).

Table 5: Dietary Reference Intake (DRI): Recommended Dietary Allowances (RDAs) for Individuals for Macronutrients and Vitamins.

Lifestage (years)	Carbohydrates (g/d)	Total Fibre (g/d)	Fat (g/d)	Protein (g/d)	Vitamin A (g/d)	Vitamin C (g/d)	Vitamin E (g/d)
9-18	130	31-38	ND	34-52	600-900	45-75	11-15
19-70	130	30-38	ND	56	900	90	15
>70	130	30	ND	56	900	90	15

Macronutrients (S, N, P, K, Na, Mg, Fe and Ca) are essential elements or minerals required by the body in high amounts (but lower than the major nutrients) and these play a major role in the human body for different metabolic activities. Micronutrients (Zn, Co, F, Mn, B and Si) are essential elements or minerals required in lower amounts but these nutrients are also vital for proper functioning of the body (Rengel et al., 1999). Table 6 provides the Dietary Reference Intake (DRI) and Recommended Dietary Allowances (RDAs) for essential elements and Table 7 provides the Tolerable Upper Intake Levels (ULs) for elements.

Table 6: Dietary Reference Intake (DRI): Recommended Dietary Allowances (RDAs) for Individuals for Essential Elements.

Lifestage (years)	Ca (mg/d)	Cr (µg/d)	Cu (µg/d)	Fe (mg/d)	Mg (mg/d)	Mn (mg/d)	Se (µg/d)	Zn (mg/d)
9-18	1 300	23 -25	700-890	8-11	240-410	1.9-2.2	40-55	8-11
19-70	1 000	35	900	8	400-420	2.3	55	11
>70	1 200	30	900	8	420	2.3	55	11

Table 7: Dietary Reference Intake (DRI): Tolerable Upper Intake Levels (ULs) for Individuals Essential Elements.

Lifestage (years)	Ca (mg/d)	Cr (μg/d)	Cu (μg/d)	Fe (mg/d)	Mg (mg/d)	Mn (mg/d)	Se (μg/d)	Zn (mg/d)
9-18	3000	ND	8000	40-45	350	6-9	400	23-34
19-70	2500	ND	10000	40	350	11	400	40
>70	2000	ND	10000	40	350	11	400	40

2.10 INSTRUMENTATION

The basic techniques used for the preparation and analysis of the fruits and soil samples in this study were:

- **Digestion -** the microwave accelerated reaction system (MARS 6, CEM Corporation, USA) was used for the digestion of all samples.
- **Inductively Coupled Plasma - Mass Spectrometry (**ICP-MS) – ICP-MS was used to determine the concentrations of all elements in all samples.

2.10.1 Microwave Digestion

The two main systems that are used for decomposing samples are open and closed vessel systems. Microwave digestion (closed vessel) (Figure 5) is the preferred method of decomposing both organic and inorganic samples instead of heating the sample directly on a hotplate (open vessel) (Levine et al., 1999). Microwaves come in different forms but their principal is common (Matusiewicz, 2003). Microwave digestion uses sealed bombs that allow complete digestion of both organic and inorganic samples in TFM reaction vessels that operate at high temperatures and high pressures (Skoog et al., 2004).

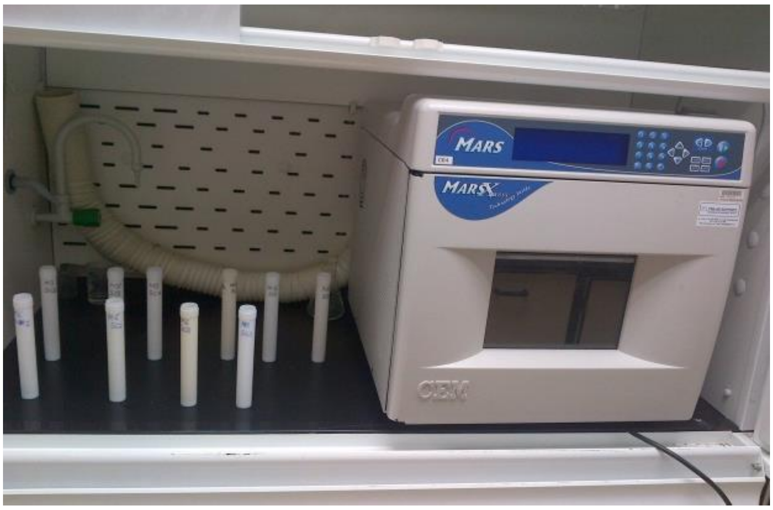


Figure 5: CEM MARS 6 microwave.

Some of the advantages of this type of digestion are.

- Fast decomposition times - 10-30 minutes compared to 1-2 hours using open vessels.
- Closed vessels prevent the loss of volatile compounds.
- Contamination is not possible since nothing can come in and out of the system.
- High temperatures allow complete digestion and high precision with replicate analyses.
- Less expensive chemicals are needed – HNO_3 is good enough for digestion.
- Smaller sample size can be run.

2.10.2 Inductively Coupled Plasma - Mass Spectrometry (ICP-MS)

Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) was introduced commercially to the market back in 1983 as an analytical technique used for analysis of trace, minor and major elements in the field of analytical chemistry (Agilent Manual, 2008). Some of the advantages that makes ICP-MS the preferred method of analysis over other spectroscopic methods is that it is sensitive, can analyse at low parts per trillion (ppt) levels, can analyse a wide range of elements in the periodic table, has a wide linear range, can analyse isotopes of elements and high sample throughput (Figure 6) (Agilent manual 2008). The ICP-MS process involves the introduction of sample, generation of ion, plasma interface, focusing of ion, separation and measurement of ion.

Samples analysed using ICP-MS enter through a simple pneumatic nebuliser as liquid then it is converted into an aerosol. The large aerosol droplets that are formed are removed by a spray chamber. Inside the spray chamber, the temperature is controlled by a thermoelectric device that prohibits the drift in the signal and also reduces solvent loading on the plasma. The remaining small droplets are focused through the central channel of the argon plasma

(Novak et al., 2003). The aerosol droplets formed are dried, decomposed, vaporised and atomised then ionised by means of the high temperature plasma (10 000 K).

Figure 6: ICP-MS used in this study.

The resulting ions formed are then positioned through the spectrometer interface (Agilent Manual, 2008). In this region, positively charged ions produced in the plasma are extracted through the vacuum system through an interface (cones). The ions pass through these cones that have a small diameter (1 mm) to ensure that high vacuum is constant in this region. The interface region produces ions which are then directed to the main vacuum chamber where the MS and detector are housed (Novak et al., 2003). The ion lenses play a major role in separating the ions from other material that is present in the system.

The ions containing the analyte enter a region where separation occurs. The commonly used mass analyser is quadrapole which uses both DC and AC electrical field for separating ions based on the ratio between mass and charge (Novak et al., 2003). The ion passes the quadrapole based on their mass to charge ratio; the single charged ions exit the quadrapole first to the detector. The widely used detector in MS is the Electron Multiplier detector (EM). The detector detects each ion and gives a signal for each mass (m/z). The height of the signal/peak is directly proportional to the amount of element present in the sample (Agilent Manual, 2008).

REFERENCES

1. Agilent Manual, Part Number G3270-90106 Edition Rev. A, September 2008 Printed in USA Agilent Technologies, Inc. 9-1 Takakura-cho, Hachioji-shi, Tokyo 192-8510 Japan.
2. Akram, S, Najam, R, Rizwani G, Abbas S. 2015. Determination of heavy metal content by atomic absorption spectroscopy (AAS) in some medicinal plants from Pakistan and Malaysian origin. Pakistan Journal of Pharmaceutical Science, 28 (5): 1781-1787.
3. Anderson, C, Deram, A, Petit, D, Brooks, R, Stewart, R, Simcock, R. 2001. Trace Elements in Soils: Bioavailability, Flux and Transfer, (I. Iskandar, M. Kirkham, eds.), vol. 4, Lewis Publishers, Boca Raton, Florida, USA, pp. 63–76.
4. Baby, J, Raj, SJ. 2010. Pharmacognostic and Phytochemical Properties of *Aloe vera* Linn-an overview. International Journal of Pharmaceutical Science Revision, 4(2):106-110.
5. Baker, DE, Amacher, MC. 1982. Nickel, Copper, Zinc and Cadmium. In: Page, A.L., Millers, R.H. and Keeney, D.R., Eds., Methods of Soil Analysis Part 2: Chemical and Microbiological Properties, 2nd Edition, Agronomy Monograph No. 9, American Society of Agronomy, Madison, 323-334.
6. Beckett, PHT. 1989. The use of extractants in studies on trace metals in soil, sewage sludge and sludge-treated soils. Advances in Soil Science, 9, 143-176.
7. Boon, R. 2010. Pooleys tress of Eastern South Africa. Flora & Fauna Publications Trust, Durban.
8. Botha, J, Shackleton, C, Emanuel, PL. 2003. Productivity and abundance of *Sclerocarya birrea* subsp. *caffra* in and around rural settlements and protected areas of the Bushbuckridge Lowveld, South Africa. Forests Trees and Livelihoods, 13; 217-232.
9. Brady, NC, Weil, RR. 2002. The Nature and Properties of Soils, Pearson Education, New Jersey. American Journal of Plant Science, 5 (9): 1258-1269.

10. Callahan, DL, Baker, AJ, Kolev, SD, Wedd AG. 2006. Metal ion ligands in hyper-accumulating plants, Journal of Biological Inorganic Chemistry, 11, 2–12.

11. Chapman, HD. 1965. Cation-exchange capacity. In: C.A. Black (ed.). Methods of soil analysis - Chemical and microbiological properties. Agronomy, 9: 891-901.

12. Chen, Y, Li, X, Shen, Z. 2004. Leaching and uptake of heavy metal by ten different species of plants during an EDTA-assisted phytoextraction process. Chemosphere, 57, 187–196.

13. Chen, CW, Kao, CM, Chen, CF, Dong, CD. 2007. Distribution and accumulation of heavy metals in sediments of Kaohsiung Harbour. Taiwan. Chemosphere, 66: 1431-1440.

14. Chong, K, Wang, WX. 2001. Comparative studies on the biokinetics of Cd, Cr, and Zn in the Green mussel *Perna viridis* and the Manila clam *Ruditapes philippinarum*. Journal of Environmental Pollution, 115: 107-121.

15. Chunilall, V, Kindness, A, Jonnalagadda SB. 2004, Heavy metal uptake by spinach leaves grown on contaminated soils with lead, mercury, cadmium, and nickel, Journal of Environmental Science and Health, 39 (3): 473 – 481.

16. Eliton, C, Davidson, B, Pretorius, B, Erlwanger, K. 2011. Proximate, mineral, amino acid, fatty acid, vitamin E, phytate phosphate and fibre composition of *Mimusops zeyheri* (Red Milkwood) seed, International. Journal of Food Science and Technology, 46, 555–560.

17. Gami, B, Pathak, S, Parabia, M. 2012. Ethnobotanical, phytochemical and pharmacological review of *Mimusops elengi* Linn. Asian Pac Trop Boimed, 2 (9):743-748.

18. Gerrard, J. 2000. Fundamentals of soils. Taylor & Francis Group. New York. USA, pp 1-22.

19. Horneck, DA, Hart, JM, Topper, K, Koepsell, B. 1989. Potentially mineralizable nitrogen. In: Methods of soil analysis used in the soil testing laboratory at Oregon State University. SM 89:4. Agricultural Experiment Station. Oregon State University. Corvallis, OR.

20. Hu, F, van Dam, R, Liu S. 2001. Diet and risk of Type II diabetes: the role of types of fat and carbohydrate. Journal of Diabetologia, 44: 805-817.
21. Leister, O.A. 2005. Seed plants of Southern Africa: families and genera. Strelitzia 10. National Biodiversity Pretoria.
22. Levine, KE, Batchelor, JD, Rhoades, CB, Jones, BT. 1999. Evaluation of a high pressure, high temperature microwave digestion system. Journal of Analytical Atomic Spectrometry, 14: 49-59.
23. Manjeshwar, SB, Ramakrishana, JP, Harshith, PB, Princy, LP, Rekha B. 2011. Chemistry and medicinal properties of the Bakul (*Mimusops elengi* Linn.): A review. Food Res International, 44 (7):1823-1829.
24. Mannan, A, Mustari, F, Hossain D, Hassan, K, Zahan, M, Haque, H, Zaman, S. 2016. Determination of Essential and Harmful Heavy Metals in Some Typical Medicinal Plants Grown in Bangladesh. Journal of Chemical Biology and Physical. Science, 6 (3): 911-920.
25. Mashela, PW, Mollel, N. 2001. Farmer – identified and selected indigenous fruit tree with suitable attributes for smallholder farming systems in South Africa's Northern Province. South African Journal of Famers, 30:1-12.
26. Matusiewicz, H. 2003. Chapter 6: Wet digestion methods. In: Sample preparation for trace element analysis. Mester, Z, Sturgeon, R. (eds). Elsevier: Amsterdam, Vol 41: pp 193-233.
27. Mengel, K, Kirkby, EA, Kosegarten, H, Appel, T. 2001. The soil as a plant nutrient medium. In Principles of plant nutrition, 5th edition. Kluwer Academic Publishers: Netherlands, pp. 15-34.
28. McCauley, A, Jones, C, Jacobsen, J. 2003. Soil pH and organic matter. Nutrient Management Module 8. www.landresources.montana.edu. (Date accessed: 12 May 2017).
29. Mishra, G, Mitra, R. 1967. Constituents of fruit and seed of *Mimusops Elengi*. J. Phytochem, 6:453

30. Mngadi, S, Moodley, R, Jonalaggadda, S. 2015. Elemental distribution and chemical composition of Amadumbe (*Colocasia esculanta*) leaf and impact of soil quality. Fresenius Environmental Bulletin, 24(4), 1250-1257.

31. Moustafa, H, Amal, M, Mohamed, R, Eman, G. 2016. A Review on Phenolic Compounds from Family Sapotaceae. Journal of Pharmacognosy and Phytochemistry, 5(2): 280-287.

32. Naicker, C, Kindness, A, Pillay, L. 2016. Identification of Chelidonic Acid as the Predominant Ligand involved in Ni Uptake in the Hyperaccumulator *Berkheya coddii*, S. African Journal of Chemistry, 69, 201–207

33. Njukeng, NJ, Nkeng, EG, Ehabe, EE, Schnug, E. 2013. A Comparative study on the use of calcium acetate lactate, Calcium chloride and acidic ammonium acetate-ethylenediaminetetraacetic acid (AAAc-EDTA) for the quantification of extractable, P, K and Mg from acidic soils. International Research Journal of Pure & Applied Chemistry, 3(1): 22-31.

34. Novák M, Emmanuel S, Vile M, Erel Y, Véron A, Pačes T, Wieder KR, Vaněček M, Štěpánová M, Břízová E, Hovorka J. 2003. ICP-MS measurements of lead isotopic ratios in soils heavily contaminated by lead smelting: tracing the sources of pollution, Environ Sci Technol 37:437–445.

35. Okatchi, H, Raletamo, K, Marobela, K. 2012. Determination of potentially toxic heavy metals in traditionally used medicinal plants for HIV/AIDS opportunistic infections in Ngamiland District in Northern Botswana, Journal of Analytica Chimica Acta, 730 (4): 42-48.

36. Palmer, E, Pitman, N. 1972. Trees of southern Africa, vol. 3. Balkema, Cape Town.

37. Podlesakova, E, Nemecek, J, Vacha, R. 2001 Mobility and bioavailability of trace metals in soils. In Trace Elements in Soils: Bioavailability, Flux and Transfer; Iskandor, I.K.; Kirkham, M.B., Eds. Lewis Publishers: Boca Raton, Florida.

38. Pooley, E. 1993. The complete field guide to trees of Natal, Zululand and Transkei. Natal Flora Publications Trust, Durban.

39. Pooley, E. 2003. The complete field guide to trees of Natal, Zululand and Transkei. Natal Flora Publication Durban, KwaZulu-Natal.

40. Rengel, Z, Batten, GD, Crowley, DE. 1999. Agronomic approaches for improving the micronutrient density in edible portions of field crops. Field Crops Res., 60, 27-40.

41. Schlickmann, F, da Silva, L. 2015. Gastroprotective bio-guiding study of fruits from *Mimusops balata*, Naunyn-Schmiedeberg's Archives of Pharmacology, 388 (11): 1187–1200.

42. Simelane, B. 2014. Antimalarial activity of *Hypoxis colchicifolia*, bak, *Mimusops caffra*, E. Mey. ex A.DC and *mimusops obtusifolia* Lam. from South Africa, PHD Thesis, pp 4-7.

43. Skoog, DA, West, DM, Holler, FJ, Crouch, SR. 2004. Fundamentals of analytical chemistry; Brooks/Cole Publications: USA, 258-265.

44. South African National Biodiversity Institute (SANBI). Webpage: PlantZ Africa.. http://www.plantzafrica.com/. Accessed June 2017.

45. Stoffers, P, Glasby, GP, Wilson, CJ, Davis, KR, Watter, P. 1986. Heavy metal pollution in Wellington Harbour. New Zealand Journal of Marine Freshwater Research, 20: 495-512.

46. Stover, RC, Sommers, LE, Silveira, J. 1976. Evaluation of metals in waste water sludge. Journal of Water Pollution Control Federation, 48: 2165-2175.

47. Ure, A. M. 1996. Single extraction schemes for soil analysis and related applications. The Science of the Total Environment, 178: 3-10.

48. Walkley, A, Black, IA. 1934. An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Science, 37: 29-37.

49. Webb, R, Rosenzweig, C, Levine E. 1993. Specifying land surface characteristics in general circulation models: Soil profile data set and derived water-holding capacities, Global Biogeochemical Cycles, 7(1): 97–108.

50. Wei, W, Wang, Y, Wei, ZG, Zhao, HY, Li HX, Hu F. 2009. Roles of organic acids and nitrate in the long-distance transport of cobalt in xylem saps of *Alyssum murale* and *Trifolium subterraneum*, Biol. Trace Elem. Res., 131, 165–176.

51. WHO. 2005. Malnutrition-The Global Picture. World Health Organization. Available athttp://www.who.int/home-page/. Accessed on 01/06/ 2017.

52. Wood, JM, Wang, HK. 1983. Microbial resistance to heavy metals. Environmental Science and Technology 17, 582-590.

53. www.pmfias.com%25252Fsoil-profile-soil-horizon-soil-types-sandy-clayey-loamy%25252 (accessed on 25/06/2017).

54. Yaqin, JI. 2008. Using geoaccumulation index to study source profiles of soil dust in China. Journal of Environmental Sciences, 20, 571–578.

55. Zhang, WJ, Jiang, FB, Ou, JF. 2011. A review on heavy metal contamination in the soil worldwide: Situation, impact and remediation techniques, Environmental Sceptics and Critics, 2014, 3(2): 24-38

CHAPTER THREE - ELEMENTAL COMPOSITION AND NUTRITIONAL VALUE OF THE EDIBLE FRUITS OF COASTAL RED MILKWOOD (*MIMUSOPS CAFFRA*) AND IMPACT OF SOIL QUALITY ON THEIR CHEMICAL CHARACTERISTICS

3.1 ABSTRACT

In this study, the elemental distribution of essential and toxic elements in the soil and fruits of the indigenous plant species, *Mimusops caffra*, from ten sites along the KwaZulu-Natal east coast was investigated using inductively coupled plasma-optical emission spectrometry. This was done to determine the nutritional value of the fruits as well as to evaluate the impact of soil quality on elemental uptake by the plant. The elemental concentrations in the fruits (in descending order) were found to be K > Na> Ca > Mg > Si > Al > Fe > Zn > Mn > Ni > Cr > Cu > Pb > Mo > Sb > As > Se > V > Cd > Co. The results show that approximately 10 g of fruit would contribute more than 85% towards the recommended dietary allowance for Fe and Si for most adults. The proximate chemical composition revealed the fruits to contain approximately 84% moisture, 4.7% ash, 6.9% protein, 1.7% oil and 2.7% carbohydrates.
The study indicates that the fruits of this indigenous plant species are good source of essential elements with low levels of potentially toxic elements (Pb, As and Cd) which makes the plant a good indigenous food source especially for vulnerable communities that need food security.

Keywords: Geoaccumulation index, toxic elements, nutritional value, synergy, antagonism.

3.2 INTRODUCTION

South Africa, like other developing countries faces the problem of poor dietary intake, food insecurity and poor health care services that prevail within the context of the HIV/AIDS pandemic (Goggin et al., 2009). Recent research has shown that approximately 14 million people live with food insecurity and 1.5 million children suffer from malnutrition (Gqaleni et al., 2007). This is because most people from rural communities are not eating a balanced diet as they cannot afford to buy commercially available fruit and vegetable due to economic and physical inaccessibility Moodley et al., 2007). In light of this, there should be more focus on promoting indigenous, locally grown fruit and vegetable as these are easily accessible and could possibly be of high nutritional value.

The accumulation of trace metals in food is of special concern to developing countries like South Africa as they undergo industrialisation while maintaining the tradition of eating locally grown food such as Amadumbe and sweet potatoes (WHO, 2005). It is therefore important to determine the nutritional quality of these foods to ensure that they comply with standards set by health organisations like the World Health Organisation (Botha et al., 2003).

Mimusops caffra, of the plant family Sapotaceae, is commonly known as Coastal Red Milkwood (English), umThunzi (isiZulu), umHlophe (Xhosa) and Moepel (Afrikaans). *Mimusops caffra*, is an indigenous, small to medium-sized, evergreen tree that grows in large, uniform groups in the dune forests of the KwaZulu-Natal coast, Southern Africa and Mozambique (Botha et al., 2003). The tree normally grows to a height of about 10 m and occasionally reaches a height of 25 m. The bark is dark grey in colour and contains milky latex. The leaves are hardy and leathery, with a blue-green upper colour and have whitish hairs below (Palmer and Pitman, 1972). The bark is used in traditional medicine to treat wounds and bruises (Pooley, 1993). The plant produces berry-like, sweet, pulpy fruits which are eaten by local South Africans and is also used in the production of jelly and alcoholic beverages.

Despite the well-documented ethno-botanical literature in South Africa, very little information on the chemical and nutritional quality of the edible fruits in *Mimusops caffra* is available.

Previous research reported on the elemental concentrations in the fruit of the indigenous species, *Carissa macrocarpa* and *Harpephyllum caffrum* (Moodley et al., 2012; 2013). This paper reports on the elemental concentrations in the fruit the indigenous plant *Mimusops caffra* found on the east coast of KwaZulu-Natal, South Africa that was investigated, as a function of soil quality parameters and to assess nutritional value.

3.3 MATERIALS AND METHODS

3.3.1 Sampling

Sampling was conducted along the east coast of KwaZulu-Natal. Plant material and soil samples were collected from ten different sites, namely: Site A–Umhlanga, Site B–Durban, Site C–Isipingo Beach, Site D–Amanzimtoti, Site E–Warner Beach, Site F–Winlkelspruit, Site G–Umgababa, Site H–Ilfracombe, Site I–Scottsburgh and Site J–Margate (Figure 7). Sampling was conducted in December 2011 during the summer harvest period. The weather during the collection period was moderate (25°C) without rainfall or the wind. The landscape where the sampling sites were located, showed flat topography and the soil type was sandy (Sites A, C, D, E, I, J) or sandy loam (Sites B, F, G, H). Soil samples were systematically collected from 6 points along the dripline of the trees. Soil cores (3.8 cm height, 5 cm diameter, approximately 150 g) were extracted using a 20 cm long thin walled stainless steel coring tube from a depth of approximately 15 cm from six points around the tree. Soil aliquots were composited; the extraneous material such as leaves and rocks were removed and samples were then manually homogenised using a plastic spoon. The composited soil volume was reduced by coning and quartering and the final aliquot was packed in polyethylene bags and refrigerated at 4 ^{0}C to limit microbial manifestations. Plant samples were also stored in polyethylene bags and refrigerated at 4 ^{0}C.

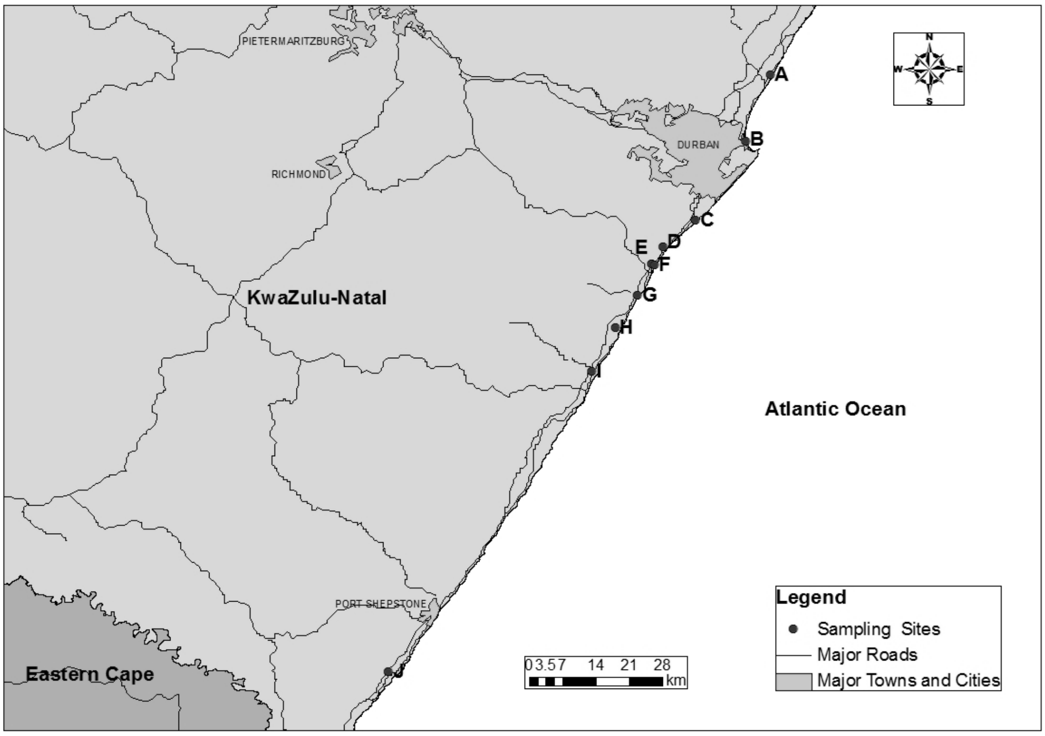


Figure 7: Sampling sites for *Mimusops caffra* plant at the ten different sites.

3.3.2 Reagents and standards

All chemicals used were of analytical-reagent grade supplied by either Merck (Darmstadt, Germany) or Sigma-Aldrich (St. Louis, United States of America). For preparation of standards and dilution, double distilled water was used. All glassware was cleaned with 3 M HNO_3 to prevent contamination. The certified reference material (CRM) *lyophilized brown* bread (BCR 191), from the Community Bureau of Reference of the Commission of the European Communities, was analysed to evaluate the accuracy of the analytical method due to matrix similarities with the plant samples. The analytical procedure used for the CRM was the same as that for the plant samples.

3.3.3 Sample preparation

Fruit samples were washed with double distilled water then cut into smaller pieces with a stainless steel knife. Thereafter, samples were dried, overnight, in an oven at 50°C, crushed into a fine powder using a food processor (Russell Hobbs range) and stored in labelled polyethylene bags. Soil samples were dried in an oven at 50°C, overnight, sieved through a 2 mm sieve to obtain the soil fraction then placed into labelled polyethylene bags until analysed.

3.3.4 Digestion of samples

Digestions were performed according to the method as described by Moodley et al. (2013). Samples were digested using a Mars 6 Microwave Sample Preparation System with patented Xpress™ technology (closed vessel technique). Five replicates of each sample were digested. Both fruit (0.5 g) and soil (0.25 g) samples with 10 mL of 70% HNO_3 were loaded into the 50 mL microwave vessels and digested after standing for 30 min to pre- digest. Digests were filtered through Whatman No. 1 filter papers into 50 mL volumetric flasks and made up to the mark with double distilled water. All samples were stored in a refrigerator in polyethylene bottles until analysed.

3.3.5 Analytical instruments

All elements were analysed using the Agilent ICP-MS 770X with an AS 93 plus auto-sampler. Working standards were made up from elemental standards (1000 mg L^{-1}, Sigma Aldrich (St Louis, USA)) with double distilled water and 10 mL of 70% HNO_3 to match the sample matrix. All analyses were done in triplicate. The Radiometer MeterLab pHM 220 was used for the analysis of soil samples.

3.3.6 Extraction of exchangeable metals

An extractant solution (1 L) using a combination of chemical extractants (ammonium acetate (38.542 g, 0.5 M), ethylenediaaminetetraacetic acid (EDTA) (37.225 g, 0.1 M) and acetic acid (25 mL, 96%)) was prepared and used to release available metals from soil fractions as described by Moodley et al (2012). Approximately 5 g of dry soil sample was extracted with 50 mL of extractant solution in polyethylene bottles at room temperature. For good reproducibility, the soil/extractant ratio, temperature and duration of extraction were kept constant. The extracting solution was then filtered, to remove particulate matter, using a 0.45μm Millipore filter (membrane type, High Volume Low Pressure). Thereafter, the filtrate was stored in labelled polyethylene bottles in a refrigerator until analyzed.

3.3.7 Soil organic matter (SOM), cation exchange capacity (CEC) and soil pH

The pH of the soil was determined using the method as described by Mckenzie et al. (2003) and Skoog et al. (2004). A 1:1 soil-water suspension was measured using a pH meter fitted with a glass electrode. The Walkley-Black method was used to determine the soil organic matter (SOM) (Wakley et al., 1934). For determination of the cation exchange capacity (CEC) of soil, the pH 7.0 ammonium acetate method as described by Chapman was employed (Chapman, 1965).

3.3.8 Bioaccumulation factor (BAF)

Bioaccumulation refers to the tendency of a living organism to absorb substances or chemicals in its body (Reddy et al, 2011). Bioaccumulation of heavy metals such as Hg, Pb, Cd and As can cause detrimental effects to the organism. These heavy metals are discharged into the environment mainly due to human activities and have become a major concern since they can be taken up by plants and introduced into the human food chain (Allan, 1999). The bioaccumulation factor (BAF) is the relative accumulation of elements taken up by the plant. The ratio of the concentration of the metal in the fruit to the concentration of the metal in the soil is referred to as Bioaccumulation factor (BAF). This can be for both total and exchangeable soil concentrations.

$$\text{BAF} = \frac{[Element]_{plant}}{[Element]_{soil}}$$

The BAF can indicate exclusion (BAF < 1), accumulation (BAF > 1) or hyper-accumulation (BAF >>> 1).

3.3.9 Geoaccumulation Index

Geoaccumulation indices are used for assessing contamination in soil and it builds on the background level of natural fluctuations including very low anthropogenic input (Yaqin, 2008). In this study, the I_{geo} of a metal in sediment was calculated as follows:

$$I_{geo} = \log_2 \frac{C_n}{B_n \times 1.5}$$

C_n is the concentration of the heavy metal, B_n is the geoaccumulation background concentration which is multiplied by a factor of 1.5 to account for possible variation in background data due to lithological variation. The doubling of this value provides the upper limit of the next higher class and subsequent doubling provides the upper limit of a higher class (Yaqin, 2008).

3.3.10 Exchangeable percentage

The exchangeable percentage (Ex%) gives the percentage of each element from soil that is available for plant uptake and is a percentage of the ratio of the exchangeable concentration of the element in the soil to the total concentration of the element in the soil. This can be calculated as follows:

$$Ex\% = \frac{[Element]_{exchangeable}}{[Element]_{soil\ total}} \times 100$$

3.3.11 Statistical analysis

Pearson's product-moment correlation coefficients were used to assess the plant-soil relationships and soil competition effects for soil parameters and concentration of elements in the fruit. All statistical analyses were done using the Statistical Package for the Social Sciences (PASW Statistics 19, IBM Corporation, Cornell, New York).

3.3.12 Quality assurance

Certified Reference Material (CRM), *lyophilized brown bread* (BCR 191) sample was analysed and the data statistically evaluated using the statistical mean and standard deviation. The statistical data obtained from the analysis of the CRM was compared with the target values from the Certificate of Analysis (COA). The standard deviation together with the mean was used to compute the acceptable range to determine the accuracy of the developed test method. The test results obtained on analysis of the CRM are summarized in Table 8. The investigation showed that the test results are within the acceptable range of that stipulated for the CRM, meaning that the method is accurate at the 95% confidence interval.

Table 8: Comparison of measured and certified/indicative values (Mean ± SD at 95% confidence interval, n = 5), based on dry mass, in the certified reference material, *lyophilized brown bread* (BCR 191).

Element	Certified/Indicative concentration[a] mg kg^{-1}	Measured concentration mg kg^{-1}
As	0.023 [a]	0.023 ± 0.43
Ca	0.41 [a]	0.36 ± 0.11
Cu	2.60 ± 0.1	2.16 ± 0.6
Fe	40.7 ± 2.3	34.3 ± 5.7
Mg	0.50 [a]	0.45 ± 0.98
Mn	20.3 ± 0.7	20.0 ±3.2
Zn	19.5 ± 0.5	20.6 ± 5.3

[a] Indicative values are those without uncertainties.

3.4 RESULTS AND DISCUSSION

3.4.1 Elemental analysis

The chemical composition of the fruit of *Mimusops caffra* was found to contain 84% moisture, 4.7% ash, 6.9% protein, 1.7% oil and 2.7% carbohydrates.

The elemental concentrations for the twenty selected elements in the fruit collected from ten sampling sites and corresponding soil samples as per site are summarised in Table 9. In this study, Ca, K, Mg, Na and Si were found to be the major elemental components of the fruit (Fig. 8). Plant K ranged from 6599 to 14039 mg kg^{-1}. All sites showed total and exchangeable soil concentrations of K to be lower than plant concentrations (Table 9). In this study, K was the element found in highest concentrations in the fruit compared to other elements. The plant appeared to accumulate K, with BAFs (total and exchangeable) between 9 and 227.

Table 9: Elemental concentrations in mg kg^{-1} (Mean (SD), n=5) of selected elements in the fruit of *Mimusops caffra.*

Site	Element	Soil (T)	Soil (Ex)	Fruit	Ex%	BAF(T)	BAF(Ex)
A	Al	9583(1356)	112(25)	167(2)	1.2	0.0	1.5
B		1923(2224)	64(24)	119(6)	3.3	0.1	1.9
C		4031(48)	95(32)	176(6)	2.4	0.0	1.9
D		2389(527)	973(32)	191(10)	40.7	0.1	0.2
E		3055(766)	1275(10)	91(11)	41.7	0.0	0.1
F		3851(1875)	1911(11)	172(5)	49.6	0.0	0.1
G		3520(1099)	1542(25)	135(10)	43.8	0.0	0.1
H		2959(460)	1142(10)	317(20)	38.6	1.1	2.7
I		3346(335)	1230(20)	173(9)	36.8	0.1	0.1
J		6274(739)	2341(25)	96(8)	37.3	0.0	0.0
A	As	2.79(0.06)	0.21(0.1)	0.06(0.1)	7.5	0.0	0.3
B		2.79(0.14)	2.05(0.07)	0.1(0)	73.5	0.0	0.0
C		3.93(0.1)	2.16(0.14)	0.09(0)	55.0	0.0	0.0
D		3.5(0.1)	2.93(0.1)	0.1(0)	83.7	0.0	0.0
E		3.24(0.1)	0.34(0.07)	1.42(0)	10.5	0.4	4.2
F		8.62(0.1)	0.11(0.09)	0.1(0.01)	1.3	0.0	0.9
G		2.84(0.1)	0.12(0.06)	0.06(0)	4.2	0.0	0.5
H		2.21(0.37)	0.14(0.06)	0.1(0)	6.3	0.0	0.7
I		3.45(0.1)	0.03(0.06)	0.07(0)	0.9	0.0	2.3
J		12.4(1.02)	0.83(0.06)	0.02(0.01)	6.7	0.0	0.0
A	Ca	7118(122)	5225(20)	2343(815)	73.4	0.3	0.4
B		28243(1122)	10567(98)	3848(1596)	37.4	0.1	0.4
C		21761(6449)	2321(282)	6020(324)	10.7	0.3	2.6
D		12920(1675)	1675(23)	7203(268)	13.0	0.6	4.3
E		14172(1043)	10432(20)	5810(200)	73.6	0.4	0.6
F		4266(427)	3814(17)	4846(122)	89.4	1.1	1.3
G		32591(2932)	29316(15)	4286(359)	90.0	0.1	0.1
H		21867(1804)	18036(33)	3785(421)	82.5	0.2	0.2
I		1343(134)	905(3)	3476(283)	67.4	2.6	3.8
J		8852(885)	7685(9)	4743(570)	86.8	0.5	0.6

A	Cr	30.9(2.3)	ND	0(0.3)	1.5	0.0	ND
B		61.9(2.2)	ND	1.9(1.9)	0.0	0.0	ND
C		9.6(11.1)	ND	4.6(3.1)	0.0	0.5	ND
D		16.8(11.5)	ND	5.2(0.5)	0.0	0.3	ND
E		33(8.3)	ND	0.6(0.4)	0.0	0.0	ND
F		23.4(27.1)	ND	10.2(0.3)	0.0	0.4	ND
G		19.3(22.3)	ND	5.3(0.8)	0.0	0.3	ND
H		12.8(8.5)	ND	14.1(0.8)	0.0	1.1	ND
I		8.1(9.6)	ND	4(0.5)	0.0	0.5	ND
J		0(4.3)	ND	1.2(0.4)	ND	ND	ND
A	Cu	16.2(3.5)	4.7(1.2)	6.7(1.3)	29.0	0.4	1.4
B		10.5(6.1)	5.1(0.3)	5.1(2.2)	48.6	0.5	1.0
C		8.1(1.5)	3.4(0.5)	4.9(1.4)	42.0	0.6	1.4
D		10.3(5.2)	9.3(0.5)	3.9(0.8)	90.3	0.4	0.4
E		26.2(40.2)	2.5(0)	5(1)	9.5	0.2	2.0
F		36.2(5.8)	3.5(0.5)	3(0.9)	9.7	0.1	0.9
G		4.3(0.9)	2.4(0.8)	6.2(2.5)	55.8	1.4	2.6
H		7.3(1.1)	5.6(0.5)	1.7(0.2)	76.7	0.2	0.3
I		6.7(1.2)	3.9(0.5)	4.1(2)	58.2	0.6	1.1
J		20.8(2.5)	3.2(0.9)	2.3(0.6)	15.4	0.1	0.7
A	Fe	11345(709)	341(10)	170(14)	3.0	0.0	0.5
B		4662(3117)	145(23)	139(12)	3.1	0.0	1.0
C		6099(251)	127(24)	164(9)	2.1	0.0	1.3
D		6451(808)	222(15)	197(7)	3.4	0.0	0.9
E		7118(852)	254(8)	70(5)	3.6	0.0	0.3
F		8528(889)	174(25)	202(17)	2.0	0.0	1.2
G		4960(742)	233(2)	162(10)	4.7	0.0	0.7
H		5589(761)	144(12)	225(19)	2.6	0.0	1.6
I		4396(313)	142(12)	120(4)	3.2	0.0	0.8
J		6235(937)	153(10)	78(3)	2.5	0.0	0.5
A	K	797(4)	331(48)	8339(88)	41.5	10.5	25.2
B		637(3)	366(90)	10441(119)	57.5	16.4	28.5
C		521(3)	51(64)	11599(118)	9.8	22.3	227.4

D		348(1)	284(6)	14039(4)	81.6	40.3	49.4
E		365(4)	72(8)	7027(2)	19.7	19.3	97.6
F		616(2)	65(2)	10567(1)	10.6	17.2	162.6
G		348(1)	197(17)	12458(4)	56.6	35.8	63.2
H		475(3)	148(11)	11098(10)	31.2	23.4	75.0
I		254(2)	130(5)	11541(7)	51.2	45.4	88.8
J		727(5)	270(18)	6599(9)	37.1	9.1	24.4
A	Mg	1516(59)	149(82)	1225(6)	9.8	0.8	8.2
B		1664(95)	522(23)	1723(8)	31.4	1.0	3.3
C		1709(17)	317(3)	1479(5)	18.5	0.9	4.7
D		1251(116)	328(120)	1567(1)	26.2	1.3	4.8
E		1271(105)	291(48)	1032(2)	22.9	0.8	3.5
F		1453(111)	425(8)	1786(4)	29.2	1.2	4.2
G		1498(243)	110(19)	1167(6)	7.3	0.8	10.6
H		1478(214)	365(34)	1117(3)	24.7	0.8	3.1
I		161(11)	59(4)	1697(3)	36.6	10.5	28.8
J		1016(118)	200(10)	677(2)	19.7	0.7	3.4
A	Mn	221(13)	164(8)	12(1)	74.2	0.1	0.1
B		125(49)	70(5)	8(1)	56.0	0.1	0.1
C		111(4)	43(3)	9(1)	38.7	0.1	0.2
D		103(9)	81(8)	9(2)	78.6	0.1	0.1
E		112(19)	64(9)	5(1)	57.1	0.0	0.1
F		235(21)	57(3)	7(1)	24.3	0.0	0.1
G		70(9)	26(1)	13(1)	37.1	0.2	0.5
H		103(13)	91(5)	12(3)	88.3	0.1	0.1
I		66(6)	55(8)	43(1)	83.3	0.7	0.8
J		97(10)	70(5)	8(1)	72.2	0.1	0.1
A	Na	6330(398)	519(26)	5352(768)	8.2	0.8	10.3
B		6550(144)	576(88)	6205(220)	8.8	0.9	10.8
C		6093(245)	431(195)	5844(582)	7.1	1.0	13.6
D		6330(429)	251(64)	6641(240)	4.0	1.0	26.5
E		6017(38)	237(15)	4291(49)	3.9	0.7	18.1
F		5985(41)	408(105)	7403(255)	6.8	1.2	18.1

G		6033(50)	465(79)	4473(111)	7.7	0.7	9.6
H		6057(124)	371(60)	2485(198)	6.1	0.4	6.7
I		6015(27)	66(7)	4341(332)	1.1	0.7	65.8
J		5618(105)	325(40)	3903(767)	5.8	0.7	12.0
A	Ni	1.5(0.3)	0.9(0.1)	1.9(0.4)	60.0	1.3	2.1
B		9(1.3)	ND	3.2(0.3)	ND	0.4	ND
C		30.8(1)	ND	2.9(2)	ND	0.1	ND
D		5.2(0.2)	0.5(0.1)	4.6(1.8)	9.6	0.9	9.2
E		5.5(0.6)	ND	1.8(1.3)	ND	0.3	ND
F		7.1(0.9)	ND	7.2(2.7)	ND	1.0	ND
G		10(1)	ND	6.5(2.5)	ND	0.7	ND
H		3.6(0.6)	ND	13.2(6.2)	ND	3.7	ND
I		4.3(2.9)	0.6(0.1)	6.5(2.4)	14.0	1.5	10.8
J		2(0.4)	1.1(0.1)	1(0.4)	55.0	0.5	0.9
A	Pb	20.9(7.32)	0.12(0.02)	5.99(0.34)	0.6	0.3	49.9
B		14.94(1.77)	2.02(0.15)	0.94(1.13)	13.5	0.1	0.5
C		29.5(3.73)	1.23(0.02)	0.44(0.51)	4.2	0.0	0.4
D		5.59(0.26)	0.25(0.02)	1.05(0.08)	4.5	0.2	4.2
E		3.42(2.36)	0(0)	1.41(0.39)	0.0	0.4	ND
F		6.63(2.1)	0(0)	0.24(0.07)	0.0	0.0	ND
G		28.62(3.11)	3.33(0.14)	0.51(0.06)	11.6	0.0	0.2
H		2.23(0.25)	1.23(0.22)	0.87(0.24)	55.2	0.4	0.7
I		8.66(0.38)	0(0)	0.74(0.22)	0.0	0.1	ND
J		5.91(0.45)	1.25(0.11)	0.7(0.22)	21.2	0.1	0.6
A	Si	606(10)	480(9)	1619(223)	79.2	2.7	3.4
B		665(38)	304(8)	1193(75)	45.7	1.8	3.9
C		683(7)	237(6)	998(142)	34.7	1.5	4.2
D		500(46)	211(4)	715(112)	42.2	1.4	3.4
E		509(42)	108(5)	497(13)	21.2	1.0	4.6
F		581(45)	28(6)	459(29)	4.8	0.8	16.4
G		599(97)	32(2)	406(23)	5.3	0.7	12.7
H		591(86)	40(10)	342(15)	6.8	0.6	8.6
I		64(4)	45(5)	325(42)	70.3	5.1	7.2

J		406(47)	54(10)	257(36)	13.3	0.6	4.8
A	V	2.75(0.23)	0.13(0)	0.17(0)	4.7	0.1	1.3
B		0.68(0.01)	0.09(0)	0.1(0)	13.2	0.1	1.1
C		0.56(0.01)	0.05(0.01)	0.14(0)	8.9	0.3	2.8
D		1.37(0.13)	0.11(0.01)	0.11(0.04)	8.0	0.1	1.0
E		0.28(0.01)	0.12(0)	0.03(0.01)	42.9	0.1	0.3
F		0.28(0.03)	0.1(0)	0.18(0.05)	35.7	0.6	1.8
G		1.37(0.04)	0.08(0.01)	0.12(0.06)	5.8	0.1	1.5
H		0.22(0.02)	0.06(0.04)	0.1(0.06)	27.3	0.5	1.7
I		0.31(0.07)	0.06(0)	0.08(0.01)	19.4	0.3	1.3
J		0.23(0.06)	0.07(0)	0.01(0.01)	30.4	0.0	0.1
A	Zn	121.5(0.4)	18.4(1.2)	18.3(0.1)	15.1	0.2	1.0
B		130.7(0.4)	23(2)	41.8(0.2)	17.6	0.3	1.8
C		68.6(0.1)	15.1(3.5)	27.5(0.1)	22.0	0.4	1.8
D		70.9(0.1)	20.9(0.3)	34.6(0.1)	29.5	0.5	1.7
E		52.1(0.1)	7.2(0.6)	26.3(0.1)	13.8	0.5	3.7
F		114.6(0.2)	13.8(5.3)	24.3(0.1)	12.0	0.2	1.8
G		49.2(0.1)	10.9(2.3)	38.5(0.3)	22.2	0.8	3.5
H		50.6(0.4)	6.7(1.4)	13.4(0.1)	13.2	0.3	2.0
I		67(0.2)	36.6(5)	20.5(0.1)	54.6	0.3	0.6
J		67.5(0.1)	26.7(2)	41.7(0.4)	39.6	0.6	1.6

Site A – Umhlanga, Site B – Durban, Site C – Isipingo Beach, Site D – Amanzimtoti, Site E – Warner Beach, Site F – Winlkelspruit, Site G – Umgababa, Site H – Ilfracombe, Site I – Scottsburgh and Site J – Margate.
*Soil(T)- Soil Total, Soil (Ex)-Soil exchangeable, BAF-Bioaccumulation factor.

Sodium is known to be present at higher concentrations in most plants as it is essential for their growth (Maschner, 2002). Plant Na ranged from 2485 to 7403 mg kg^{-1}. Total soil Na ranged from 5618 to 6550 mg kg^{-1}, of which, between 66 to 576 mg kg^{-1} was in exchangeable form (average Ex% = 6). This indicates that Na is tightly bound to soil lattice thereby restricting exchangeability.

The typical concentration of Ca in plants is 5000 mg kg^{-1} (Akenga et al., 2014). Although total soil Ca ranged from 1343 to 32591 mg kg^{-1}, plant Ca ranged from 2343 to 7203 mg kg^{-1} (which is close to typical concentrations for most plants). This indicated the plant's ability to control uptake. The plant tended to accumulate Ca when soil concentrations were low (Site I), BAF = 2.6 (total) and 3.8 (exchangeable) and exclude it when soil concentrations were high (Site G), BAF = 0.1 (total and exchangeable). The amount of Ca in exchangeable form was between 10 and 90%.

Total soil Mg ranged from 161 to 1709 mg kg^{-1} with the Ex% being less than 37. The plant tended to accumulate Mg with BAFs (total) being close to 1 in most cases. This indicates that plant Mg is more closely related to total than exchangeable soil Mg and that the plant will accumulate Mg to meet physiological needs.

Total soil Si concentrations were within a narrow range (64 to 683 mg kg^{-1}), of which, 4.8 to 79.2 was in exchangeable form. At most sites, the plant tended to accumulate Si (Average BAF = 1.6 (total) and 6.9 (exchangeable)) but this accumulation by the plant tended to decrease southwards along the coast of KwaZulu-Natal from Umhlanga (Site A) to Margate (Site J) as shown in Figure 8.

The growth of most plants is promoted by the accumulation of trace elements. Elements such as Cu, Mn, Ni and Zn are required by most living organisms mostly humans and if these are found at higher concentration they can cause detrimental effects to human health causing serious illness. Table 9 and Figure 9 show that the soil at all sites is rich in Al (1923 to 9583 mg kg^{-1}) but less than 50% of this Al is in an available form. The average concentration of Al in the fruit was 163.7 mg kg^{-1}. Total soil Cu ranged from 4.3 to 36.2 mg kg^{-1} and 9.5 to 90.3% of this was in available form. Copper in the plant appears to be restricted to the narrow range of 1.7 to 6.7 mg kg^{-1}.

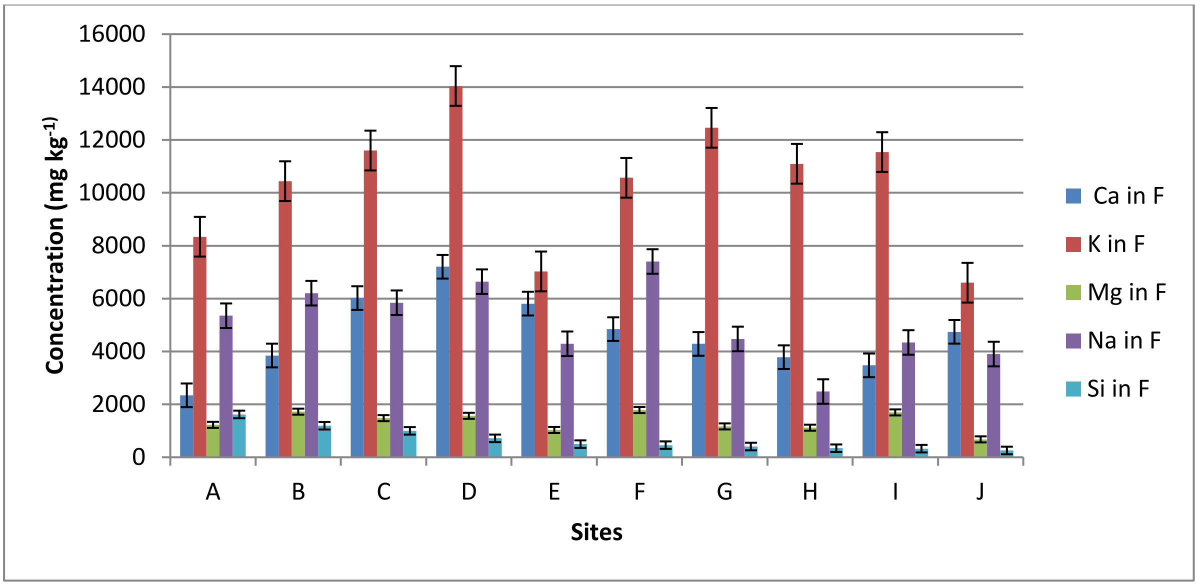


Figure 8: Distribution of the major elements in *Mimusops caffra* fruit (F) at the 10 different sites.

Site A – Umhlanga, Site B – Durban, Site C – Isipingo Beach, Site D – Amanzimtoti, Site E – Warner Beach, Site F – Winlkelspruit, Site G – Umgababa, Site H – Ilfracombe, Site I – Scottsburgh and Site J – Margate

Iron uptake and transport is influenced by a variety of factors such as soil pH, root morphology and salinity (Morrissey and Guerinot, 2009). The availability and uptake of Fe is a complex process as many factors can influence Fe levels in the plant (Morrissey and Guerinot, 2009). The growth of plants may be slow if Fe in the soil is at high concentrations. Exchangeable Fe was observed to be extremely low compared to total soil Fe (Ex% < 4.7). This is because Fe may be bound to Al-Mn-hydroxide complexes or contained in mineral lattices within the soil, rendering it unavailable. Iron in the plant ranged from 70 to 225 mg kg^{-1} with an average BAF (bioavailable) of 0.8 indicating that the Fe (in available from) was sufficient to meet the plant's physiological requirement levels.

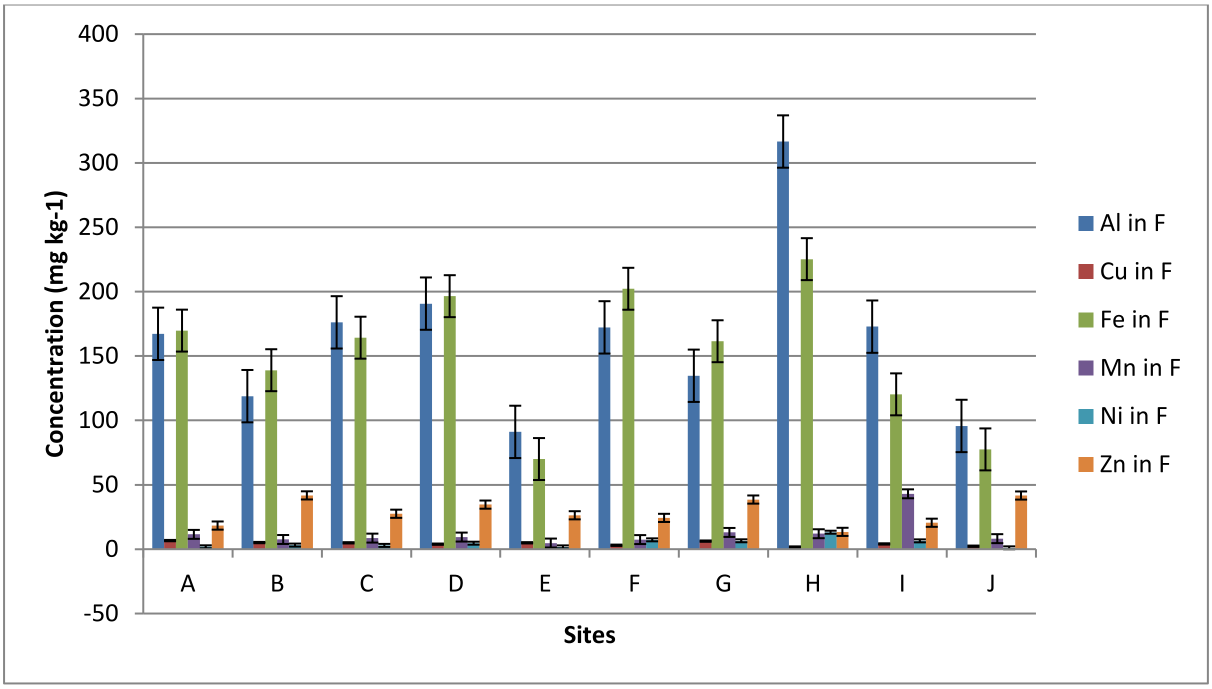


Figure 9: Distribution of the minor elements in *Mimusops caffra* fruit (F) at the 10 different sites.

Site A – Umhlanga, Site B – Durban, Site C – Isipingo Beach, Site D – Amanzimtoti, Site E – Warner Beach, Site F – Winlkelspruit, Site G – Umgababa, Site H – Ilfracombe, Site I – Scottsburgh and Site J – Margate

Manganese concentrations in the fruit ranged from 5 to 43 mg kg^{-1} which was lower than available concentrations at all sites thereby indicating exclusion of this element to meet physiological needs (average BAF = 0.2). Both the soil and plant concentration of Ni in this study were low with total soil Ni ranging from 1.5 to 30 mg kg^{-1} and Ni in the plant ranging from 1 to 13.2 mg kg^{-1}. At most sites, exchangeable Ni was low (below the instrument detection limit) which indicates that the plant has mechanisms to increase the availability of Ni for uptake.

Exchangeable Zn ranged from 6.7 to 36.6 mg kg^{-1} and Zn in the fruit ranged from 13.4 to 41.8 mg kg^{-1}. Zn concentrations in the fruit reveal that the plant has a tendency to accumulate the element (BAF exchangeable from 0.6 to 3.7).

Total soil As is at very low concentrations (2.21 to 12.4 mg kg^{-1}) thereby indicating non-contamination and As levels in the fruit are considered to be safe for human consumption (0.02 to 1.42 mg kg^{-1}). Total soil Cr was less than 62 mg kg^{-1} however none of this was in available form. Chromium (0.6 to 14.1 mg kg^{-1}) and Mo (0.05 to 0.45 mg kg^{-1}) concentrations in the plant were within a small range of variation however, for both these elements, exchangeable concentrations were below the instrument detection limits.

Total soil Pb ranged from 3.42 to 28.62 mg kg^{-1}. At 50% of the sites, soil concentrations are higher than the maximum permissible concentration of 6.6 mg kg^{-1} set by the Water Research Commission for South African Agricultural soils (Snyman et al., 1998). However, these are still lower than the maximum permissible levels for other countries such as the United Kingdom and Canada (100 mg kg^{-1}) (WHO, 2011). In South Africa, the maximum level of Pb in small fruits is set at 0.2 mg kg^{-1} wet weight due to its negative impact on human health (Department of health, 2007). The concentration of Pb in the fruits ranged from 0.24 to 5.99 mg kg^{-1} (dry weight) and with the fruit having a moisture content of 84%, this would make the fruits safe for human consumption.

Although the soil concentrations of Sb and Se were below the detection limit of the instrument at all sites, Sb was present in fruit samples in the range of 0.01 to 1.38 mg kg^{-1}. Vanadium was detected both in soil and plant samples although at low concentrations. Vanadium in the plant ranged from 0.03 to 0.18 mg kg^{-1}.

3.4.2 Estimated contribution of *Mimusops caffra* fruit to the human diet

Table 10 shows the dietary reference intakes (DRIs) in the fruit. This is expressed as mg/day of the average concentration of each essential element studied. Of the twenty elements studied, five are considered non-essential (Al, Cd, Co, Pb and Sb) and these are excluded from the table as there are no known official DRIs for these elements. About 10 g of fruits (dry mass), which is equivalent to about one handful (normal serving size), contributes adequately to the recommended dietary allowances (RDAs) for the nutrients Ca, Cu, K, Mg, Mn, Mo, Na, V and Zn.

Table 10: Dietary Reference Intake (DRIs), Recommended Dietary Allowances (RDAs) and Tolerable Upper Intake Levels (ULs) of elements for most individuals and average concentration of elements (n = 5) in *Mimusops caffra* fruits.

Element	Average Concentration (mg/10 g dry mass)	DRI[a] (mg/day)		Estimated Contribution to RDA (%)
		RDA	UL	
As	0.19	ND[b]	ND	ND
Ca	464	1000-1300	2500	35.7
Cu	0.43	0.9	8	47.8
Cr	0.47	0.024-0.035	ND	1342
Fe	15.3	8-18	45	85
K	1037	1000-3500	4000	29.6
Mg	134.7	310-320	350	42.1
Mn	1.25	1.6-2.3	9	54.4
Mo	0.03	0.1-0.5	1	6.0
Na	509.3	1000-2400	2500	21.2
Ni	0.49	ND	1.0	ND
Si	68.09	50-80	100	85.1
V	0.01	0.006-0.18	0.1	5.6
Zn	2.87	8-11	34	26.1
Se	ND	0.05	0.4	ND

.[a] Institute of Medicine of the National Academies: Dietary Reference Intakes (2001).

[b] ND = not determined due to lack of data.

The consumption of 10.0 g of fruits may contribute 85% towards the RDA for Fe for most adults. Iron is found in haemoglobin. Its deficiency causes anaemia and increases susceptibility to infection. On the other hand, an excess of Fe may increase a person’s risk of cancer and heart diseases (Calabrese et al., 1985). Adults and children in South Africa suffering from anaemia can eat these fruits as an alternative to those available in the market with high Fe content such as apricots, beetroot and olives to help overcome Fe deficiency.

The consumption of 10.0 g of fruits may contribute 85.1% towards the RDA for Si for most adults. High levels of Si are present in vegetables such as alfalfa, bell peppers and root vegetables and in fruits such as apples and red raspberry. This element is essential for bone formation and digestive system disorder (Lela et al., 2013). Studies have shown a correlation between improvements in Alzheimer's disease and hair loss with ingestion of fruits and vegetables with high Si content (Davenward et al., 2013; Jugdaohsingh, 2007).

Chromium is considered to be an important microelement for normal carbohydrate, lipid and protein metabolism in humans (Kobla and Volpe, 2000). The consumption of 10 g of fruits exceeds the RDA for this element (1342%). Mngadi et al. (2014) found similar results in *Amadumbe* leaves where the Cr levels exceeded the RDA for humans. However, high intake of Cr is not linked to adverse health effects therefore the Institute of Medicine has not established a tolerable upper intake level (UL) for this element (Davenward et al., 2013).

3.4.3 Soil properties (pH, SOM and CEC)

Soil properties (pH, SOM and CEC) were investigated and the results are shown in Table 11. The results show that Mimusops caffra plants thrive in slightly basic soil conditions (pH range (6.01 to 9.81). According to Mahlangeni et al. (2016) the availability of some heavy elements in soil is caused by high soil pH (basic conditions). SOM is an important determinate in soil since it acts as a pool of nutrients for plants and also increases the nutrient holding capacity of soil. Recent research has shown that metal availability is positively correlated to SOM (Yemeni et al., 2006). In this study, there was a wide range of variation in SOM from the different sites (1.19 to 5.92%). Most cations adsorb to the negative surface charges as a result of an increase in SOM which causes an increase in the soils CEC. The CEC is important because it provides the approximate quantities of available Ca2+, Mg2+ and K+ ions in soil. The CEC ranged between 1.72 and 12.99. Cations like Ca and Mg that are usually bound to the surface of soil particles are more available for cation exchange within a pH range of 6.5 to 8. From Table 11 we see that most pH values greater than 6.5 have the highest CECs.

Table 11: pH, SOM and CEC of soil samples from the ten different sites in KwaZulu-Natal.

Sites[b]	**pH ($CaCl_2$)**[a]	**SOM**[a] (%)	**CEC**[a] (meq/100 g)
A	6.77	3.59	10.36
B	9.81	3.27	6.34
C	8.19	4.23	10.59
D	7.53	3.82	7.98
E	7.57	4.09	8.88
F	8.16	5.92	11.78
G	6.76	2.57	12.99
H	7.37	1.19	1.72
I	6.01	4.05	8.23
J	7.57	5.29	9.89

[a] Expressed as mean, n = 5

[b] Sites: Site A – Umhlanga, Site B – Durban, Site C – Isipingo Beach, Site D – Amanzimtoti, Site E – Warner Beach, Site F – Winlkelspruit, Site G – Umgababa, Site H – Ilfracombe, Site I – Scottsburgh and Site J – Margate.

3.4.5 Geoaccumulation index

Table 12 shows the total concentration of each element in soil and by calculation of the geoaccumulation indices (I_{geo}), the extent of contamination, if there is any, is then estimated. According to (Müller, 1986) I_{geo} values below zero would mean that the soil is uncontaminated. The I_{geo} values found in this study at all sites indicated non-contamination in the soil. Evidence to this is the I_{geo} values which are all negative indicating lack of contamination (Yaqin, 2008).

3.4.6 Statistical analysis

A correlation matrix for the elemental concentration in *Mimusops caffra* fruit and soil (total and exchangeable) is given in Table 13. The statistical analysis indicates the extent to which the interactions are closely related where $r > 0.8$ indicates a strong positive relationship (synergistic) and $r < -0.8$ indicates a strong negative relationship (antagonistic).

A synergistic effect is observed as a result of an increase in the levels of one or more interacting elements leading to an increase in the exchangeability of another (Marshner, 2002). A positive correlation was observed between exchangeable Cr and total soil Al, Fe and V. This means that increased concentrations of these elements in soil allow Cr to become more exchangeable probably by displacing adsorbed Cr since competing for the same soil adsorption site. This could be due to charge similarities between these elements. The same relationship is observed between exchangeable Na and total soil Mg and Si.

A strong correlation between fruit and exchangeable/total concentration for a particular element indicates that the plant has a tendency to accumulate or exclude the particular element. This was evident for Si, where, there was a strong positive correlation between Si in the fruit with exchangeable Si ($r = 1$). Although, no similarity exists in chemical nature of the metals, a strong positive relationship existed between Pb in the fruit with exchangeable Cr, Fe, Mn and Si indicating that high exchangeable concentrations of these elements promote uptake of Pb, but mechanism by which the synergy occurs is not clear. Correlations also existed between fruit concentrations between Sb, As, Se and Co. While Sb and As are metalloids, Se is a non-metal and Co is a metal. Analogous to earlier observation, not much similarity was observed in the chemical properties of those elements.

A positive correlation between the elements and SOM was observed. Total soil As and Cu correlated positively with SOM. On the other hand, exchangeable Ca correlated negatively with SOM. Aluminium in the fruit correlated negatively to both SOM (r = -0.7) and CEC (r = - 0.8). No further significant correlations between SOM with the elements were obtained.

The pH of the soil was found to correlate strongly (r = 1.0) with exchangeable Mg in the soil. Ca and Mg are known to be more available at pH levels between 6 and 8 (Bagamba et al., 2007). The correlation justifies this since the pH of the soil at all sites is between 6 and 8, hence more available for plant uptake.

3.5 CONCLUSION

The elemental composition and nutritional value of *Mimusops caffra* fruit from ten different geographic locations along the east coast of KwaZulu-Natal was determined. Twenty elements were assessed for nutritional value in the fruits and elemental concentrations, in descending order, were found to be K > Na> Ca > Mg > Si > Al > Fe > Zn > Mn > Ni > Cr > Cu > Pb > Mo > Sb > As > Se > V > Cd > Co. The study showed that the fruits of the indigenous plant, *Mimusops caffra*, are a rich source of essential elements such as K, Na, Ca and Mg with low levels of the toxic elements studied thereby making it nutritious and safe for human consumption. The high concentration of Ca and Mg in the fruits supports the medicinal use of *Mimusops caffra* which is used to treat wounds and sores.

Table 12: Total Baseline Concentrations (TBC, mg kg^{-1}) of metals in South African soils, total soil concentration (S(T), mk kg^{-1}), and geoaccumulation index (I_{geo}) for each sampling site.

Metal	**TBC**	**Site A**		**Site B**		**Site C**		**Site D**		**Site E**		**Site F**		**Site G**		**Site H**		**Site I**		**Site J**	
		S(T)	**I_{geo}**	**S(T)**	**I_{geo}**	**S(T)**	**I_{geo}**	**S(T)**	**I_{geo}**	**S(T)**	**I_{geo}**	**S(T)**	**I_{geo}**	**S(T)**	**I_{geo}**	**S(T)**	**I_{geo}**	**S(T)**	**I_{geo}**	**S(T)**	**I_{geo}**
Cd	2.7	0.18	-4.5	0.04	-6.7	0.11	-5.2	0.11	-5.2	0.16	-4.7	0.19	-4.4	0.18	-4.5	0.12	-5.1	0.06	-6.1	0.00	-
Co	69	1.55	-6.1	0.65	-7.3	0.27	-8.6	0.59	-7.5	0.19	-9.1	0.05	-11.0	1.63	-6.0	0.17	-9.2	0.09	-10.2	0.66	-7.3
Cr	353	30.95	-4.1	61.90	-3.1	9.61	-5.8	16.84	-5.0	33.04	-4.0	23.45	-4.5	19.35	-4.8	12.76	-5.4	8.11	-6.0	0.00	-
Cu	117	16.25	-3.4	10.51	-4.1	8.11	-4.4	10.28	-4.1	26.19	-2.7	36.20	-2.3	4.29	-5.4	7.28	-4.6	6.67	-4.7	20.8	-3.1
Pb	66	20.90	-2.2	14.94	-2.7	29.50	-1.7	5.59	-4.1	3.42	-4.9	6.63	-3.9	28.62	-1.8	2.23	-5.5	8.66	-3.5	5.91	-4.1
Zn	115	121.5	-0.5	130.7	-0.4	68.60	-1.3	70.9	-1.3	52.1	-1.7	114.6	-0.6	49.2	-1.8	50.6	-1.8	67.0	-1.4	67.5	-1.4

Sites: Site A – Umhlanga, Site B – Durban, Site C – Isipingo Beach, Site D – Amanzimtoti, Site E – Warner Beach, Site F – Winlkelspruit, Site G – Umgababa, Site H – Ilfracombe, Site I – Scottsburgh and Site J – Margate.

Table 13: Correlation matrix for the elemental concentration in *Mimusops caffra* fruits (F) and soil (total (T) and exchangeable (E).

	AlF	CoF	CrE	CrF	FeT	FeE	FeF	MgE	MnT	MnE	MnF	NaE	NiT	NiF	PbE	PbF	SbT	SbE	SbF	SeF	SiT	SiE	SiF	VT	VE	VF
pH	-0.1	0.0	-0.3	0.0	-0.1	-0.4	0.0	0.9*	0.2	-0.1	-0.6	0.6	0.3	-0.2	0.2	-0.3	0.4	0.1	0.0	0.0	0.6	0.2	0.3	-0.3	0.0	0.1
CEC	-0.8*	0.1	0.2	-0.4	0.3	0.3	-0.2	-0.4	0.2	-0.3	-0.1	0.1	0.3	-0.5	0.1	0.1	0.1	0.2	0.1	0.0	0.0	0.0	0.1	0.3	0.2	0.3
AlT	-0.2	-0.2	0.9*	-0.4	0.8*	0.6	-0.1	-0.5	0.5	0.7*	-0.1	0.3	-0.2	-0.4	-0.2	0.8*	-0.2	0.0	-0.2	-0.2	0.0	0.5	0.4	0.6	0.3	0.2
AlF		-0.2	-0.1	0.8*	-0.2	-0.3	0.5	0.2	-0.1	0.2	0.0	0.0	-0.2	0.8*	0.1	-0.1	-0.4	-0.3	-0.2	-0.1	0.1	-0.3	-0.3	-0.2	-0.3	0.0
AsE		-0.2	-0.2	-0.2	-0.2	-0.2	0.1	0.5	-0.2	-0.1	-0.3	0.1	0.4	-0.3	0.1	-0.2	0.9*	0.7*	-0.1	-0.2	0.3	0.4	0.3	0.1	0.0	0.0
ASF		1.0*	-0.1	-0.3	0.1	0.3	-0.6	0.0	-0.1	-0.1	-0.2	-0.3	-0.1	-0.3	-0.3	0.0	0.0	-0.3	1.0*	1.0*	0.0	-0.1	-0.1	-0.2	0.4	-0.5
CaT					-0.5	-0.1	0.2	0.3	-0.4	-0.3	-0.4	0.6	0.4	0.2	0.9*	-0.3	0.5	0.4	0.1	0.0	0.6	0.0	0.1	0.0	-0.2	0.0
CoT					0.3	0.7*	0.0	-0.4	0.1	0.3	-0.2	0.5	-0.2	-0.3	0.5	0.6	0.3	0.6	-0.1	-0.2	0.3	0.4	0.4	0.8*	0.3	0.2
CoF					0.2	0.3	-0.5	0.1	0.1	-0.1	-0.3	-0.3	-0.1	-0.2	-0.4	0.0	-0.2	-0.4	0.9*	1.0*	0.0	-0.2	-0.2	-0.3	0.5	-0.4
CrE					0.8*	0.8*	0.1	-0.3	0.6	0.9*	0.0	0.4	-0.3	-0.3	-0.3	1.0	-0.1	0.1	-0.1	-0.1	0.2	0.8*	0.7*	0.8*	0.5	0.4
CrF					-0.2	-0.4	0.8*	0.3	0.0	-0.2	0.0	0.0	0.0	0.9*	0.1	-0.4	-0.3	-0.3	-0.3	-0.3	0.1	-0.5	-0.4	-0.4	-0.4	0.3
FeT						0.7*	0.2	0.0	0.9*	0.7*	-0.4	0.3	-0.2	-0.3	-0.5	0.8*	-0.2	-0.1	0.0	0.1	0.3	0.5	0.5	0.6	0.7*	0.5
FeE												0.2	-0.4	-0.3	-0.2	0.8*	0.1	0.2	0.4	0.3	0.2	0.5	0.5	0.8*	0.8*	0.2
FeF												0.3	0.1	0.7*	0.0	0.0	0.0	0.1	-0.5	-0.6	0.4	0.1	0.2	0.3	-0.1	0.8*
MgT												0.9*	0.4	0.0	0.4	0.1	0.4	0.3	0.1	0.0	1.0*	0.4	0.5	0.3	0.2	0.4
MnE													-0.5	-0.1	-0.4	0.9*	-0.1	0.0	-0.2	-0.1	0.1	0.7*	0.6	0.6	0.6	0.2
MoT													0.9*	-0.2	0.1	-0.1	0.3	0.1	-0.1	-0.1	0.1	0.2	0.2	0.0	-0.4	0.2
NaE																		0.3	-0.2	-0.3	0.9*	0.5	0.6	0.4	0.2	0.5
PbF																		0.1	0.0	0.0	0.1	0.8*	0.7*	0.8*	0.6	0.3
SbT																		0.9*	0.1	-0.1	0.4	0.4	0.4	0.3	0.2	0.0
SbF																				1.0*	0.1	-0.1	-0.1	-0.1	0.5	-0.4
SiE																							1.0*	0.7*	0.5	0.4

Pearson correlation (r); * Correlation is significant at the 0.05 level (2-tailed).

XT= Total soil concentration of element X, XE= Exchangeable soil concentration of element X, XF= concentration of element X in fruit CEC = cation exchange capacity.

3.6 REFERENCES

1. Akenga, P, Salim, A, Onditi, A, Yusuf A. 2014. Determination of selected micro and macronutrients in sugarcane growing soils at Kakamega North District. Journal of Applied Chemistry, 7, 2278-5736.
2. Allan, RJ. 1999. Atmospheric mercury contamination of remote aquatic ecosystems in Canada. Journal of Water, Science and Technology, 39: 173–177.
3. Bagamba, F, Ruben, R, Rufino, M. 2007. Determinants of banana productivity and technical efficiency in Uganda. In An Economic Assessment of Banana Genetic Improvement and Innovation in the Lake Victoria Region of Uganda and Tanzania; Smale Eds.; International Food Policy Research Institute: Washington, D.C., 113.
4. Botha, J, Shackleton, C, Emanuel, PL. 2003. Productivity and abundance of *Sclerocarya birrea* subsp. *caffra* in and around rural settlements and protected areas of the bushbuckridge Lowveld, South Africa. Forests Trees and Livelihoods, 13, 217-232.
5. Calabrese, EJ, Canada, AT, Sacco, C. 1985. Trace elements in public health. Annual Review - Public Health, 6, 131-146.
6. Chapman, HD. Cation exchange capacity. 1965. In: Methods of Soil Analysis Part 2– Chemical and Microbiological Properties; Black, C.A., Eds.; American Society of Agronomy: Madison, 891–901.
7. Davenward, S, Bentham, P, Wright, J, Crome, P, Job, D, Polwart, A, Exley, C. 2013. Silicon-rich mineral water as a non-invasive test of the 'aluminum hypothesis' in Alzheimer's disease, Journal of Alzheimers Dis, 33(2), 423-30.
8. Department of Health. 2007. Foodstuffs, Cosmetics and Disinfectants Act (Act No. 54 of 1972). Government Gazette: Pretoria, South Africa, 1-15.

9. Goggin, K, Pinkston, M, Gqaleni, N, Puoane, T, Wilson, D, Berkley-Patton, J, Martinez, DA. 2009. The role of South African traditional health practitioners in HIV/AIDS prevention and treatment. In: HIV/AIDS Global Frontiers in Prevention/ Intervention; Pope, C.; White, R.T.; Malow, R., Eds.; Taylor and Francis: New York, 1-5.
10. Gqaleni, N, Moodley, I, Kruger, H, Ntuli, A, McLeod, H. 2007. Traditional and complementary medicine. In: South African Health Review; Harrison, S.; Bhana, R.; Ntuli, A., Eds.; Health Systems Trust: Durban, South Africa, 175-185.
11. Jugdaohsingh, R. 2007. Silicon and Bone health. Journal Nutritional Health and Aging, 11(2), 99–110.
12. Kobla, HV, Volpe, SL. 2000. Chromium, exercise and body composition. Critical Reviews in Food Science and Nutrition, 40, 291–308.
13. Lela, MJ, Ivica, C, Sandra, KP, Krešimir, P. 2013. Biological and therapeutic effects of ortho- silicic acid and some ortho-silicic acid-releasing compounds: New perspectives for therapy. Nutrition & Metabolism, 10(2), 1-12.
14. Mahlangeni, N Moodley, R, Jonnalagadda, SB. 2016. Heavy metal distribution in *Laportea peduncularis* and growth soil from the eastern parts of KwaZulu-Natal, South Africa. Environmental Monitoring and Assessment, 188(2), 76.
15. Marschner, H. 2002. Mineral Nutrition of Higher Plants; Academic Press Harcourt Brace and Company Publishers: London, 2744-7325.
16. McKenzie, RH. 2003. Soil pH and Plant Nutrients. Agri-Facts: Agdex, 531-4, 1-2.
17. Mngadi, S, Moodley, R, Jonalaggadda, S. 2014. Elemental distribution and chemical composition of Amadumbe (*Colocasia esculanta*) leaf and impact of soil quality. Fresenius Environmental Bulletin, 24(4), 1250-1257.

18. Moodley, R, Kindness, A, Jonnalagadda, SB. 2007. Chemical composition of Macadamia nuts (*Macadamia integrifolia*) and impact of soil quality. Journal of Environmental Science and Health Part A, 42, 2097-2104.

19. Moodley, R, Koorbanally, N, Jonnalagadda, SB. 2012. Elemental composition and fatty acid profile of the edible fruits of Amatungula (*Carissa macrocarpa*) and impact of soil quality on chemical characteristics. Analytica Chimica Acta, 42, 2097-2104.

20. Moodley, R, Koorbanally, N, Jonnalagadda, SB. 2013. Elemental composition and nutritional value of the edible fruits of *Harpephyllum caffrum* and impact of soil quality on their chemical characteristics. Journal of Environmental Science and Health, Part B, 48, 539-547

21. Morrissey, J, Guerinot, M. 2009. Iron uptake and transport in plants: The good, the bad, and the ionome. Chemical Reviews, 109(10), 4553–4567.

22. Müller, G. 1986. Index of geoaccumulation in sediments of the Rhine River. Geology Journal, *2*, 109-188.

23. Palmer, E, Pitman, N. 1972. Trees of Southern Africa; AA Balkema: Cape Town, South Africa, 258-269.

24. Pooley, E. 1993. The Complete Field Guide to Trees of Natal, Zululand and Transkei; Natal Flora Publications Trust: Durban, South Africa, 311-322.

25. Reddy, M, Moodley, R, Kindness, A, Jonnalagadda, SB. 2011. Impact of soil quality on the elemental uptake and distribution of *Colocasia esculenta* (Amadumbe), an edible root. Journal of Environmental Science and Health, Part B, 46, 247-256.

26. Skoog, DA, West, DM, Holler, FJ, Crouch, SR. 2004. Fundamentals of analytical chemistry; Brooks/Cole Publications: USA, 258-265.

27. Snyman, H.G, Herselman, JE. 1998. Permissible Utilization and Disposal of Sewage Sludge Report Number: TT 261/06; Water Research Commission: Pretoria, 1-62.

28. Walkley, A, Black, IA. 1934. An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Science, *37*, 29-37.

29. World Health Organisation. 2005 National Policy on Traditional Medicine and Regulation of Herbal Medicine; Report of a WHO global survey: Geneva, 1-156.

30. World Health Organisation. 2011. Lead in Drinking Water, Background Document for Development of WHO Guidelines for Drinking-Water Quality; WHO Press: Geneva, Switzerland, 1-19.

31. Yaqin, JI. 2008. Using geoaccumulation index to study source profiles of soil dust in China. Journal of Environmental Sciences, 20, 571–578.

32. Yemeni, MN, Hashem, AR. 2006. Bacterial diversity of soil samples of saline sites in Kharaghoda, International Journal of Research in Pure and Applied Microbiology, 13(2), 129-133.

CHAPTER FOUR - ELEMENTAL COMPOSITION AND NUTRITIONAL VALUE OF THE EDIBLE FRUITS OF TRANSVAAL RED MILKWOOD (*MIMUSOPS ZEYHERI*) AND IMPACT OF SOIL QUALITY

4.1 ABSTRACT

Mimusops Zeyheri, which is known to be rich in vitamin C, is widely used in traditional medicine and as a source of nourishment. The fruits of this plant were assessed for heavy metal contamination, since it is picked from wherever it grows by the local people in South Africa, irrespective of proximity to contaminated sites. *M. zeyheri* fruits grow abundantly in industrial and mining areas in Gauteng and North-West province of South Africa, where sampling was done. The results showed concentration of metals in the soil to be in decreasing order of K > Na> Ca > Mg > Fe > Al > Zn > Mn > Cu > Cr > Sr > Pb > As > Li > Ni ≈ Co > Rb > U > Bi > Ga > Be > Tl > Mo > Ba > Ag > Cd. The fruits were shown to contain adequate levels of essential macro and microelements to contribute positively to the diet and was also shown to be rich in Cr and Mn. Geoaccumulation indices of toxic metals showed moderate contamination by Cd in soil which did not accumulate in the fruits thereby making them safe for human consumption. Principal component and cluster analysis of soil showed Co, Cr, Ga, Mg, Pb, Rb, and Tl to come from the same source which are mining activities.

Keywords: heavy metal contamination, geoaccumulation index, bioaccumulation.

4.2 INTRODUCTION

The problem of food insecurity is one of the biggest concerns in the world. Research conducted in 2008 has shown that over the period 1990 to 2007, a high percentage of people were experiencing hunger due to increased food prices and reduced agricultural activities (FAO, 2004). This is exacerbated by poverty, high unemployment rate and high tariffs. In most African countries, approximately 35% of a country's population is exposed to food insecurity, about 1.5 million of children mostly in rural areas have problems associated with malnutrition and more than 1 million deaths that occur annually in children are due to malnutrition (Pingali et al., 2005).

Indigenous edible fruits such as *Carissa macrocarpa*, *Rhoicissus digitata* and *Mimusops caffra* that are picked from the wild by the local people in South Africa, have been shown to have high nutritional value (Moodley, 2012; Mlambo et al., 2014; Mngadi et al., 2016). Furthermore, it has been reported that most rural households in South Africa rely on wild fruits for dietary diversity and food security (Moodley et al., 2008). However, the problem with consuming these fruits is potential exposure to heavy metals that come from contaminated soil. Plants take up nutrients from soil for growth and development and toxic heavy metals that are present in soil are speciously taken up through various mechanisms. This may either lead to phytotoxicity or may become a route of exposure to toxic substances through ingestion that can have detrimental effects to human health (Street et al., 2008; Liu et al., 2013). Heavy metals such as As, Cd, Cr and Pb are introduced into soil in different ways such as industrial and anthropogenic activities and they are known to be major contaminants since they are non-biodegradable and are highly toxic on long term exposure (Chunilall et al., 2004). Therefore, an assessment of the heavy metal content in food that are both commercial and indigenous is crucial to appraise for toxicity and associated risks to human health.

Mimusops zeyheri, of the family Sapotaceae, is known as Transvaal red milkwood or Mmupudu and is indigenous to the northern parts of South Africa and most of the other African countries. The yellow oval fruits that are known for their high vitamin C content, usually ripen from April to September (Mashela and Mollel, 2001). Ripe fruits are picked and eaten directly from the tree, sold in street and open markets, used for making jams, jellies and fermented juices, and used for the production of different beverages (Mashela and Mollel, 2001; Eliton et al., 2011).

Previous studies have reported on the elemental distribution in edible plant species found in South Africa as a function of soil quality (Mngadi et al., 2016; Moodley et al., 2012; Mahlangeni et al., 2016). In this study, we investigate the distribution of metals in *M. Zeyheri* fruits collected from various sites in Gauteng and North West Province, South Africa. From the eight sampling sites, twenty four elements (Li, Be, B, V, Cr, Mn, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Mo, Ag, Cd, Re, Ba, Tl, Pb, Bi and U) were selectively investigated. The impact of soil quality on elemental uptake was evaluated to assess for metal toxicity in the fruits and geoaccumulation indices were calculated to assess for metal contamination in the soil.

4.3 MATERIALS AND METHODS

4.3.1 Sampling

Sampling was conducted in Gauteng and North West Province, South Africa which is dominated by different mining companies. Plant material and soil samples were collected from eight different sites namely: Site A - Atteridgeville, Site B - Hamanskraal, Site C - Mamelodi, Site D - Soshanguve, Site E - Silverton, Site F - Mabopane, Site G - Garankuwa and Site H - Rustenburg. Sampling was conducted in April 2017 during the harvest period. The weather during the collection period was cold (19ºC) without rainfall or the wind. Soil samples were systematically obtained by extracting a diameter of 3.8 cm soil cores using a 20 cm long thin-walled stainless steel coring tube from a depth of approximately 15 cm from six points around the dripline of the tree. Soil aliquots were manually homogenised using a plastic spoon after removal of extraneous material such as leaves and rocks. The composited soil volume was reduced by coning and quartering and the final aliquot was packed in polyethylene bags and refrigerated at 4ºC to limit microbial manifestations. Plant samples were also stored in polyethylene bags and refrigerated at 4ºC.

4.3.2 Reagents and standards

Analytical grade chemicals from Merck were used for this research. Double distilled water was used for the preparation of standards and for dilution of samples. HNO_3 (3M) was used to thoroughly clean all glassware to prevent contamination. The certified reference material (CRM), *lyophilized brown bread* (BCR 191, Community Bureau of Reference of the Commission of the European Communities), was analysed to evaluate the accuracy of the analytical method due to matrix similarities with the plant samples. The analytical procedure used for the CRM was the same as that for the plant samples.

4.3.3 Sample preparation

All fruit samples were washed with distilled water several times to ensure they were clean and then cut into smaller pieces with a stainless steel knife. The samples were then dried, overnight, in an oven at 50ºC, crushed into a fine powder using a food processor (Russell Hobbs range) and stored in labelled polyethylene bags. Soil samples were dried in an oven at 50ºC, overnight, sieved through a 2 mm sieve to obtain the soil fraction then placed into labelled polyethylene bags until analysed.

4.3.4 Digestion of samples

Digestions were performed using the CEM microwave accelerated reaction system (MARS 6, CEM Corporation, USA) according to the method as described by Moodley et al. (2012). Five replicates of each sample (fruit and CRM (0.5 g), and soil (0.25 g)) were digested in 10 mL of

70% HNO_3 after pre-digestion for 30 min. Digests were filtered through Whatman No. 1 filter papers into 50 mL volumetric flasks and made up to the mark with double distilled water. All samples were stored in a refrigerator in polyethylene bottles until analysed.

4.3.5 Analytical instruments

All elements were analysed using the Agilent ICP-MS 770X with an AS 93 plus auto-sampler. Analytes were Li, Be, B, V, Cr, Mn, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Mo, Ag, Cd, Re, Ba, Tl, Pb, Bi and U. Working standards were made up from elemental standards (1000 mg L^{-1}, Merck, St Louis, USA) with double distilled water and 10 mL of 70% HNO_3 to match the sample matrix. All analyses were done in triplicate. The Radiometer MeterLab pHM 220 was used for the analysis of soil samples.

4.3.6 Extraction of exchangeable metals

An extractant solution using ammonium acetate, ethylenediaaminetetraacetic acid (EDTA) and acetic acid was prepared and used to release available metals from soil fractions as described by Moodley et al (2012). The extracts which contained the exchangeable fraction of metals in soil were stored in labelled polyethylene bottles in a refrigerator until analysed.

4.3.7 Soil pH, Soil organic matter (SOM) and cation exchange capacity (CEC)

Soil pH was obtained by measuring a soil to 0.01 M $CaCl_2$ suspension (1:1) using a pH meter. SOM was determined according to the method as described by Walkley and Black (1934) and CEC was determined according to the method as described by Chapman (1965). All determinations were done in quadruplicate.

4.3.8 Bioaccumulation factor (BAF)

Bioaccumulation is the accumulation of the metal in the plant from the surrounding area and the bioaccumulation factor (BAF) is calculated as follows:

$$BAF = \frac{[\text{Element}]_{\text{plant}}}{[\text{Element}]_{\text{soil}}}$$

4.3.9 Geoaccumulation Index

The geo-accumulation index (Igeo) assesses the extent to which metal contamination has occurred by comparing measured metal concentrations to that of the earth's crust (Yaqin, 2008) and is calculated as follows:

$$I_{geo} = \log_2 \frac{C_n}{B_n \times 1.5}$$

C_n is the concentration of the heavy metal in the soil and B_n is the geoaccumulation background concentration in the earth's crust.

4.3.10 Quality assurance

The CRM, *lyophilized brown bread* (BCR 191), was analysed and the data statistically evaluated using the statistical mean and standard deviation. The test results obtained on analysis of the CRM are summarized in Table 14. The investigation showed that the test results compared well to certified values thereby validating the method.

Table 14: Comparison of measured and certified/indicative values (Mean ± SD at 95% confidence interval, n = 5), based on dry mass, in the certified reference material, *lyophilized brown bread* (BCR 191).

Element	Certified/Indicative concentration mg kg^{-1}	Measured concentration mg kg^{-1}
As	0.023[a]	0.020 ± 0.23
Ca	0.41[a]	0.39 ± 0.21
Cu	2.60 ± 0.1	2.66 ± 0.9
Fe	40.7 ± 2.3	40.3 ± 7.9
Mg	0.50 [a]	0.51 ± 0.50
Mn	20.3 ± 0.7	20.7 ±4.3
Zn	19.5 ± 0.5	19.8 ± 0.3

[a] Indicative values are those without uncertainties.

4.3.11 Statistical analysis

Multivariate statistical analyses (principal component analysis (PCA) and cluster analysis (CA)) were performed to determine the relationship between input variables. An analysis of covariance was performed to assess for significant differences between plant and soil at the different sites. Pearson's correlation coefficients (r) were obtained to determine the relationship between the concentrations of the elements in the plant and soil (total and exchangeable). All statistical analyses were performed using the Statistical Package for the Social Science (PASW Statistics, Version 23, IBM Corporation, Cornell, New York).

4.4 RESULTS AND DISCUSSION

4.4.1 Elemental analysis

The fruits of *M. Zeyheri* were found to contain 2.0 % carbohydrate, 92.7% moisture, 6.2% protein, 4.1% ash and 0.4% oil.

The concentration of the elements in the fruits and soil (total and exchangeable) and BAF are presented in Table 15. The macronutrients in the fruit were found to be Ca, K, Mg and Na (Figure 10).

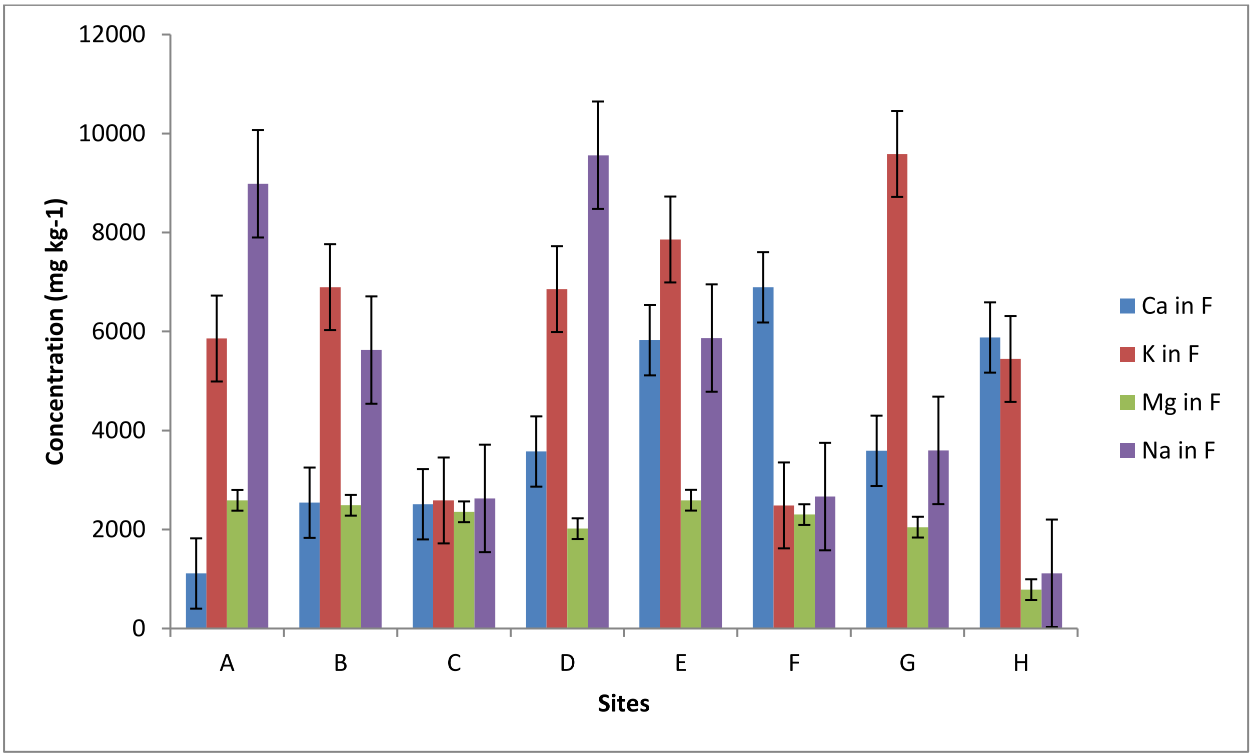


Figure 10: Distribution of the major elements in *Mimusops zeyheri* fruits at the 8 sites.

Site A- Atteridgeville, Site B Hamanskraal, Site C- Mamelodi, Site D- Soshanguve, Site E-Silverton, Site F-Mabopane, Site G- Garankuwa and Site H- Rustenburg

Potassium was the macronutrient found in highest concentration in the fruit (Table 15, Figure 10) compared to the other elements in this study. Total soil Mg was highest at Rustenburg (2425 mg kg^{-1}) but was low in the fruit (785 mg kg^{-1}) while total soil Mg was lowest at Silverton

(1153 mg kg^{-1}) with high concentration in the fruit (2591 mg kg^{-1}). On average, 35% of Mg in the soil was available for plant uptake and high amount of this was accumulated by the plant with high BAF ranging between 2.2 to 20.7 %. High BAFs observed for Ca, Mg and Na show that the plant has a tendency to accumulate these elements to meet physiological requirement levels, similar to other studies (Mngadi et al., 2016; Mahlangeni et al., 2016; Moodley et al., 2017).

Table 15: Elemental concentrations in mg kg^{-1} (Mean (SD), n=5) of selected elements in the fruits of *Mimusops zeyheri.*

		Concentration (mg kg^{-1})			**BAF**		**[Soil]E/[Soil]T**
Site*	**Element**	**Soil (T)**	**Soil (E)**	**Fruit**	**[Fruit]/[Soil]T**	**[Fruit]/[Soil]E**	**%**
A	Ag	4.31 (0.2)	0.8 (0.02)	ND	-	-	18
B		1.08 (0.1)	ND	ND	-	-	-
C		3.20 (0.5)	0.2 (0.01)	ND	-	-	5
D		2.27 (0.1)	0.6 (0.02)	ND	-	-	26
E		2.80 (0.2)	0.4 (0.05)	ND	-	-	13
F		2.77 (0.1)	1.2 (0.03)	ND	-	-	42
G		1.10 (0.2)	0.6 (0.02)	ND	-	-	56
H		2.90 (0.5)	ND	ND	-	-	-
A	Al	2503 (101)	1582 (12)	125 (12)	0.05	0.1	63.2
B		1496 (96)	526 (9)	225 (18)	0.15	0.4	35.2
C		1812 (25)	568 (8)	105 (19)	0.06	0.2	31.3
D		1850 (65)	582 (9)	95 (9)	0.05	0.2	31.5
E		1486 (42)	568 (12)	125 (16)	0.08	0.2	38.2
F		1945 (62)	925 (4)	198 (11)	0.10	0.2	47.6
G		3333 (120)	1525 (5)	389 (7)	0.12	0.3	45.8
H		1254 (58)	625 (8)	89 (18)	0.07	0.1	49.8
A	As	8.52 (0.2)	2.3 (0.1)	ND	-	-	27.0
B		3.18 (0.5)	2.5 (0.1)	ND	-	-	78.6
C		2.98 (0.09)	0.5 (0.1)	ND	-	-	16.8
D		6.17 (0.5)	0.5(0.1)	ND	-	-	8.1
E		7.26 (0.5)	3.8 (0.1)	ND	-	-	52.4
F		3.49 (0.6)	0.5 (0.1)	8.14 (0.2)	2.33	16.3	14.3
G		4.00 (0.8)	0.9 (0.1)	ND	-	-	22.5
H		8.52 (1.2)	1.87 (0.1)	10.5 (0.2)	1.23	5.6	21.9
A	Ba	11.79 (0.1)	3.1 (0.2)	ND	-	-	26.5
B		7.47 (0.1)	4.2 (0.5)	ND	-	-	55.9
C		9.69 (0.1)	9.3 (0.4)	ND	-	-	95.8
D		7.68 (0.1)	2.8 (0.09)	ND	-	-	36.3
E		7.32 (0.1)	5.4 (0.6)	ND	-	-	73.3
F		14.06(0.1)	1.3 (0.41)	ND	-	-	9.4
G		22.00 (0.2)	19.9 (1.2)	ND	-	-	90.6
H		19.00 (1)	7.5 (0.58)	ND	-	-	39.3
A	Be	0.102 (0.02)	ND	ND	-	-	-
B		0.150 (0.02)	ND	ND	-	-	-
C		0.077 (0.01)	ND	ND	-	-	-
D		0.065 (0.002)	ND	ND	-	-	-
E		0.063 (0.001)	ND	ND	-	-	-
F		0.097 (0.002)	ND	ND	-	-	-
G		1.020 (0.002)	ND	ND	-	-	-

H		0.250 (0.002)	ND	ND	-	-	-
A	Bi	32 (2.5)	ND	0.03 (0.001)	0.001	-	-
B		13 (0.8)	ND	0.05 (0.002)	0.004	-	-
C		9 (3.5)	ND	ND	-	-	-
D		23 (1.8)	ND	ND	-	-	-
E		29 (1.8)	ND	ND	-	-	-
F		13 (0.5)	ND	ND	-	-	-
G		20 (1.5)	ND	0.01 (0.002)	0.0003	-	-
H		8 (0.9)	ND	0.10 (0.001)	0.0112	1.0	1.2
A	Ca	4484 (12)	3225 (120)	1111 (58)	0.25	0.3	71.9
B		21926 (25)	1052 (95)	2541 (69)	0.12	2.4	4.8
C		3274 (14)	2325 (102)	2510 (102)	0.77	1.1	71.0
D		3376 (18)	1258 (58)	3576 (15)	1.06	2.8	37.3
E		27969 (22)	15892 (65)	5825 (255)	0.21	0.4	56.8
F		7183 (35)	5589 (12)	6893 (35)	0.96	1.2	77.8
G		5555 (0.1)	4369 (85)	3589 (36)	0.65	0.8	78.6
H		78528 (12)	38258 (103)	5879 25)	0.07	0.2	48.7
A	Cd	0.706 (1.2)	0.02 (0.8)	ND	-	-	2.7
B		0.039 (1.3)	0.01 (0.1)	ND	-	-	26.1
C		0.035 (0.5)	0.01 (0.6)	ND	-	-	32.2
D		0.035 (0.9)	0.02 (0.5)	ND	-	-	63.6
E		0.034 (0.8)	0.01 (0.4)	ND	-	-	27.2
F		2.900 (0.8)	0.01 (0.4)	ND	-	-	0.2
G		3.700 (0.1)	0.01 (0.8)	ND	-	-	0.3
H		3.000 (0.6)	0.01 (0.9)	ND	-	-	0.4
A	Cu	1.5 (1.2)	0.200 (0.2)	6.20 (0.5)	4.13	31.0	13.3
B		5.2 (1.5)	0.020 (0.5)	8.60 (0.2)	1.65	430.0	0.4
C		6.5 (1.2)	1.200 (0.12)	9.20 (0.2)	1.42	7.7	18.5
D		12.3 (1.0)	1.63 (0.1)	12.50 (0.3)	1.02	7.7	13.3
E		20.2 (1.2)	5.60 (0.3)	4.47 (0.01	0.22	0.8	27.7
F		8.5 (1.1)	2.20 (0.5)	5.01 (0.1)	0.59	2.3	25.9
G		1.2 (1.3)	0.020 (0.5)	8.98 (0.2)	7.48	449.0	1.7
H		4.56 (1)	0.100 (0.5)	22.70 (0.3)	4.98	227.0	2.2
A	Co	0.413 (4.5)	ND	ND	-	-	-
B		0.163 (5.6)	ND	0.64 (0.08)	3.94	-	-
C		0.149 (0.49)	ND	0.82 (0.06)	5.49	-	-
D		0.268 (0.85)	ND	1.24 (0.06)	4.65	-	-
E		0.229 (0.6)	ND	0.01 (0.04)	0.06	-	-
F		0.191 (0.2)	ND	6.14 (0.05)	32.12	-	-
G		0.368 (0.1)	ND	0.79 (0.01)	2.14	-	-
H		0.558 (0.5)	ND	1.02 (0.02)	1.82	-	-
A	Cr	22 (0.1)	ND	ND	0.00	-	-
B		12 (2)	ND	1.50 (0.02)	0.12	-	-
C		8 (0.2)	ND	2.50 (0.03)	0.33	-	-
D		15 (0.3)	ND	10.60 (0.00)	0.68	-	-
E		14 (0.2)	ND	12.50 (0.02)	0.91	-	-
F		14 (0.2)	ND	5.60 (0.01)	0.41	-	-
G		12 (0.1)	ND	8.90 (0.01)	0.74	-	-
H		35(0.1)	0.7 (0.05)	22.80 (0.02)	0.65	31.2	2.1
A	Fe	3670 (111)	125.0 (12)	150.0 (1.5)	0.04	1.2	3.4
B		1111 (125)	526.0 (15)	241.4 (2.5)	0.22	0.5	47.3
C		9589 (98)	258.0 (25)	246.9 (1.6)	0.03	1.0	2.7
D		2365 (52)	136.0 (25)	89.0 (1.8)	0.04	0.7	5.8
E		2958 (36)	155.0 (36)	256.0 (1.7)	0.09	1.7	5.2
F		1003 (112)	89.0 (12)	489.0 (1.6)	0.49	5.5	8.9
G		5252 (85)	42.0 (9)	685.0 (1.5)	0.13	16.3	0.8
H		4589 (65)	78.0 (25)	130.0 (1.8)	0.03	1.7	1.7
A	Ga	0.92 (0.05)	ND	0.01 (0.004)	0.01	1.1	0.5
B		0.51 (0.01)	ND	ND	0.01	1.0	0.9
C		0.49 (0.02)	ND	0.01 (0.004)	0.01	1.1	0.9

D		0.63(0.03)	ND	ND	0.01	1.0	0.7
E		0.47 (0.02)	ND	ND	0.01	1.0	1.0
F		0.51 (0.02)	ND	0.02 (0.004)	0.03	3.8	0.9
G		1.00 (0.01)	ND	0.01 (0.004)	0.01	1.2	0.5
H		1.90 (0.013)	ND	ND	0.00	1.0	0.2
A	K	397 (12.5)	29.4 (0.1)	5858 (20.5)	14.76	199.3	7.4
B		211 (107)	10.7 (0.6)	6897 (35)	32.76	644.4	5.1
C		890 (9.2)	10.8 (0.6)	2587 (36)	2.91	240.1	1.2
D		243 (8)	23.9 (0.54)	6857 (35)	28.21	286.7	9.8
E		197 (7)	12.3 (0.5)	7859(25.6)	39.89	637.3	6.3
F		665 (10.3)	25.6 (0.59)	2487 (35)	3.74	97.1	3.8
G		524 (10.2)	23.8 (0.54)	9587 (12.2)	18.30	403.5	4.5
H		852 (9.8)	7.6 (0.56)	5445 (9.8)	6.39	712.5	0.9
A	Li	3.90 (0.1)	ND	1.20 (0.04)	0.31	-	-
B		2.00 (0.2)	ND	0.50 (0.04)	0.25	-	-
C		1.12 (0.2)	ND	0.30 (0.04)	0.27	-	-
D		2.63 (0.3)	ND	1.50 (0.08)	0.57	-	-
E		2.04 (0.2)	ND	1.60 (0.04)	0.78	-	-
F		2.27 (0.3)	ND	2.50 (0.04)	1.10	-	-
G		5.21 (0.3)	1.2 (0.005)	0.60 (0.04)	0.12	0.5	22.2
H		4.58 (0.21)	2.6 (0.005)	2.90 (0.05)	0.63	1.1	56.1
A	Mg	1510 (12.3)	688 (2.5)	2589 (12.3)	1.71	3.8	45.6
B		1310 (0.25.3)	524 (6.6)	2489 (8.6)	1.90	4.8	40.0
C		1311 (612)	259 (5.5)	2358 (20..3)	1.80	9.1	19.7
D		1380 (0.1)	854 (6.6)	2017 (8.9)	1.46	2.4	61.9
E		1153 (2.3)	125 (8.7)	2591 (9.5)	2.25	20.7	9.5
F		1234 (5.6)	658 (6.5)	2301 (10.5)	1.86	3.5	53.3
G		1452 (9.5)	528 (5.6)	2047 (10.2)	1.41	3.9	36.4
H		2425 (4.5)	359 (3.5)	785 (4.9)	0.32	2.2	14.8
A	Mn	480 (12)	89.0 (0.2)	10.0 (0.05)	0.02	0.1	18.6
B		347 (25)	58.0 (0.5)	12.9 (0.08)	0.04	0.2	16.7
C		239 (21)	36.0 (1.3)	49.0 (0.1)	0.21	1.4	15.1
D		370 (9.5)	75.0 (1.5)	6.5 (0.6)	0.02	0.1	20.3
E		400 (10)	66.0 (2.5)	28.0 (0.6)	0.07	0.4	16.5
F		678 (20.5)	87.0 (1.32)	8.9 (0.8)	0.01	0.1	12.8
G		560 (12.3)	47.0 (0.5)	40.2 (0.9)	0.07	0.9	8.4
H		740 (12.1)	ND	78.9 (0.54)	0.11	0.4	25.0
A	Mo	2.6 (0.2)	ND	ND	-	1.0	0.1
B		1.4 (0.3)	0.01 (0.004)	0.01 (0.04)	-	1.0	0.4
C		1.9 (0.2)	ND	ND	-	1.0	0.2
D		1.7 (0.1)	ND	ND	-	1.0	0.2
E		2.3 (0.2)	ND	ND	-	1.0	0.2
F		2.6 (0.3)	0.07 (0.004)	0.07 (0.04)	-	1.0	2.7
G		2.3 (0.2)	ND	ND	-	1.0	0.2
H		4.7 (0.1)	ND	ND	-	1.0	0.1
A	Na	7895 (235)	439.3 (102)	8985.0 (98)	1.14	20.5	5.6
B		8763 (12)	559.8 (9)	5625.0 (58)	0.64	10.0	6.4
C		7598 (2.6)	450.3 (8)	2628.0 (25)	0.35	5.8	5.9
D		7526 (35)	713.2 (7)	9562.0 (65)	1.27	13.4	9.5
E		7821 (12)	545.1 (65)	5868.0 (85)	0.75	10.8	7.0
F		8369 (18)	444.2 (10)	2665.0 (41)	0.32	6.0	5.3
G		8215 (25)	1223.0 19.9)	3598.0 (25)	0.44	2.9	14.9
H		7301 (2.4)	854.0 (15)	1115.0 (65)	0.15	1.3	11.7
A	Ni	844 (11.5)	631.7 (18)	0.13 (0.01)	0.0001	0.0002	74.8
B		353 (25.6)	224.9 (15)	0.50 (0.05)	0.0014	0.002	63.8
C		354 (11.6)	250.6 (16)	0.74 (0.06)	0.0021	0.003	70.8
D		561 (10.5)	277.9 (19)	1.18 (0.02)	0.0021	0.004	49.5
E		559 (9.8)	140.8 (12)	ND	-	-	25.2
F		517 (8.6)	108.3 (11)	5.89 (0.1)	0.0114	0.054	20.9
G		984 (9.5)	751.7 (10)	0.69 (0.1)	0.0007	0.001	76.4

H		254 (10)	120.7 (11)	0.97 (0.1)	0.0038	0.008	47.5
A	Pb	125 (1.2)	ND	0.50 (0.02)	-	-	0.0
B		29 (1.5)	6.7(0.2)	1.50 (0.06)	0.05	0.2	23.2
C		24 (2.6)	ND	2.50 (0.08)	0.10	-	-
D		62 (2.5)	ND	1.02 (0.05)	0.02	-	-
E		89 (3.6)	ND	2.22 (0.06)	0.02	-	-
F		20 (5.6)	ND	2.50 (0.05)	0.12	-	-
G		58 (5.6)	ND	0.10 (0.02)	0.00	-	-
H		133 (1.2)	ND	8.90 (0.1)	0.07	-	-
A	Rb	68 (5.6)	2.7 (0.1)	0.05 (0.004)	0.001	0.019	3.9
B		47 (8.6)	1.5 (0.2)	0.15 (0.004)	0.003	0.099	3.2
C		30 (6)	4.0 (0.5)	0.12 (0.004)	0.004	0.030	13.2
D		51 (4.2)	1.6 (0.1)	0.25 (0.004)	0.005	0.158	3.1
E		65 (8.6)	1.3 (0.1)	0.05 (0.004)	0.001	0.037	2.1
F		20 (10.5)	3.4 (10.6)	0.65 (0.004)	0.033	0.190	17.4
G		52 (2.6)	3.0 (00.5)	0.03 (0.004)	0.0001	0.008	5.8
H		74 (6)	5.5 (0.6)	0.95 (0.004)	0.013	0.173	7.4
A	Sr	240 (10.5)	4.6 (0.5)	0.15 (0.02)	0.001	0.03	1.9
B		641 (1.5)	2.5 (0.3)	1.25 (0.01)	0.002	0.50	0.4
C		181(2.3)	4.1 (0.8)	1.54 (0.1)	0.008	0.38	2.3
D		325 (15.6)	3.0 (0.5)	20.50 (1.2)	0.063	6.91	0.9
E		385 (10.2)	2.7 (0.6)	2.56 (0.2)	0.007	0.94	0.7
F		599 (8.9)	3.9 (0.9)	6.20 (0.5)	0.010	1.61	0.6
G		341 (5.5)	3.5 (0.4)	5.00 (0.1)	0.015	1.43	1.0
H		353 (2.1)	5.5 (0.05)	6.60 (0.25)	0.019	1.20	1.6
A	Te	0.92 (0.05)	ND	0.00	-	-	-
B		0.35 (0.06)	ND	0.00	-	-	-
C		0.22 (0.05)	ND	0.00	0.01	-	-
D		0.57 (0.09)	ND	0.00	-	-	-
E		0.64 (0.05)	ND	0.00	-	-	-
F		0.46 (0.087)	ND	0.02	0.04	-	-
G		0.36 (0.05)	ND	0.00	-	-	-
H		0.58 (0.08)	ND	0.00	-	-	-
A	Tl	2.85(0.03)	0.5 (0.08)	0.0	-	-	18.0
B		0.99 (0.05)	ND	0.0	-	-	-
C		1.30 (0.02)	0.8 (0.07)	0.0	-	-	61.5
D		1.67 (0.03)	ND	0.0	-	-	-
E		3.20 (0.04)	2.3 (0.09)	0.0	-	-	71.9
F		1.52 (0.05)	1.0 (0.02)	0.0	-	-	67.6
G		1.80 (0.25)	ND	0.0	-	-	-
H		2.90 (0.205)	0.4 (0.08)	0.0	-	-	14.1
A	U	441 (7.8)	5.60 (0.1)	0.15 (0.01)	-	-	1.3
B		96 (0.1)	ND	ND	-	-	-
C		29 (0.8)	ND	ND	-	-	-
D		153(1.6)	ND	ND	-	-	-
E		152 (9.2)	ND	ND	-	-	-
F		217 (5.8)	ND	ND	-	-	-
G		68 (01.8)	ND	ND	-	-	-
H		225 (4.9)	12.08 (0.8)	0.24 (0.01)	-	-	5.4
A	Zn	229 (2.6)	85.3 (8.9)	35.00 (2.7	0.15	0.4	37.3
B		111 (7.6)	65.8 (12.6)	96.00 (6.6)	0.86	1.5	59.2
C		116 (8.9)	58.9 (18.3)	84.00 (8.7)	0.73	1.4	50.9
D		112 (1.5)	99.1 (10)	65.00 (9.9)	0.58	0.7	88.7
E		143(6.5)	25.9 (9.8)	25.00 (6.6)	0.18	1.0	18.2
F		91 (5.8)	88.6 (14)	35.00 (5)	0.39	0.4	97.9
G		133 (6.1)	23.8(10)	85.00 (7)	0.64	3.6	17.9
H		85(10.8)	25.7 (8)	65.00 (8)	0.76	2.5	30.2

Site A- Atteridgeville, Site B Hamanskraal, Site C- Mamelodi, Site D- Soshanguve, Site E-Silverton, Site F-Mabopane, Site G- Garankuwa and Site H- Rustenburg.ND- Not determinable

Fe is one of the most abundant elements in soil (Knezek et al., 1971). The average of Fe and Al in the fruit was 169 and 286 mg kg^{-1}, respectively. Mamelodi (9589 mg kg^{-1}) was found to have the highest total soil Fe while the lowest was observed at Mabopane (1003 mg kg^{-1}). At all sites, Fe in the fruit was higher than Al expect at Soshanguve where Fe was 89 mg kg^{-1} while Al was 95 mg kg^{-1}. The percentage Fe available for plant uptake was in a narrow range (0.8 – 47.3%). The lowest amount of Fe in the fruit was at Soshanguve while higher Fe concentrations in the fruit was at Garankuwa with low BAF <1 at all sites. The highest concentration of Al in the fruit was at Garankuwa (389 mg kg^{-1}) and lowest at Rustenburg (89 mg kg^{-1}) (Figure 11a). This means that plant concentrations of Al are dependent on soil concentrations. When Al in soil is too high, then the plant concentration also increases; when Al in soil is low then the plant concentration also decreases.

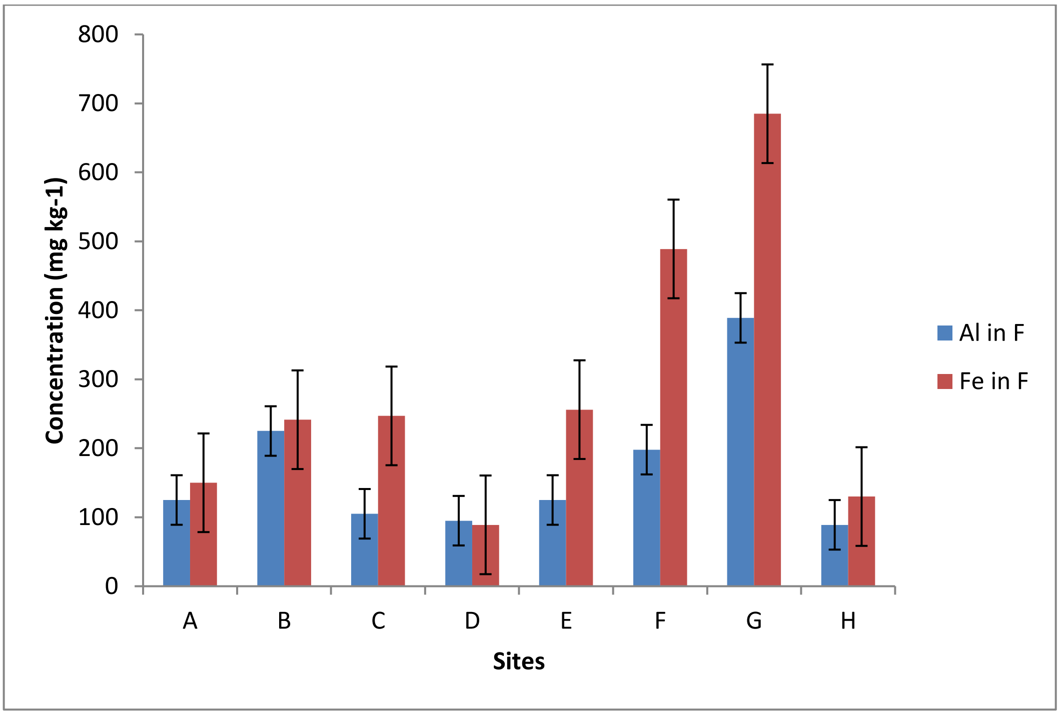


Figure 11a: Distribution of the minor elements in *Mimusops zeyheri* fruit at the 8 sites.

Site A- Atteridgeville, Site B Hamanskraal, Site C- Mamelodi, Site D- Soshanguve, Site E-Silverton, Site F-Mabopane, Site G- Garankuwa and Site H- Rustenburg

Zinc is one of the microelements that is essential for plant growth (Marschner, 1993). Total soil Zn ranged from 85 - 229 mg kg^{-1} which is the typical range for most uncontaminated soils (10 – 300 mg kg^{-1}) (Marschner, 1993). The average concentration of Zn in the fruit was 61 mg kg^{-1}, average Ex% is 65% and BAF > 1 at most sites indicating that the plant tends to accumulate Zn. The concentration of Zn in the fruit was in a small range of variation (25 – 96 mg kg^{-1}) with Hammanskraal being the highest and Silverton the lowest (Figure 11b).

Although copper is essential for both plants and humans, it is potentially toxic if ingested at high concentrations (Marschner, 1993). On average, only 12.9% of Cu was available for plant uptake. Total soil Cu was highest at Silverton (20.2 mg kg^{-1}), of which 5.60 mg kg^{-1} was in exchangeable form and concentration in the fruits was 4.47 mg kg^{-1}. The plant tended to accumulate Cu with high BAF > 1 at all sites.

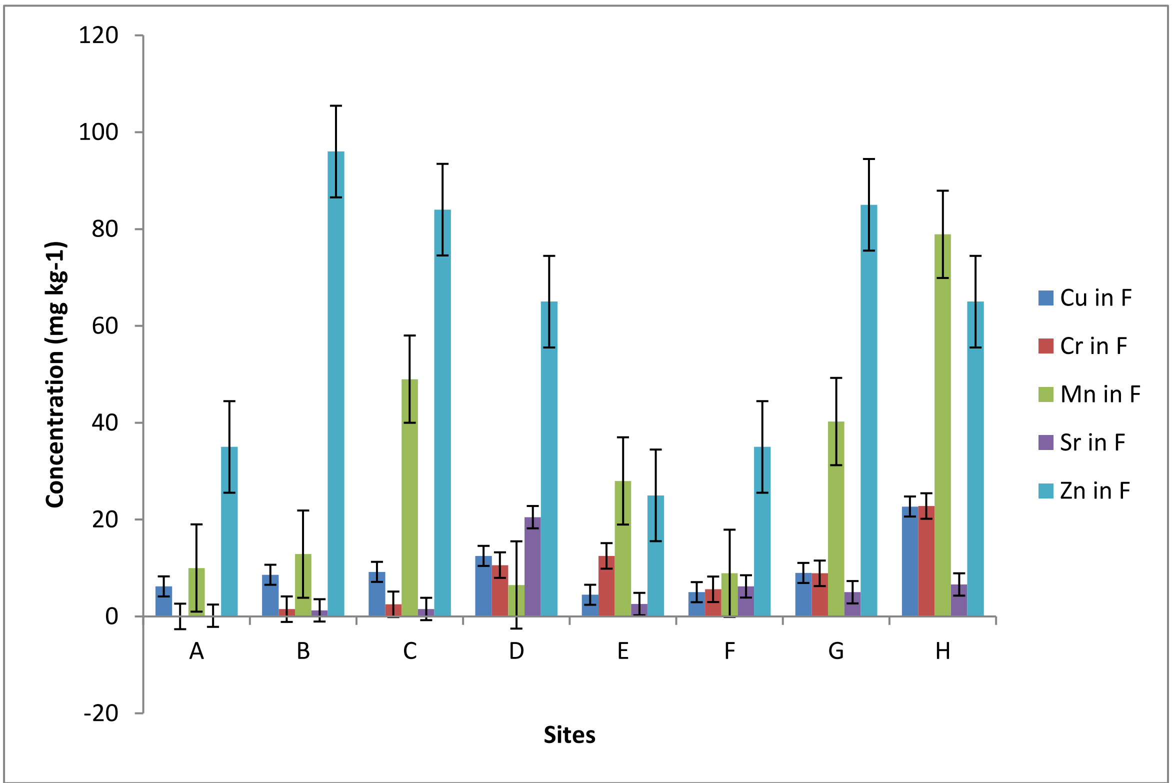


Figure 121b: Distribution of the minor elements in *Mimusops zeyheri* fruit at the 8 sites.

Site A- Atteridgeville, Site B Hamanskraal, Site C- Mamelodi, Site D- Soshanguve, Site E-Silverton, Site F-Mabopane, Site G- Garankuwa and Site H- Rustenburg

Strontium is mostly found in soil and plants at lower concentrations (Schneider et al., 2005). In this study, total soil Sr was between 181 to 641 mg kg^{-1}. Low concentrations of Sr was available for plant uptake (Ex%= 0.4 -1.9%), however, the plant tended to accumulate Sr at most sites (Soshanguve, Mabopane, Garankuwa and Rusternburg). Total soil Mn ranged from 239 to 740 mg kg^{-1}. Manganese in the fruit ranged between 6.5 and 78.9 mg kg^{-1}. Exchangeable Cr was below the detection limit for most sites except Rustenburg however, Cr in the fruit ranged between 1.5 and 22.8 mg kg^{-1}. Total soil Co was higher at Rustenburg and lowest at Mamelodi and exchangeable Co was below the detection limit at all sites. The fruit tended to accumulate Co even though at relatively low concentrations (0.01 to 6.14 mg kg^{-1}).

Amongst the toxic metals, As in the fruit was found at Mabopane and Rustenburg only. Total soil As ranged between (3.0 – 8.5 mg kg^{-1}). At Mabopane and Rustenburg, As concentration in the fruit was higher than the total soil concentrations indicating plant accumulate As due to industrial activities in the area. No Cd was detected in any of the fruit samples. The estimation of Cd in non-polluted soils ranges between 0.04 to 0.32 mg kg^{-1} (Lagerwerff et al., 1971). In this study, total soil Cd was above this limit (0.03 – 3.7 mg kg^{-1}).

The concentration of Ga, Mo and Rb in the fruit was low (Table 15). Although Ga in soil was present at all sites none was available for plant uptake. Rubidium is generally found in soils at 100 mg $kg^{-1}$and in most land plants at 20 mg kg^{-1} (Liu et al., 2013). Total soil Rb was highest at Rustenburg and lowest at Mabopane and Rb in the fruit was within the range 0.03 to 0.95 mg kg^{-1} which is considered safe.

Uranium is a naturally occurring element found in low levels within rocks, soil and water. Humans can also add U to the environment by mining or melting processes (Liu et al., 2013). In this study, total soil U ranged between 29 to 441 mg kg^{-1}. The high concentration of U at most sites is due to mining activities taking place in the Gauteng and North West Province and surroundings. Only two sites were found to contain U in the fruit. Atteridgeville had 0.15 mg kg^{-1} in the fruit, total soil U was 441 mg kg^{-1} with 5.6 mg kg^{-1} in exchangeable form. At Rustenburg, 12.1 mg kg^{-1} was available and only 0.24 mg kg^{-1} was detected in the fruits.

4.4.2 Soil pH, SOM and CEC

The soil was analysed for soil pH, SOM and CEC for all soil samples collected at 8 sites chosen in this study (Table 16). The results showed that the pH of the soil ranged from 5.96 to 7.14 indicating that the plant grows in a slightly acidic soil. SOM ranged from 2.02 to 8.00% and the CEC ranged between 10.0 and 14.7 meq/100g. CEC is higher than SOM at all sites. An increase in SOM would increase soils CEC since mineral cations adsorb to the negative surface charges of organic soil particles.

Table 16: pH, SOM and CEC of soil samples obtained from the eight different sites.

Sites^{b}	***pH*** ($CaCl_2$)^{a}	***SOM***^{a} ***(%)***	***CEC***^{a} ***(meq/100g)***
A	6.02	2.02	13.3
B	6.37	4.04	10.8
C	7.14	5.08	10.0
D	6.22	6.99	14.7
E	6.04	6.20	12.8
F	5.96	7.88	11.7
G	6.32	8.00	12.5
H	6.98	5.96	11.9

[a] Expressed as mean, n = 5

Site A- Atteridgeville, Site B Hamanskraal, Site C- Mamelodi, Site D- Soshanguve, Site E-Silverton, Site F-Mabopane, Site G- Garankuwa and Site H- Rustenburg

4.4.3 Chemical composition of *Mimusops zeyheri* fruits

4.4.3.1 Contribution to the diet

It is important to study the contribution of the nutrients in the fruits of *M. zeyheri* to recommended dietary allowances (RDAs) since these fruits are picked and eaten raw by the people in rural communities. The results are summarised and presented in Table 17 below. Most nutrients in this study were within Tolerable Upper Intake Levels (ULs).

Table 17: Dietary Reference Intake (DRIs), Recommended Dietary Allowances (RDAs) and Tolerable Upper Intake Levels (ULs) of elements for most individuals and average concentration of elements (n = 5) in *Mimusops zeyheri* fruits.

Element	Average Concentration (mg/10 g dry mass)	DRI[a] (mg/day)		Estimated Contribution to RDA (%)
		RDA	UL	
As	0.023	ND[b]	ND	ND
Al	1.69	100-200	100	0.60
Ca	39.91	1000-1300	2500	3.1
Cu	0.097	0.9	8	10.8
Cr	0.081	0.024-0.035	ND	231
Fe	2.86	8-18	45	15.9
K	59.47	1000-3500	4000	1.7
Mg	21.47	310-320	350	6.7
Mn	2.93	1.6-2.3	9	127
Mo	ND	0.1-0.5	1	-
Na	50.5	1000-2400	2500	21
Ni	0.013	ND	1.0	1.3
Zn	0.61	8-11	34	5.5

[a] Institute of Medicine of the National Academies: Dietary Reference Intakes (2001).
[b] ND = not determined.

For the toxic elements (Ag, As, Bi, Cd, Ga, Pb, Sr and U) the concentrations in the fruit were within safe levels. The consumption of a handful of the fruits (approximately 10.0 g) would contribute 3.1, 1.7, 6.7 and 21% towards the RDA for the micronutrients Ca, K, Mg and Na, respectively (Table 17). Similar results were observed by Mngadi et al. (2016) in the fruits of *M. caffra*. The results showed that 10.0 g of fruits would contribute 231% towards the RDA for Cr for most adults which exceeds the threshold limit. Similar results were observed by Mlambo et al (2015) and Mngadi et al (2016) in the fruits of *Rhoicissus digitata* and *M. caffra*, respectively. However, high intake of Cr is not linked to adverse health effects therefore the Institute of Medicine has not established a tolerable upper intake level (UL) for this element (Davenward et al., 2013).

Mn is known to promote healthy bone structure and formation and also to create enzymes for bone building (Kuo et al., 1981). In this study, the consumption of 10.0 g of the fruits exceeds the RDA for this element (127%) contributing 0.055 mg/day towards the RDA. However, this contribution is within the UL for Mn (9 mg/day). This means that consumption of *M. zeyheri* fruits will not have a negative impact on human health associated with Mn toxicity. The information provided by this study show that *M. zeyheri* fruits are not associated with toxicity but appear to be good for human health.

4.4.4 Geoaccumulation index

The I_{geo} values of heavy metals in soil samples from eight different sites are shown in Table 18. For most elements (Co, Cr, Cu, Ni and Pb) in soil, I_{geo} was less than 0 indicating no contamination by the metals except for Cd which showed contamination at three sites. At Garankuwa there was moderate contamination by Cd (I_{geo} = 1.04) and Mabopane and Rustenburg were uncontaminated to moderately contaminated (Table 18).

Table 18: Total baseline concentration (TBC) of metals in South African soils (mg kg^{-1}) and geoaccumulation index (I_{geo}) for eight sites.

Element	TBT	**Site A**	**Site B**	**Site C**	**Site D**	**Site E**	**Site F**	**Site G**	**Site H**
Cd	2.7	-2.47	-6.67	-6.07	-7.07	-7.07	**0.67**	**1.04**	**0.78**
Co	69	-7.08	9.32	-9.45	-8.60	-8.83	-9.09	-8.13	-7.54
Cr	353	-4.61	-5.47	-5.97	-5.14	-5.24	-5.24	-5.47	-36.9
Cu	117	-4.38	-3.89	-4.23	-3.42	-3.39	-3.66	-2.42	-2.79
Ni	159	-4.83	-6.16	-6.01	-5.41	-5.42	-5.53	-4.60	-6.55
Pb	66	-6.35	-1.78	-2.06	-4.01	-3.50	-2.30	-4.06	-2.90
Zn	115	-0.97	-0.64	-0.94	-1.0	-1.60	-0.94	-0.94	-1.03

Site A- Atteridgeville, Site B Hamanskraal, Site C- Mamelodi, Site D- Soshanguve, Site E-Silverton, Site F- Mabopane, Site G- Garankuwa and Site H- Rustenburg

4.4.5 Statistical analysis

The importance of using principal component analysis is the reduction of the dimensionality of a set of datasets consisting of a large number of related variables, whilst retaining as much as possible, the variation present in the dataset. From the original datasets the new sets of variables are extracted and these are called principal components. The principal components points to the direction where the larger variation is achieved. A component loading higher than 0.71 is considered excellent (Nowak, 1998). Multivarite PCA and CA analysis were performed to assess if the heavy metals in the soil are from a common source. The component matrix and the rotated component matrix of the element in the soil are shown in Table 19.

Table 19: Principal component loadings of metals in soil (Rotation method).

	Rotated component matrix		
	1	2	3
Eigenvalue	7.979	5.405	4.661
Percentage of total variance	29.553	20.019	17.263
Percentage of cumulative variance	29.553	49.573	66.835
AgT	0.154	-0.053	-0.051
AlT	-0.189	0.324	**0.875**
AsT	**0.860**	-0.007	0.087
BaT	0.227	**0.882**	0.210
BeT	0.080	0.525	0.555
BiT	0.255	-0.349	**0.752**
CaT	**0.716**	0.286	-0.587
CdT	0.085	**0.970**	0.116
CoT	**0.829**	0.473	0.105
CrT	**0.815**	0.374	-0.295
CuT	-0.042	-0.362	-0.189
FeT	-0.069	0.007	-0.002
GaT	**0.760**	0.538	-0.180
KT	-0.135	0.598	-0.414
LiT	0.603	0.595	0.442
MgT	**0.729**	0.442	-0.377
MnT	0.330	**0.880**	-0.092
MoT	0.622	0.647	-0.331
NaT	-0.486	-0.028	0.212
NiT	-0.015	0.175	**0.982**
PbT	**0.917**	0.094	0.132
RbT	**0.968**	-0.122	0.185
SrT	-0.255	0.130	-0.292
TeT	0.592	-0.084	0.322
TlT	**0.738**	0.116	0.168
UT	0.415	0.092	0.207
ZnT	0.215	-0.333	**0.704**

In soil, three principal components were extracted with eigenvalues >1 explaining 67% of the total variance. The first principal component (29.5% of the variance) indicated high loadings of As, Ca, Co, Cr, Ga, Mg, Pb, Rb, and Tl which could be from anthropogenic sources such as mining which is highly dense in the Gauteng ad North West Province (Rustenburg, Mabopane and Hamanskraal). The second principal component (20.0% of the variance) showed significant loadings of Ba, Cd and Mn. The high loadings of Ba and Cd indicate that these elements could be from the same source such as industrial applications of casting steel and iron (Rustenburg and Mabopane) while Mn could be from a natural source. A study conducted by Karadas et al. (2012) revealed principal components for Cd, Mn, Ba and Sr in soil, similar to this study. The third principal component (17.3% of the variance) revealed high loadings of Al, Bi, Ni and Zn which could result from anthropogenic input such as production of alloys in the nearby industries (Rustenburg and Mabopane).

To indicate the degree of association between metals in the soil, Ward’s method was applied shown by the Euclidean distance (Figure 12). In this study, cluster analysis was used to further analyse the possible source of elements based on the similarities of their chemical properties. In soil there were three main clusters A, B and B1 (sub cluster of B). Cluster A showed close associations between Al, Bi, Ni and Zn, similar to PCA. Cluster B showed close associations between Co, Cr, Ga, Mg, Pb, Rb, and Tl while cluster B1 showed close associations between Ba and Mn, similar to PCA therefore confirming that these elements are from the same source.

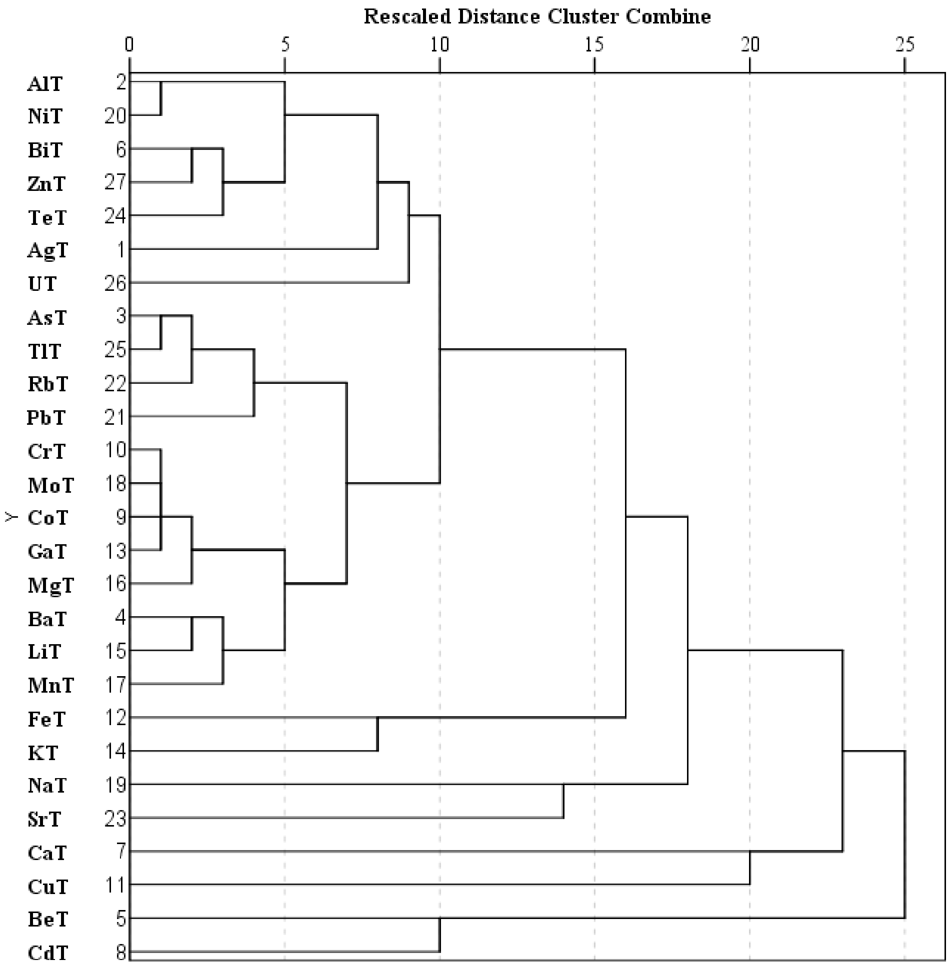


Figure 132: Cluster analysis using Ward's method of elements in soil measured by Euclidean distance.

4.5 CONCLUSION

The presence of heavy metals can be a problem to humans if introduced into the environment (food chain) at higher levels. However, the results indicate that though there was moderate contamination of Cd in the soil, the plant tended to exclude this metal, therefore posing no risk of Cd toxicity, if consumed. Principal component and cluster analysis revealed that Co, Cr, Ga, Mg, Pb, Rb, and Tl were from the same source. The fruits were shown to contain adequate levels of essential nutrients to contribute positively to the diet and was also shown to be rich in Cr and Mn.

4.6 REFERENCES

1. Chapman, HD. 1965. Cation-exchange capacity. In: C.A. Black (ed.). Methods of soil analysis - Chemical and microbiological properties. Agronomy, 9: 891-901.
2. Chunilall, V, Kindness, A, Jonnalagadda SB. 2004, Heavy metal uptake by spinach leaves grown on contaminated soils with lead, mercury, cadmium, and nickel, Journal of Environmental Science and Health, 39 (3): 473 - 481.
3. Davenward, S, Bentham, P, Wright, J, Crome, P, Job, D, Polwart, A, Ex;eyu, C. 2013. Silicon-rich mineral water as a non-invasive test of the aluminium hypothesis in Alzheimer's diseases. Journal of Alzheimer's Dis, 33(2), 423 – 430.
4. Eliton, C, Davidson, B, Pretorius, B, Erlwanger, K. 2011. Proximate, mineral, amino acid, fatty acid, vitamin E, phytate phosphate and fibre bcomposition of *Mimusops zeyheri* (Red Milkwood) seed International Journal of Food Science and Technology, 46, 555–560
5. Food and Agriculture Organisation. 2004. The state of food insecurity in the world. 2. Monitoring progress towards the world Food Summit and Millennium Development Goals. Rome: Food and Agriculture Organisation available from (accessed from 02 June 2017)
6. Hinsinge, P, Plassard, C, Tang, C, Jaillard B. 2003. Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: A review, Plant and Soil 248: 43–59.
7. Kalavrouziotis, IK, Koukoulakis, P, Papadopoilos, AH, Pantazis, V. 2008. Interrelationship of heavy metals macro and micronutrients, and properties of soil cultivated with Brassica oleracea var. italic (Broccoli), under the effect of treated municipal wastewater. Journal of Water Air and Soil Pollution, 190 (4): 309-321.
8. Knezek, BD, Greinert, H. 1971. Influence of Soil Fe and MnEDTA Interactions upon the Fe and Mn Nutrition of Bean Plants. Journal of Soil Science, 39 (3): 473 – 481.

9. Karadaş, C, Kara, D. 2012. Chemometric approach to evaluate trace metal concentrations in some spices and herbs. Journal of Agricultural and Food Chemistry, 49 (10): 4775-4779.
10. Kuo, S, Heilman, PE, Baker, AS. 1981. Distribution and forms of copper, zinc, cadmium, iron, and manganese in soils near a copper smelter. Journal of Soil Science 12: 345-356.
11. Lagerwerff, JV, Specht, A.W. 1970. Lead and zinc levels and chemical fractionation in road-side soils of Caracas, Venezuela Environmnental of Science Technology 4, 583 589.
12. Liu, X, Song, Q, Tang, Y, Li, W, Xu, J, Wu, J, Wang, F, Brookes, PC. 2013. Human health risk assessment of heavy metals in soil-vegetable system: A multi-medium analysis. Science of the Total Environment, 463-464: 530-540.
13. Mahlangeni, N, Moodley, R, Jonnalagadda, S.B. 2016. Heavy metal distribution in Laportea peduncularis and growth soil from the eastern parts of KwaZulu-Natal, South Africa. Environmental Monitoring and Assessment. 188(2), 76.
14. Marschner, H. 1993. Influence of phosphocompost application on phosphorus availability and uptake by maize grown in red soil of Ishigaki Island, Journal of Agricultural Science, 4 (2), 258- 269
15. Mashela, PW, Mollel, N. 2001. Farmer – identified and selected indigenous fruit tree with suitable attributes for smallholder farming systems in South Africa’s Northern Province. South African Journal of Farmers, 30:1-12.
16. Mlambo, L, Moodley, R, Koorbanally N. 2014. Elemental composition of the fruits of Baboon grape (*Rhoicissus digitata*) and impact of soil quality on chemical characteristics. Journal of Food and Nutrition Research, 325-368.
17. Mngadi, S, Moodley, R, Jonalaggadda, S. 2016. Elemental distribution and chemical composition of Amadumbe (*Colocasia esculanta*) leaf and impact of soil quality. Fresenius Environmental Bulletin, 24(4), 1250-1257.

18. Moodley, R, Kindness, A, Jonnalagadda, SB. 2008. Chemical composition of Macadamia nuts (*Macadamia integrifolia*) and impact of soil quality. Journal of Environmental Science and Health Part A, 42: 2097-2104.

19. Moodley, R, Koorbanally, N, Jonnalagadda, SB. 2012. Elemental composition and fatty acid profile of the edible fruits of Amatungula (*Carissa macrocarpa*) and impact of soil quality on chemical characteristics. Analytica Chimica Acta, 42, 2097-2104.

20. Nowak, B. 1998. Contents and relationship of elements in human hair for a non-industrialised population in Poland. Science of Total Environment, 209(1): 59-68.

21. Pingali, P, Alinovi, L, Sutton, J. 2005. Food Security in Complex Emergencies: Enhancing Food System Resilience. Journal of Disaster Volume 29, Issue, Pages S5–S24.

22. Pooley, E. 1993. The Complete Field Guide to Trees of Natal, Zululand and Transkei; Natal Flora Publications Trust: Durban, South Africa, 311-322.

23. Schneider, A, Changa, C, Paulsen, K. 2015. The changing spatial form of cities in Western China Annemarie. Journal of Landscape and Urban Planning, 135 40–61.

24. South African National Biodiversity Institute (SANBI). Webpage: PlantZAfrica.. http://www.plantzafrica.com/. Accessed 02/04/2017.

25. Street, RA, Stirk, WA, van Staden, J. 2008. South African traditional medicinal plant trade-Challenges in regulating quality, safety and efficacy. Journal of Ethnopharmacology, 119: 705-710.

26. Walkley, A, Black, IA. 1934. An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Science, 37: 29-37.

27. World Health Organisation. 2011. Lead in Drinking Water, Background Document for Development of WHO Guidelines for Drinking-Water Quality; WHO Press: Geneva, Switzerland, 1-19.

28. Yaqin, JI. 2008. Using geoaccumulation index to study source profiles of soil dust in China. Journal of Environmental Sciences, 20, 571–578.

CHAPTER 5 - CONCLUSIONS AND RECOMMENDATIONS

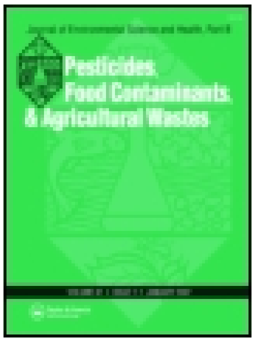
5.1 GENERAL

South Africa, experiencing high cases of malnutrition and food insecurity, needs to exploit alternative sources of nutrition that could help alleviate this problem. There are numerous edible plants that need to be investigated for their chemical composition to overcome malnutrition and hunger. Both *M. caffra* and *M. zeyheri* have been reported to be edible. *M. caffra* is used for making alcoholic beverages and for the production of jelly. *M. zeyheri* is known for containing high vitamin C and is also used widely for making different products. These two plants were investigated for distribution of nutrients and heavy metals and the impact of soil quality on uptake by the plants was evaluated to assess for potential metal toxicities. The research showed that both plants were good sources of essential elements. According to the findings from *M. caffra*, the elemental concentrations in the fruits (in descending order) were found to be K > Na> Ca > Mg > Si > Al > Fe > Zn > Mn > Ni > Cr > Cu > Pb > Mo > Sb > As > Se > V > Cd > Co. According to the findings from *M. zeyheri*, the elemental concentrations in the fruits (in descending order) were found to be K > Na> Ca > Mg > Fe > Al > Zn > Mn > Cu > Cr > Sr > Pb > As > Li > Ni ≈ Co > Rb > U > Bi > Ga > Be > Tl > Mo > Ba > Ag > Cd. The nutritional value of *M. caffra* showed that the fruit is a rich source of Fe, Si and Cr contributing 85.1%, 85% and 1324%, respectively towards the RDA for these elements. The fruits of *M. zeyheri* was shown to be a rich source of Cr (231%) and Mn (127%). The elements (both essential and toxic) in both fruits were found to be within acceptable limits making them safe for human consumption. Statistical analyses showed that contamination in soil by the various heavy metals came from various sources however soil contamination did not affect the concentration of heavy metals in the fruits thereby indicating the plants ability to take up metals to meet metabolic needs and exclude metals if at elevated levels for survival.

5.2 RECOMMENDATIONS FOR FUTURE WORK

- Phytochemistry of both plants since these are used for medicinal purposes.
- Isolation and structural elucidation of all phytocompounds in both fruits
- Study the microbial activity of the isolated compounds.
- Speciation analysis of the elements can also be undertaken.

APPENDICES



Journal of Environmental Science and Health, Part B

Pesticides, Food Contaminants, and Agricultural Wastes

ISSN: 0360-1234 (Print) 1532-4109 (Online) Journal homepage: http://www.tandfonline.com/loi/lesb20

Elemental composition and nutritional value of the edible fruits of coastal red-milkwood (Mimusops caffra) and impact of soil quality on their chemical characteristics

Sihle Mngadi, Roshila Moodley & Sreekantha B. Jonnalagadda

JOURNAL OF ENVIRONMENTAL SCIENCE AND HEALTH, PART B
2017, VOL. 0, NO. 0, 1–11
http://dx.doi.org/10.1080/03601234.2017.1301753

Taylor & Francis
Taylor & Francis Group

Elemental composition and nutritional value of the edible fruits of coastal red-milkwood (*Mimusops caffra*) and impact of soil quality on their chemical characteristics

Sihle Mngadi[a,b], Roshila Moodley[a], and Sreekantha B. Jonnalagadda[a]

[a]School of Chemistry and Physics, University of KwaZulu-Natal, Westville campus, Chiltern Hills, Durban, South Africa; [b]Council for Geoscience, Private Bag X112, Pretoria, South Africa

ABSTRACT
In this study, the elemental distribution of essential and toxic elements in the soil and fruits of the indigenous plant species, *Mimusops caffra*, from ten sites along the KwaZulu-Natal east coast was investigated using inductively coupled plasma-optical emission spectrometry. This was done to determine the nutritional value of the fruits as well as to evaluate the impact of soil quality on elemental uptake by the plant. The elemental concentrations in the fruits (in descending order) were found to be K > Na > Ca > Mg > Si > Al > Fe > Zn > Mn > Ni > Cr > Cu > Pb > Mo > Sb > As > Se > V > Cd > Co. The results show that approximately 10 g of fruit would contribute more than 85% towards the recommended dietary allowance for Fe and Si for most adults. The proximate chemical composition revealed the fruits to contain approximately 84% moisture, 4.7% ash, 6.9% protein, 1.7% oil and 2.7% carbohydrates. The study indicates that the fruits of this indigenous plant species are a good source of essential elements with low levels of potentially toxic elements (Pb, As and Cd) which makes the plant a good indigenous food source especially for vulnerable communities that need food security.

ARTICLE HISTORY
Received 28 September 2016
Accepted 16 January 2017

KEYWORDS
Geo-accumulation index; toxic elements; nutritional value; synergy; antagonism

Introduction

South Africa, like other developing countries faces the problem of poor dietary intake, food insecurity, and poor health care services that prevail within the context of the HIV/AIDS pandemic.[1] Recent research has shown that approximately 14 million people live with food insecurity and 1.5 million children suffer from malnutrition.[2] This is because most people from rural communities are not eating a balanced diet as they cannot afford to buy commercially available fruit and vegetable due to economic and physical inaccessibility.[3] In light of this, there should be more focus on promoting indigenous, locally grown fruit and vegetable as these are easily accessible and could possibly be of high nutritional value.

The accumulation of trace metals in food is of special concern to developing countries like South Africa as they undergo industrialisation while maintaining the tradition of eating locally grown food such as Amadumbe and sweet potatoes.[4] It is therefore important to determine the nutritional quality of these foods to ensure that they comply with standards set by health organisations like the World Health Organisation.[5]

Mimusops caffra, of the plant family Sapotaceae, is commonly known as Coastal Red Milkwood (English), umThunzi (isiZulu), umHlophe (Xhosa) and Moepel (Afrikaans). *Mimusops caffra*, is an indigenous, small to medium-sized, evergreen tree that grows in large, uniform groups in the dune forests of the KwaZulu-Natal coast, Southern Africa and Mozambique.[6] The tree normally grows to a height of about 10 m and occasionally reaches a height of 25 m. The bark is dark grey in colour and contains milky latex. The leaves are hardy and leathery, with a blue-green upper colour and have whitish hairs below.[7] The bark is used in traditional medicine to treat wounds and bruises.[8] The plant produces berry-like, sweet, pulpy fruits which are eaten by local South Africans and is also used in the production of jelly and alcoholic beverages.

Despite the well-documented ethno-botanical literature in South Africa, very little information on the chemical and nutritional quality of the edible fruit in *Mimusops caffra* is available. Previous research reported on the elemental concentrations in the fruit of the indigenous species, *Carissa macrocarpa* and *Harpephyllum caffrum*.[9,10] This paper reports on the elemental concentrations in the fruit of the indigenous plant *Mimusops caffra* found on the east coast of KwaZulu-Natal, South Africa that was investigated, as a function of soil quality parameters and to assess nutritional value.

Materials and methods

Sampling

Sampling was conducted along the east coast of KwaZulu-Natal. Plant material and soil samples were collected from ten different sites, namely: Site A – Umhlanga, Site B – Durban, Site C – Isipingo Beach, Site D – Amanzimtoti, Site E – Warner Beach, Site F – Winlkelspruit, Site G – Umgababa, Site H – Ilfracombe, Site I – Scottsburgh and Site J – Margate (Fig. A1).

CONTACT Sreekantha B. Jonnalagadda jonnalagaddas@ukzn.ac.za School of Chemistry, University of KwaZulu-Natal, Westville Campus, Private Bag X54001, University Road, Chiltern Hills, Durban 4000, South Africa.

Sampling was conducted in December 2011 during the summer harvest period. The weather during the collection period was moderate (25°C) without rainfall or the wind. The landscape where the sampling sites were located, showed flat topography and the soil type was sandy (Sites A, C, D, E, I, J) or sandy loam (Sites B, F, G, H). Soil samples were systematically collected from 6 points along the dripline of the trees. Soil cores (3.8 cm height, 5 cm diameter, approximately 150 g) were extracted using a 20 cm long thin walled stainless steel coring tube from a depth of approximately 15 cm from six points around the tree. Soil aliquots were composited; the extraneous material such as leaves and rocks were removed and samples were then manually homogenised using a plastic spoon. The composited soil volume was reduced by coning and quartering and the final aliquot was packed in polyethylene bags and refrigerated at 4°C to limit microbial manifestations. Plant samples were also stored in polyethylene bags and refrigerated at 4°C.

Reagents and standards

All chemicals used were of analytical-reagent grade supplied by either Merck (Darmstadt, Germany) or Sigma-Aldrich (St. Louis, MO, USA). For preparation of standards and dilution, double distilled water was used. All glassware was cleaned with 3 M HNO_3 to prevent contamination. The certified reference material (CRM) *lyophilized brown* bread (BCR 191), from the Community Bureau of Reference of the Commission of the European Communities, was analysed to evaluate the accuracy of the analytical method due to matrix similarities with the plant samples. The analytical procedure used for the CRM was the same as that for the plant samples.

Sample preparation

Fruit samples were washed with double distilled water then cut into smaller pieces with a stainless steel knife. Thereafter, samples were dried, overnight, in an oven at 50°C, crushed into a fine powder using a food processor (Russell Hobbs range) and stored in labelled polyethylene bags. Soil samples were dried in an oven at 50°C, overnight, sieved through a 2 mm sieve to obtain the soil fraction then placed into labelled polyethylene bags until analysed.

Digestion of samples

Digestions were performed according to the method described by Moodley et al.[9] Samples were digested using a Mars 6 Microwave Sample Preparation System with patented Xpress^{TM} technology (CEM, Matthews, NC, USA, closed vessel technique). Five replicates of each sample were digested. Both fruit (0.5 g) and soil (0.25 g) samples with 10 mL of 70% HNO_3 were loaded into the 50 mL microwave vessels and digested after standing for 30 min to pre- digest. The plant CRM and soil matrix were digested using the method described by Mahlangeni et al.[11] Digests were filtered through Whatman No. 1 filter papers (Sigma-Aldrich) into 50 mL volumetric flasks and made up to the mark with double distilled water. All samples were stored in a refrigerator in polyethylene bottles until analysed.

Analytical instruments

Trace elements were analysed using the Agilent ICP-MS 770X with an AS 93 plus auto-sampler (Agilent, Santa Clara, CA, USA). The Varian ICP-OES 5100 (Palo Alto, CA, USA) was used for the analysis of elements (Al, As, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Si, V and Zn). Working standards were made up from elemental standards (1000 mg L^{-1}, Sigma-Aldrich) with double distilled water and 10 mL of 70% HNO_3 to match the sample matrix. All analyses were done in triplicate. The Radiometer MeterLab pHM 220 was used for the analysis of soil samples.

Extraction of exchangeable metals

An extractant solution (1 L) using a combination of chemical extractants (ammonium acetate (38.542 g, 0.5 M), ethylenediaminetetraacetic acid (EDTA) (37.225 g, 0.1 M) and acetic acid (25 mL, 96%)) was prepared and used to release available metals from soil fractions as described by Moodley et al.[9] Approximately 5 g of dry soil sample was extracted with 50 mL of extractant solution in polyethylene bottles at room temperature. For good reproducibility, the soil/extractant ratio, temperature and duration of extraction were kept constant. The extracting solution was then filtered, to remove particulate matter, using a 0.45μm Millipore filter (Billerica, MA, USA, membrane type, High Volume Low Pressure). Thereafter, the filtrate was stored in labelled polyethylene bottles in a refrigerator until analysed.

Soil organic matter (SOM), cation exchange capacity (CEC) and soil pH

The pH of the soil was determined using the method as described by Mckenzie and Skoog.[12,13] A 1:1 soil–water suspension was measured using a pH meter fitted with a glass electrode. The Walkley-Black method was used to determine the soil organic matter (SOM).[14] For determination of the cation exchange capacity (CEC) of soil, the pH 7.0 ammonium acetate method as described by Chapman was employed.[15]

Bioaccumulation factor

Bioaccumulation is the gradual accumulation over a period of time of a substance such as pesticides or metals in a living organism to meet metabolic needs.[16] The ratio of the concentration of the metal in the fruit to the concentration of the metal in the soil is referred to as Bioaccumulation factor (BAF). This can be for both total and exchangeable soil concentrations (Eq. 1).

$$BAF = \frac{[Element]_{plant}}{[Element]_{soil}} \quad (1)$$

The BAF can indicate exclusion (BAF < 1), accumulation (BAF > 1) or hyper-accumulation (BAF >>> 1).

Geoaccumulation index

The use of geoaccumulation indices date back to the 1960s where it was used for assessing contamination in soil and it builds on the background level of natural fluctuations including very low anthropogenic input.[17] In this study, the I_{geo} of a metal in sediment can be calculated using Eq. (2).

$$I_{geo} = \log_2 \frac{C_n}{B_n \times 1.5} \qquad (2)$$

C_n is the concentration of the heavy metal. B_n is the geoaccumulation background concentration which is multiplied by a factor of 1.5 to account for possible variation in background data due to lithological variation. The doubling of this value provides the upper limit of the next higher class and subsequent doubling provides the upper limit of a higher class.[17]

Exchangeable percentage

The exchangeable percentage (Ex%) gives the percentage of each element from soil that is available for plant uptake and is a percentage of the ratio of the exchangeable concentration of the element in the soil to the total concentration of the element in the soil. This can be calculated using Eq. (3).

$$Ex\% = \frac{[Element]_{exchangeable}}{[Element]_{soil\ total}} \times 100 \qquad (3)$$

Statistical analysis

Pearson's product-moment correlation coefficients were used to assess the plant-soil relationships and soil competition effects for soil parameters and concentration of elements in the fruit. All statistical analyses were done using the Statistical Package for the Social Sciences (PASW Statistics 19, IBM Corporation, Cornell, NY, USA).

Quality assurance

Certified Reference Material (CRM), *lyophilized brown bread* (BCR 191) sample was analysed and the data statistically evaluated using the statistical mean and standard deviation. The statistical data obtained from the analysis of the CRM was compared with the target values from the Certificate of Analysis (COA). The standard deviation together with the mean was used to compute the acceptable range to determine the accuracy of the developed test method. The test results obtained on analysis of the CRM are summarized in Table 1. The investigation showed that the test results are within the acceptable range of that stipulated for the CRM, meaning that the method is accurate at the 95% confidence interval.

Results and discussion

Elemental analysis

The chemical composition of the fruit of *Mimusops caffra* was found to contain 84% moisture, 4.7% ash, 6.9% protein, 1.7% oil and 2.7% carbohydrates.

Table 1. Comparison of measured and certified/indicative values (Mean ± SD at 95% confidence interval, n = 5), based on dry mass, in the certified reference material, *lyophilized brown bread* (BCR 191).

Element	Certified/Indicative concentration [a] mk kg^{-1}	Measured concentration mk kg^{-1}
As	0.023 [a]	0.023 ± 0.43
Ca	0.41 [a]	0.36 ± 0.11
Cu	2.60 ± 0.1	2.16 ± 0.56
Fe	40.7 ± 2.3	34.3 ± 5.67
Mg	0.50 [a]	0.45 ± 0.98
Mn	20.3 ± 0.7	20.0 ±3.19
Zn	19.5 ± 0.5	20.6 ± 5.24

[a]Indicative value are those without uncertainties.

The elemental concentrations for the twenty selected elements in the fruit collected from ten sampling sites and corresponding soil samples as per site are summarised in Table A1.

In this study, Ca, K, Mg, Na and Si were found to be the major elemental components of the fruit (Fig. 1). Plant K ranged from 6599 to 14039 mg kg^{-1}. All sites showed total and exchangeable soil concentrations of K to be lower than plant concentrations (Table A1). In this study, K was the element found in highest concentrations in the fruit compared to other elements. The plant appeared to accumulate K, with BAFs (total and exchangeable) between 9 and 227.

Sodium is known to be present at higher concentrations in most plants as it is essential for their growth.[18] Plant Na ranged from 2485 to 7403 mg kg^{-1}. Total soil Na ranged from 5618 to 6550 mg kg^{-1}, of which, between 66 to 576 mg kg^{-1} was in exchangeable form (average Ex% = 6). This indicates that Na is tightly bound to soil lattice thereby restricting exchangeability.

The typical concentration of Ca in plants is 5000 mg kg^{-1}.[19] Although total soil Ca ranged from 1343 to 32591 mg kg^{-1}, plant Ca ranged from 2343 to 7203 mg kg^{-1} (which is close to typical concentrations for most plants). This indicated the plant's ability to control uptake. The plant tended to accumulate Ca when soil concentrations were low (Site I), BAF = 2.6 (total) and 3.8 (exchangeable) and exclude it when soil concentrations were high (Site G), BAF = 0.1 (total and exchangeable). The amount of Ca in exchangeable form was between 10% and 90%.

Total soil Mg ranged from 161 to 1709 mg kg^{-1} with the Ex% being less than 37. The plant tended to accumulate Mg with BAFs (total) being close to 1 in most cases. This indicates that plant Mg is more closely related to total than exchangeable soil Mg and that the plant will accumulate Mg to meet physiological needs.

Total soil Si concentrations were within a narrow range (64 to 683 mg kg^{-1}), of which, 4.8 to 79.2 was in exchangeable form. At most sites, the plant tended to accumulate Si (Average BAF = 1.6 (total) and 6.9 (exchangeable)) but this accumulation by the plant tended to decrease southwards along the coast of KwaZulu-Natal from Umhlanga (Site A) to Margate (Site J) as shown in Figure 1.

The growth of most plants is promoted by the accumulation of trace elements. Elements such as Cu, Mn, Ni and Zn are required by most living organisms mostly humans and if these are found at higher concentration they can cause detrimental effects to human health causing serious illness. Table A1 and

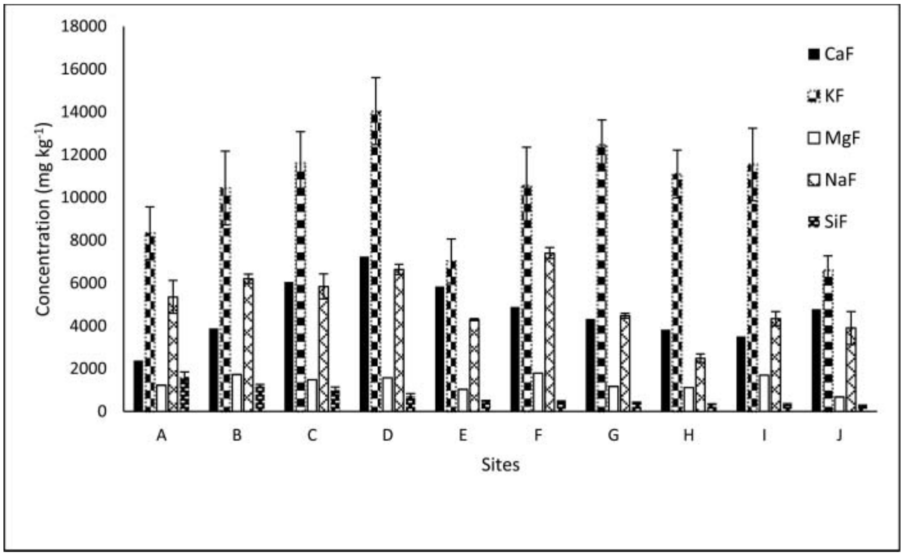


Figure 1. Distribution of the major elements in *Mimusops caffra* fruit (F) at the 10 different sites. Site A – Umhlanga, Site B – Durban, Site C – Isipingo Beach, Site D – Amanzimtoti, Site E – Warner Beach, Site F – Winlkelspruit, Site G – Umgababa, Site H – Ilfracombe, Site I – Scottsburgh and Site J – Margate. *F= Fruit.

Figure 2 shows that the soil at all sites is rich in Al (1923 to 9583 mg kg^{-1}) but less than 50% of this Al is in an available form. The average concentration of Al in the fruit was 163.7 mg kg^{-1}. Total soil Cu ranged from 4.3 to 36.2 mg kg^{-1} and 9.5 to 90.3% of this was in available from. Copper in the plant appears to be restricted to the narrow range of 1.7 to 6.7 mg kg^{-1}.

Iron uptake and transport is influenced by a variety of factors such as soil pH, root morphology and salinity.[20] The availability and uptake of Fe is a complex process as many factors can influence Fe levels in the plant.[20] The growth of plants may be slow if Fe in the soil is at high concentrations. Exchangeable Fe was observed to be extremely low compared to total soil Fe (Ex% < 4.7). This is because Fe may be bound to Al-Mn-hydroxide complexes or contained in mineral lattices within the soil, rendering it unavailable. Iron in the plant ranged from 70 to 225 mg kg^{-1} with an average BAF (bioavailable) of 0.8 indicating that the Fe (in available from) was sufficient to meet the plant's physiological requirement levels.

Manganese concentrations in the fruit ranged from 5 to 43 mg kg^{-1} which was lower than available concentrations at all sites thereby indicating exclusion of this element to meet physiological needs (average BAF = 0.2). Both the soil and plant concentration of Ni in this study were low with total soil Ni ranging from 1.5 to 30 mg kg^{-1} and Ni in the plant ranging from 1 to 13.2 mg kg^{-1}. At most sites, exchangeable Ni was low (below the instrument detection limit) which indicates that the plant has mechanisms to increase the availability of Ni for uptake.

Exchangeable Zn ranged from 6.7 to 36.6 mg kg^{-1} and Zn in the fruit ranged from 13.4 to 41.8 mg kg^{-1}. Zn concentrations in the fruit reveal that the plant has a tendency to accumulate the element (BAF exchangeable from 0.6 to 3.7).

Total soil As is at very low concentrations (2.21 to 12.4 mg kg^{-1}) thereby indicating non-contamination and As levels in the fruit are considered to be safe for human consumption (0.02 to 1.42 mg kg^{-1}). Total soil Cr was less than 62 mg kg^{-1} however none of this was in available form. Chromium (0.6 to 14.1 mg kg^{-1}) and Mo (0.05 to 0.45 mg kg^{-1}) concentrations

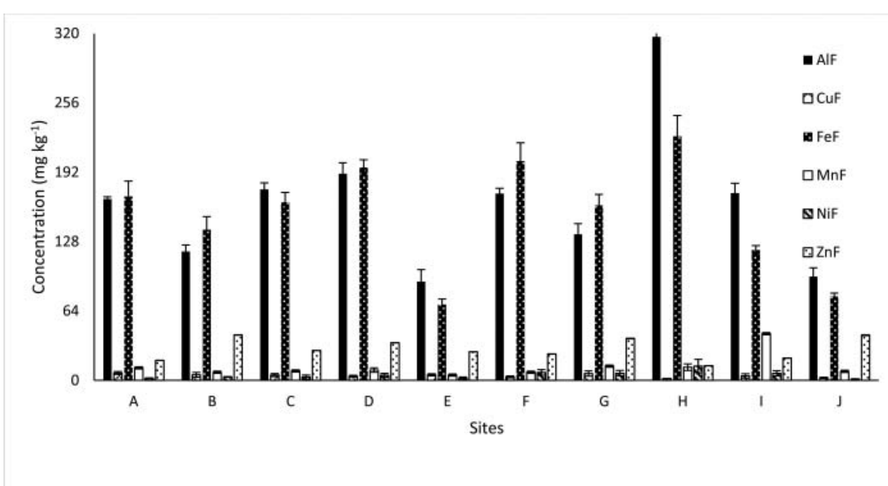


Figure 2. Distribution of the minor elements in *Mimusops caffra* fruit (F) at the 10 different sites. Site A – Umhlanga, Site B – Durban, Site C – Isipingo Beach, Site D – Amanzimtoti, Site E – Warner Beach, Site F – Winlkelspruit, Site G – Umgababa, Site H – Ilfracombe, Site I – Scottsburgh and Site J – Margate.*F= Fruit.

Table 2. Dietary Reference Intake (DRIs), Recommended Dietary Allowances (RDAs) and Tolerable Upper Intake Levels (ULs) of elements for most individuals[a] and average concentration of elements (n = 5) in *Mimusops caffra* fruits.

Element	Average Concentration (mg/10 g dry mass)	DRI[a] (mg/day) RDA	UL	Estimated Contribution to RDA (%)
As	0.19	ND[b]	ND	ND
Ca	464	1000-1300	2500	35.7
Cu	0.43	0.9	8	47.8
Cr	0.47	0.024-0.035	ND	1342
Fe	15.3	8-18	45	85
K	1037	1000-3500	4000	29.6
Mg	134.7	310-320	350	42.1
Mn	1.25	1.6-2.3	9	54.4
Mo	0.03	0.1-0.5	1	6.0
Na	509.3	1000-2400	2500	21.2
Ni	0.49	ND	1.0	ND
Si	68.09	50-80	100	85.1
V	0.01	0.006-0.18	0.1	5.6
Zn	2.87	8-11	34	26.1
Se	ND	0.05	0.4	ND

[a]Institute of Medicine of the National Academies: Dietary Reference Intakes (2001).
[b]ND = not determined due to lack of data.

Table 3. pH, SOM and CEC of soil samples obtained from the ten different sites in KwaZulu-Natal.

Sites[b]	pH ($CaCl_2$)[a]	SOM[a] (%)	CEC[a] (meq/100 g)
A	6.77	3.59	10.36
B	9.81	3.27	6.34
C	8.19	4.23	10.59
D	7.53	3.82	7.98
E	7.57	4.09	8.88
F	8.16	5.92	11.78
G	6.76	2.57	12.99
H	7.37	1.19	1.72
I	6.01	4.05	8.23
J	7.57	5.29	9.89

[a]Expressed as mean, n = 5.
[b]Sites: Site A – Umhlanga, Site B – Durban, Site C – Isipingo Beach, Site D – Amanzimtoti, Site E – Warner Beach, Site F – Winlkelspruit, Site G – Umgababa, Site H – Ilfracombe, Site I – Scottsburgh and Site J – Margate.

in the plant were within a small range of variation however, for both these elements, exchangeable concentrations were below the instrument detection limits.

Total soil Pb ranged from 3.42 to 28.62 mg kg^{-1}. At 50% of the sites, soil concentrations are higher than the maximum permissible concentration of 6.6 mg kg^{-1} set by the Water Research Commission for South African Agricultural soils.[21] However, these are still lower than the maximum permissible levels for other countries such as the United Kingdom and Canada (100 mg kg^{-1}).[22] In South Africa, the maximum level of Pb in small fruits is set at 0.2 mg kg^{-1} wet weight due to its negative impact on human health.[23] The concentration of Pb in the fruits ranged from 0.24 to 5.99 mg kg^{-1} (dry weight) and with the fruit having a moisture content of 84%, this would make the fruits safe for human consumption.

Although the soil concentrations of Sb and Se were below the detection limit of the instrument at all sites, Sb was present in fruit samples in the range of 0.01 to 1.38 mg kg^{-1}. Vanadium was detected both in soil and plant samples although at low concentrations. Vanadium in the plant ranged from 0.03 to 0.18 mg kg^{-1}.

Estimated contribution of Mimusops caffra *fruit to the human diet*

Table 2 shows the dietary reference intakes (DRIs) in the fruit. This is expressed as mg/day of the average concentration of each essential element studied. Of the twenty elements studied, five are considered non-essential (Al, Cd, Co, Pb and Sb) and these are excluded from the table as there are no known official DRIs for these elements. About 10 g of fruits (dry mass), which is equivalent to about one handful (normal serving size), contributes adequately to the recommended dietary allowances (RDAs) for the nutrients Ca, Cu, K, Mg, Mn, Mo, Na, V and Zn.

The consumption of 10.0 g of fruits may contribute 85% towards the RDA for Fe for most adults. Iron is found in haemoglobin. Its deficiency causes anaemia and increases susceptibility to infection. On the other hand, an excess of Fe may increase a person's risk of cancer and heart diseases.[24] Adults and children in South Africa suffering from anaemia can eat these fruits as an alternative to those available in the market with high Fe content such as apricots, beetroot and olives to help overcome Fe deficiency.

The consumption of 10.0 g of fruits may contribute 85.1% towards the RDA for Si for most adults. High levels of Si are present in vegetables such as alfalfa, bell peppers and root vegetables and in fruits such as apples and red raspberry. This element is essential for bone formation and digestive system disorder.[25] Studies have shown a correlation between improvements in Alzheimer's disease and hair loss with ingestion of fruits and vegetables with high Si content.[26,27]

Chromium is considered to be an important microelement for normal carbohydrate, lipid and protein metabolism in humans.[28] The consumption of 10 g of fruits exceeds the RDA for this element (1342%). Mngadi et al.[29] found similar

Table 4. Total Baseline Concentrations (TBC, mk kg^{-1}) of metals in South African soils, total soil concentration (S(T), mk kg^{-1}), and geoaccumulation index (I_{geo}) for each sampling site.

		Site A		Site B		Site C		Site D		Site E		Site F		Site G		Site H		Site I		Site J	
Metal	TBC[a]	S(T)	I_{geo}	S(T)	I_{geo}	S(T)	I_{geo}	S(T)	I_{geo}	S(T)	I_{geo}	S(T)	I_{geo}	S(T)	I_{geo}	S(T)	I_{geo}	S(T)	I_{geo}	S(T)	I_{geo}
Cd	2.7	0.18	−4.5	0.04	−6.7	0.11	−5.2	0.11	−5.2	0.16	−4.7	0.19	−4.4	0.18	−4.5	0.12	−5.1	0.06	−6.1	0.00	—
Co	69	1.55	−6.1	0.65	−7.3	0.27	−8.6	0.59	−7.5	0.19	−9.1	0.05	−11.0	1.63	−6.0	0.17	−9.2	0.09	−10.2	0.66	−7.3
Cr	353	30.95	−4.1	61.90	−3.1	9.61	−5.8	16.84	−5.0	33.04	−4.0	23.45	−4.5	19.35	−4.8	12.76	−5.4	8.11	−6.0	0.00	—
Cu	117	16.25	−3.4	10.51	−4.1	8.11	−4.4	10.28	−4.1	26.19	−2.7	36.20	−2.3	4.29	−5.4	7.28	−4.6	6.67	−4.7	20.8	−3.1
Pb	66	20.90	−2.2	14.94	−2.7	29.50	−1.7	5.59	−4.1	3.42	−4.9	6.63	−3.9	28.62	−1.8	2.23	−5.5	8.66	−3.5	5.91	−4.1
Zn	115	121.5	−0.5	130.7	−0.4	68.60	−1.3	70.9	−1.3	52.1	−1.7	114.6	−0.6	49.2	−1.8	50.6	−1.8	67.0	−1.4	67.5	−1.4

Sites: Site A – Umhlanga, Site B – Durban, Site C – Isipingo Beach, Site D – Amanzimtoti, Site E – Warner Beach, Site F – Winlkelspruit, Site G – Umgababa, Site H – Ilfracombe, Site I – Scottsburgh and Site J – Margate.

Table 5. Correlation matrix for the elemental concentration in *Mimusops caffra* fruits (F) and soil (total (T) and exchangeable (E).

	AlF	CoF	CrE	CrF	FeT	FeE	FeF	MgE	MnT	MnE	MnF	NaE	NiT	NiF	PbE	PbF	SbT	SbE	SbF	SeF	SiT	SiE	SiF	VT	VE	VF
pH	−0.1	0.0	−0.3	0.0	−0.1	−0.4	0.0	0.9*	0.2	−0.1	−0.6	0.6	0.3	−0.2	0.2	−0.3	0.4	0.1	0.0	0.0	0.6	0.2	0.3	−0.3	0.0	0.1
CEC	−0.8*	0.1	0.2	−0.4	0.3	0.3	−0.2	−0.4	0.2	−0.3	−0.1	0.1	0.3	−0.5	0.1	0.1	0.1	0.2	0.1	0.0	0.0	0.0	0.1	0.3	0.2	0.3
AlT	−0.2	−0.2	0.9*	−0.4	0.8*	0.6	−0.1	−0.5	0.5	0.7*	−0.1	0.3	−0.2	−0.4	−0.2	0.8*	−0.2	0.0	−0.2	−0.2	0.0	0.5	0.4	0.6	0.3	0.2
AlF		−0.2	−0.1	0.8*	−0.2	−0.3	0.5	0.2	−0.1	0.2	0.0	0.0	−0.2	0.8*	0.1	−0.1	−0.4	−0.3	−0.2	−0.1	0.1	−0.3	−0.3	−0.2	−0.3	0.0
AsE		−0.2	−0.2	−0.2	−0.2	−0.2	0.1	0.5	−0.2	−0.1	−0.3	0.1	0.4	−0.3	0.1	−0.2	0.9*	0.7*	−0.1	−0.2	0.3	0.4	0.3	0.1	0.0	0.0
ASF		1.0*	−0.1	−0.3	0.1	0.3	−0.6	0.0	−0.1	−0.1	−0.2	−0.3	−0.1	−0.3	−0.3	0.0	0.0	−0.3	1.0*	1.0*	0.0	−0.1	−0.1	−0.2	0.4	−0.5
CaT					−0.5	−0.1	0.2	0.3	−0.4	−0.3	−0.4	0.6	0.4	0.2	0.9*	−0.3	0.5	0.4	0.1	0.0	0.6	0.0	0.1	0.0	−0.2	0.0
CoT					0.3	0.7*	0.0	−0.4	0.1	0.3	−0.2	0.5	−0.2	−0.3	0.5	0.6	0.3	0.6	−0.1	−0.2	0.3	0.4	0.4	0.8*	0.3	0.2
CoF					0.2	0.3	−0.5	0.1	0.1	−0.1	−0.3	−0.3	−0.1	−0.2	−0.4	0.0	−0.2	−0.4	0.9*	1.0*	0.0	−0.2	−0.2	−0.3	0.5	−0.4
CrE					0.8*	0.8*	0.1	−0.3	0.6	0.9*	0.0	0.4	−0.3	−0.3	−0.3	1.0	−0.1	0.1	−0.1	−0.1	0.2	0.8*	0.7*	0.8*	0.5	0.4
CrF					−0.2	−0.4	0.8*	0.3	0.0	−0.2	0.0	0.0	0.0	0.9*	0.1	−0.4	−0.3	−0.3	−0.3	−0.3	0.1	−0.5	−0.4	−0.4	−0.4	0.3
FeT						0.7*	0.2	0.0	0.9*	0.7*	−0.4	0.3	−0.2	−0.3	−0.5	0.8*	−0.2	−0.1	0.0	0.1	0.3	0.5	0.5	0.6	0.7*	0.5
FeE												0.2	−0.4	−0.3	−0.2	0.8*	0.1	0.2	0.4	0.3	0.2	0.5	0.5	0.8*	0.8*	0.2
FeF												0.3	0.1	0.7*	0.0	0.0	0.0	0.1	−0.5	−0.6	0.4	0.1	0.2	0.3	−0.1	0.8*
MgT												0.9*	0.4	0.0	0.4	0.1	0.4	0.3	0.1	0.0	1.0*	0.4	0.5	0.3	0.2	0.4
MnE													−0.5	−0.1	−0.4	0.9*	−0.1	0.0	−0.2	−0.1	0.1	0.7*	0.6	0.6	0.6	0.2
MoT													0.9*	−0.2	0.1	−0.1	0.3	0.1	−0.1	−0.1	0.1	0.2	0.2	0.0	−0.4	0.2
NaE																		0.3	−0.2	−0.3	0.9*	0.5	0.6	0.4	0.2	0.5
PbF																		0.1	0.0	0.0	0.1	0.8*	0.7*	0.8*	0.6	0.3
SbT																		0.9*	0.1	−0.1	0.4	0.4	0.4	0.3	0.2	0.0
SbF																				1.0*	0.1	−0.1	−0.1	−0.1	0.5	−0.4
SiE																							1.0*	0.7*	0.5	0.4

Pearson correlation (r); *Correlation is significant at the 0.05 level (2-tailed).
XT = Total soil concentration of element X, XE = Exchangeable soil concentration of element X, XF = concentration of element X in fruit CEC = cation exchange capacity.

results in *Amadumbe* leaves where the Cr levels exceeded the RDA for humans. However, high intake of Cr is not linked to adverse health effects therefore the Institute of Medicine has not established a tolerable upper intake level (UL) for this element.[26]

Soil properties – soil pH, SOM and CEC

The soil properties pH, soil organic matter (SOM) and cation exchange capacity (CEC) were investigated and the results are shown in Table 3. The results show that *Mimusops caffra* plants thrive in slightly basic soil conditions (pH range – 6.01 to 9.81). According to Mahlangeni et al.[30] the availability of some heavy elements in soil is caused by high soil pH (basic conditions). Soil organic matter is an important determinate in soil since it acts as a pool of nutrients for plants and also increases the nutrient holding capacity of soil. Recent research has shown that metal availability is positively correlated to soil organic matter.[31] In this study, there was a wide range of variation in SOM from the different sites (1.19% to 5.92%). Most cations adsorb to the negative surface charges as a result of an increase in SOM which causes an increase in the soils CEC. The CEC is important because it provides the approximate quantities of available Ca^{2+}, Mg^{2+} and K^{+} ions in soil. Table 3 shows that CEC ranged from 1.72 to 11.78 meq/100g.

Geoaccumulation index

Table 4 shows the total concentration of each element in soil and by calculation of the geoaccumulation indices (I_{geo}), the extent of contamination, if there is any, is then estimated. According to Müller,[32] I_{geo} values below zero would mean that the soil is uncontaminated. The I_{geo} values found in this study at all sites indicated non-contamination in the soil. Evidence to this is the I_{geo} values which are all negative indicating lack of contamination.[17]

Statistical analysis

A correlation matrix for the elemental concentration in *Mimusops caffra* fruit and soil (total and exchangeable) is given in Table 5. The statistical analysis indicates the extent to which the interactions are closely related where $r > 0.8$ indicates a strong positive relationship (synergistic) and $r < -0.8$ indicates a strong negative relationship (antagonistic).

A synergistic effect is observed as a result of an increase in the levels of one or more interacting elements leading to an increase in the exchangeability of another.[18] A positive correlation was observed between exchangeable Cr and total soil Al, Fe and V. This means that increased concentrations of these elements in soil allow Cr to become more exchangeable probably by displacing adsorbed Cr since competing for the same soil adsorption site. This could be due to charge similarities between these elements. The same relationship is observed between exchangeable Na and total soil Mg and Si.

A strong correlation between fruit and exchangeable/total concentration for a particular element indicates that the plant has a tendency to accumulate or exclude the particular element. This was evident for Si, where, there was a strong positive correlation between Si in the fruit with exchangeable Si ($r = 1$). Although, no similarity exists in chemical nature of the metals, a strong positive relationship existed between Pb in the fruit with exchangeable Cr, Fe, Mn and Si indicating that high exchangeable concentrations of these elements promote uptake of Pb, but mechanism by which the synergy occurs is not clear. Correlations also existed between fruit concentrations between Sb, As, Se and Co. While Sb and As are metalloids, Se is a non-metal and Co is a metal. Analogous to earlier observation, not much similarity was observed in the chemical properties of those elements.

A positive correlation between the elements and SOM was observed. Total soil As and Cu correlated positively with SOM. On the other hand, exchangeable Ca correlated negatively with SOM. Aluminium in the fruit correlated negatively to both SOM ($r = -0.7$) and CEC ($r = -0.8$). No further significant correlations between SOM with the elements were obtained.

The pH of the soil was found to correlate strongly ($r = 1.0$) with exchangeable Mg in the soil. Ca and Mg are known to be more available at pH levels between 6 and 8.[33] The correlation justifies this since the pH of the soil at all sites is between 6 and 8, hence more available for plant uptake.

Conclusion

The elemental composition and nutritional value of *Mimusops caffra* fruit from ten different geographic locations along the east coast of KwaZulu-Natal was determined. A total of twenty elements were assessed for nutritional value in the fruits. The elemental concentrations in the fruit, in descending order, were found to be K > Na > Ca > Mg > Si > Al > Fe > Zn > Mn > Ni > Cr > Cu > Pb > Mo > Sb > As > Se > V > Cd > Co. The study showed that the fruits of the indigenous plant, *Mimusops caffra*, are a rich source of essential elements such as K, Na, Ca and Mg with low levels of the toxic elements studied thereby making it nutritious and safe for human consumption. The high concentration of Ca and Mg in the fruits supports the medicinal use of *Mimusops caffra* which is used to treat wounds and sores. A phytochemical analysis needs to be conducted on the plant to provide a scientific basis for its ethno-medicinal use.

Acknowledgments

The authors acknowledge the University of KwaZulu-Natal for financial support and laboratory facilities.

References

[1] Goggin, K.; Pinkston, M.; Gqaleni, N.; Puoane, T.; Wilson, D.; Berkley-Patton, J.; Martinez, D.A. The role of South African traditional health practitioners in HIV/AIDS prevention and treatment. In: *HIV/AIDS Global Frontiers in Prevention/ Intervention*; Pope, C.; White, R.T.; Malow, R., Eds.; Taylor and Francis: New York, 2009; 1–5.

[2] Gqaleni, N.; Moodley, I.; Kruger, H.; Ntuli, A.; McLeod, H. Traditional and complementary medicine. In: *South African Health Review*; Harrison, S.; Bhana, R.; Ntuli, A., Eds.; Health Systems Trust: Durban, South Africa, 2007; 175–185.

[3] Moodley, R.; Kindness, A.; Jonnalagadda, S.B. Chemical composition of Macadamia nuts (Macadamia integrifolia) and impact of soil quality. J. Environ. Sci. Health Part A. **2007**, *42*, 2097–2104.
[4] Reddy, M.; Moodley, R.; Kindness, A.; Jonnalagadda, S.B. Impact of soil quality on the elemental uptake and distribution of Colocasia esculenta (Amadumbe), an edible root. J. Environ. Sci. Health, Part B. **2011**, *46*, 247–256.
[5] World Health Organisation. National Policy on Traditional Medicine and Regulation of Herbal Medicine; Report of a WHO global survey: Geneva, 2005; 1–156.
[6] Botha, J.; Shackleton, C.; Emanuel, P.L. Productivity and abundance of *Sclerocarya* birrea subsp. caffra in and around rural settlements and protected areas of the bushbuckridge Lowveld, South Africa. Forests Trees Livelihoods. **2003**, *13*, 217–232.
[7] Palmer, E.; Pitman, N. *Trees of Southern Africa*; AA Balkema: Cape Town, South Africa, 1972; 258–269.
[8] Pooley, E. *The Complete Field Guide to Trees of Natal, Zululand and Transkei*; Natal Flora Publications Trust: Durban, South Africa, 1993; 311–322.
[9] Moodley, R.; Koorbanally, N.; Jonnalagadda, S.B. Elemental composition and fatty acid profile of the edible fruits of Amatungula (Carissa macrocarpa) and impact of soil quality on chemical characteristics. Anal. Chim. Acta. **2012**, *42*, 2097–2104.
[10] Moodley, R.; Koorbanally, N.; Jonnalagadda, S.B. Elemental composition and nutritional value of the edible fruits of Harpephyllum caffrum and impact of soil quality on their chemical characteristics. J. Environ. Sci. Health, Part B. **2013**, *48*, 539–547.
[11] Mahlangeni, N.; Moodley, R.; Jonnalagadda, S.B. Soil nutrient content on elemental uptake and distribution in sweet potatoes. J. Environ. Sci. Health, Part B. **2012**, *42*, 2097–2104.
[12] McKenzie, R.H. *Soil pH and Plant Nutrients*. Agri-Facts Agdex: Alberta, Canada, 2003; 531–4, 1–2
[13] Skoog, D.A.; West, D.M.; Holler, F.J.; Crouch, S.R. *Fundamentals of Analytical Chemistry*; Brooks/Cole: Belmont, CA, 2004; 258–265.
[14] Walkley, A.; Black, I.A. An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci. **1934**, *37*, 29–37.
[15] Chapman, H.D. Cation exchange capacity. In: *Methods of Soil Analysis Part 2– Chemical and Microbiological Properties*; Black, C.A., Eds.; American Society of Agronomy: Madison, WI, 1965; 891–901.
[16] Timperley, M.H.; Brooks, R.R.; Peterson, P.J. The significance of essential and non-essential trace elements in plants in relation to biogeochemical prospecting. J. Appl. Ecology. **1973**, *7*, 429–439.
[17] Yaqin, J.I. Using geoaccumulation index to study source profiles of soil dust in China. J. Environ. Sci. **2008**, *20*, 571–578.
[18] Marschner, H. *Mineral Nutrition of Higher Plants*; Academic Press Harcourt Brace and Company Publishers: London; 2002; 2744–7325.
[19] Akenga, P.; Salim, A.; Onditi, A.; Yusuf A. Determination of selected micro and macronutrients in sugarcane growing soils at Kakamega North District. J. Appl. Chem. **2014**, *7*, 2278–5736.
[20] Morrissey, J.; Guerinot, M. Iron uptake and transport in plants: The good, the bad, and the ionome. Chem. Rev. **2009**, *109*(10), 4553–4567.
[21] Snyman, H.G.; Herselman, J.E. Permissible Utilization and Disposal of Sewage Sludge Report Number: TT 261/06; Water Research Commission: Pretoria, 1998; 1–62.
[22] World Health Organisation. *Lead in Drinking Water, Background Document for Development of WHO Guidelines for Drinking-Water Quality*; WHO Press: Geneva, Switzerland, 2011; 1–19.
[23] Department of Health. Foodstuffs, Cosmetics and Disinfectants Act (Act No. 54 of 1972). Government Gazette: Pretoria, South Africa, 2007; 1–15.
[24] Calabrese, E.J.; Canada, A.T.; Sacco, C. Trace elements in public health. Annu. Rev. – Public Health. **1985**, *6*, 131–146.
[25] Lela, M.J; Ivica, C.; Sandra, K.P.; Krešimir, P. Biological and therapeutic effects of ortho- silicic acid and some ortho-silicic acid-releasing compounds: New perspectives for therapy. Nutrition Metabolism. **2013**, *10*(2), 1–12.
[26] Davenward, S.; Bentham, P.; Wright, J.; Crome, P.; Job, D.; Polwart, A.; Exley, C. Silicon-rich mineral water as a non-invasive test of the 'aluminum hypothesis' in Alzheimer's disease. J. Alzheimers Dis. **2013**, *33*(2), 423–30.
[27] Jugdaohsingh, R. Silicon and Bone health. J. Nutritional Health Aging. **2007**, *11*(2), 99–110.
[28] Kobla, H.V.; Volpe, S.L. Chromium, exercise and body composition. Crit. Rev. Food Sci. Nutrition. **2000**, *40*, 291–308.
[29] Mngadi, S.; Moodley, R.; Jonalaggadda, S. Elemental distribution and chemical composition of Amadumbe (Colocasia esculanta) leaf and impact of soil quality. Fresenius Environ. Bull. **2014**, *24*(4), 1250–1257.
[30] Mahlangeni, N.; Moodley, R.; Jonnalagadda, S.B. Heavy metal distribution in Laportea peduncularis and growth soil from the eastern parts of KwaZulu-Natal, South Africa. Environ. Monit. Assess. **2016**, *188*(2), 76.
[31] Yemeni, M.N.; Hashem, A.R. Bacterial diversity of soil samples of saline sites in Kharaghoda. Int. J. Res. Pure Appl. Microbiology. **2006**, *13*(2), 129–133.
[32] Müller, G. Index of geoaccumulation in sediments of the Rhine River. Geol. J. **1986**, *2*, 109–188.
[33] Bagamba, F.; Ruben, R.; Rufino, M. Determinants of banana productivity and technical efficiency in Uganda. In *An Economic Assessment of Banana Genetic Improvement and Innovation in the Lake Victoria Region of Uganda and Tanzania*; Smale, M.; Tushemereirwe, W.K., Eds.; International Food Policy Research Institute: Washington, DC, 2007; 113.

Appendix

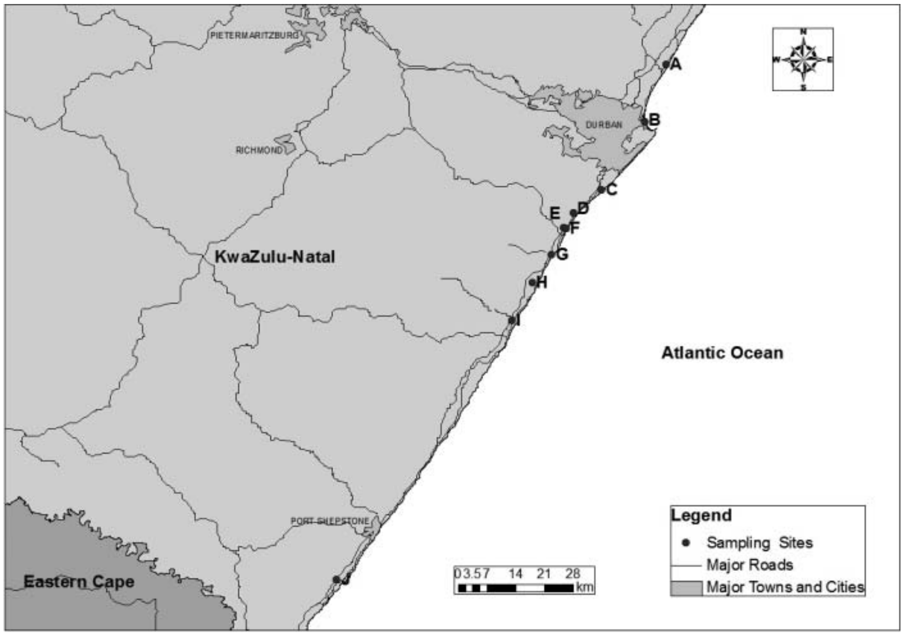


Figure A1. Sampling sites for *Mimusops caffra* plant at the 10 different sites.

Table A1. Elemental concentrations in mg kg^{-1} (Mean (SD), n = 5) of selected elements in the fruit of *Mimusops caffra.*

Site	Element	Soil (T)	Soil (Ex)	Fruit	Ex%	BAF(T)	BAF(Ex)
A	Al	9583(1356)	112(25)	167(2)	1.2	0.0	1.5
B		1923(2224)	64(24)	119(6)	3.3	0.1	1.9
C		4031(48)	95(32)	176(6)	2.4	0.0	1.9
D		2389(527)	973(32)	191(10)	40.7	0.1	0.2
E		3055(766)	1275(10)	91(11)	41.7	0.0	0.1
F		3851(1875)	1911(11)	172(5)	49.6	0.0	0.1
G		3520(1099)	1542(25)	135(10)	43.8	0.0	0.1
H		2959(460)	1142(10)	317(20)	38.6	1.1	2.7
I		3346(335)	1230(20)	173(9)	36.8	0.1	0.1
J		6274(739)	2341(25)	96(8)	37.3	0.0	0.0
A	As	2.79(0.06)	0.21(0.1)	0.06(0.1)	7.5	0.0	0.3
B		2.79(0.14)	2.05(0.07)	0.1(0)	73.5	0.0	0.0
C		3.93(0.1)	2.16(0.14)	0.09(0)	55.0	0.0	0.0
D		3.5(0.1)	2.93(0.1)	0.1(0)	83.7	0.0	0.0
E		3.24(0.1)	0.34(0.07)	1.42(0)	10.5	0.4	4.2
F		8.62(0.1)	0.11(0.09)	0.1(0.01)	1.3	0.0	0.9
G		2.84(0.1)	0.12(0.06)	0.06(0)	4.2	0.0	0.5
H		2.21(0.37)	0.14(0.06)	0.1(0)	6.3	0.0	0.7
I		3.45(0.1)	0.03(0.06)	0.07(0)	0.9	0.0	2.3
J		12.4(1.02)	0.83(0.06)	0.02(0.01)	6.7	0.0	0.0
A	Ca	7118(122)	5225(20)	2343(815)	73.4	0.3	0.4
B		28243(1122)	10567(98)	3848(1596)	37.4	0.1	0.4
C		21761(6449)	2321(282)	6020(324)	10.7	0.3	2.6
D		12920(1675)	1675(23)	7203(268)	13.0	0.6	4.3
E		14172(1043)	10432(20)	5810(200)	73.6	0.4	0.6
F		4266(427)	3814(17)	4846(122)	89.4	1.1	1.3
G		32591(2932)	29316(15)	4286(359)	90.0	0.1	0.1
H		21867(1804)	18036(33)	3785(421)	82.5	0.2	0.2
I		1343(134)	905(3)	3476(283)	67.4	2.6	3.8
J		8852(885)	7685(9)	4743(570)	86.8	0.5	0.6
A	Cr	30.9(2.3)	ND	0(0.3)	1.5	0.0	ND
B		61.9(2.2)	ND	1.9(1.9)	0.0	0.0	ND
C		9.6(11.1)	ND	4.6(3.1)	0.0	0.5	ND
D		16.8(11.5)	ND	5.2(0.5)	0.0	0.3	ND
E		33(8.3)	ND	0.6(0.4)	0.0	0.0	ND
F		23.4(27.1)	ND	10.2(0.3)	0.0	0.4	ND

(*Continued on next page*)

Table A1. (Continued).

Site	Element	Soil (T)	Soil (Ex)	Fruit	Ex%	BAF(T)	BAF(Ex)
G		19.3(22.3)	ND	5.3(0.8)	0.0	0.3	ND
H		12.8(8.5)	ND	14.1(0.8)	0.0	1.1	ND
I		8.1(9.6)	ND	4(0.5)	0.0	0.5	ND
J		0(4.3)	ND	1.2(0.4)	ND	ND	ND
A	Cu	16.2(3.5)	4.7(1.2)	6.7(1.3)	29.0	0.4	1.4
B		10.5(6.1)	5.1(0.3)	5.1(2.2)	48.6	0.5	1.0
C		8.1(1.5)	3.4(0.5)	4.9(1.4)	42.0	0.6	1.4
D		10.3(5.2)	9.3(0.5)	3.9(0.8)	90.3	0.4	0.4
E		26.2(40.2)	2.5(0)	5(1)	9.5	0.2	2.0
F		36.2(5.8)	3.5(0.5)	3(0.9)	9.7	0.1	0.9
G		4.3(0.9)	2.4(0.8)	6.2(2.5)	55.8	1.4	2.6
H		7.3(1.1)	5.6(0.5)	1.7(0.2)	76.7	0.2	0.3
I		6.7(1.2)	3.9(0.5)	4.1(2)	58.2	0.6	1.1
J		20.8(2.5)	3.2(0.9)	2.3(0.6)	15.4	0.1	0.7
A	Fe	11345(709)	341(10)	170(14)	3.0	0.0	0.5
B		4662(3117)	145(23)	139(12)	3.1	0.0	1.0
C		6099(251)	127(24)	164(9)	2.1	0.0	1.3
D		6451(808)	222(15)	197(7)	3.4	0.0	0.9
E		7118(852)	254(8)	70(5)	3.6	0.0	0.3
F		8528(889)	174(25)	202(17)	2.0	0.0	1.2
G		4960(742)	233(2)	162(10)	4.7	0.0	0.7
H		5589(761)	144(12)	225(19)	2.6	0.0	1.6
I		4396(313)	142(12)	120(4)	3.2	0.0	0.8
J		6235(937)	153(10)	78(3)	2.5	0.0	0.5
A	K	797(4)	331(48)	8339(88)	41.5	10.5	25.2
B		637(3)	366(90)	10441(119)	57.5	16.4	28.5
C		521(3)	51(64)	11599(118)	9.8	22.3	227.4
D		348(1)	284(6)	14039(4)	81.6	40.3	49.4
E		365(4)	72(8)	7027(2)	19.7	19.3	97.6
F		616(2)	65(2)	10567(1)	10.6	17.2	162.6
G		348(1)	197(17)	12458(4)	56.6	35.8	63.2
H		475(3)	148(11)	11098(10)	31.2	23.4	75.0
I		254(2)	130(5)	11541(7)	51.2	45.4	88.8
J		727(5)	270(18)	6599(9)	37.1	9.1	24.4
A	Mg	1516(59)	149(82)	1225(6)	9.8	0.8	8.2
B		1664(95)	522(23)	1723(8)	31.4	1.0	3.3
C		1709(17)	317(3)	1479(5)	18.5	0.9	4.7
D		1251(116)	328(120)	1567(1)	26.2	1.3	4.8
E		1271(105)	291(48)	1032(2)	22.9	0.8	3.5
F		1453(111)	425(8)	1786(4)	29.2	1.2	4.2
G		1498(243)	110(19)	1167(6)	7.3	0.8	10.6
H		1478(214)	365(34)	1117(3)	24.7	0.8	3.1
I		161(11)	59(4)	1697(3)	36.6	10.5	28.8
J		1016(118)	200(10)	677(2)	19.7	0.7	3.4
A	Mn	221(13)	164(8)	12(1)	74.2	0.1	0.1
B		125(49)	70(5)	8(1)	56.0	0.1	0.1
C		111(4)	43(3)	9(1)	38.7	0.1	0.2
D		103(9)	81(8)	9(2)	78.6	0.1	0.1
E		112(19)	64(9)	5(1)	57.1	0.0	0.1
F		235(21)	57(3)	7(1)	24.3	0.0	0.1
G		70(9)	26(1)	13(1)	37.1	0.2	0.5
H		103(13)	91(5)	12(3)	88.3	0.1	0.1
I		66(6)	55(8)	43(1)	83.3	0.7	0.8
J		97(10)	70(5)	8(1)	72.2	0.1	0.1
A	Na	6330(398)	519(26)	5352(768)	8.2	0.8	10.3
B		6550(144)	576(88)	6205(220)	8.8	0.9	10.8
C		6093(245)	431(195)	5844(582)	7.1	1.0	13.6
D		6330(429)	251(64)	6641(240)	4.0	1.0	26.5
E		6017(38)	237(15)	4291(49)	3.9	0.7	18.1
F		5985(41)	408(105)	7403(255)	6.8	1.2	18.1
G		6033(50)	465(79)	4473(111)	7.7	0.7	9.6
H		6057(124)	371(60)	2485(198)	6.1	0.4	6.7
I		6015(27)	66(7)	4341(332)	1.1	0.7	65.8
J		5618(105)	325(40)	3903(767)	5.8	0.7	12.0
A	Ni	1.5(0.3)	0.9(0.1)	1.9(0.4)	60.0	1.3	2.1
B		9(1.3)	ND	3.2(0.3)	ND	0.4	ND
C		30.8(1)	ND	2.9(2)	ND	0.1	ND
D		5.2(0.2)	0.5(0.1)	4.6(1.8)	9.6	0.9	9.2
E		5.5(0.6)	ND	1.8(1.3)	ND	0.3	ND
F		7.1(0.9)	ND	7.2(2.7)	ND	1.0	ND
G		10(1)	ND	6.5(2.5)	ND	0.7	ND
H		3.6(0.6)	ND	13.2(6.2)	ND	3.7	ND

(*Continued on next page*)

Table A1. (Continued).

Site	Element	Soil (T)	Soil (Ex)	Fruit	Ex%	BAF(T)	BAF(Ex)
I		4.3(2.9)	0.6(0.1)	6.5(2.4)	14.0	1.5	10.8
J		2(0.4)	1.1(0.1)	1(0.4)	55.0	0.5	0.9
A	Pb	20.9(7.32)	0.12(0.02)	5.99(0.34)	0.6	0.3	49.9
B		14.94(1.77)	2.02(0.15)	0.94(1.13)	13.5	0.1	0.5
C		29.5(3.73)	1.23(0.02)	0.44(0.51)	4.2	0.0	0.4
D		5.59(0.26)	0.25(0.02)	1.05(0.08)	4.5	0.2	4.2
E		3.42(2.36)	0(0)	1.41(0.39)	0.0	0.4	ND
F		6.63(2.1)	0(0)	0.24(0.07)	0.0	0.0	ND
G		28.62(3.11)	3.33(0.14)	0.51(0.06)	11.6	0.0	0.2
H		2.23(0.25)	1.23(0.22)	0.87(0.24)	55.2	0.4	0.7
I		8.66(0.38)	0(0)	0.74(0.22)	0.0	0.1	ND
J		5.91(0.45)	1.25(0.11)	0.7(0.22)	21.2	0.1	0.6
A	Si	606(10)	480(9)	1619(223)	79.2	2.7	3.4
B		665(38)	304(8)	1193(75)	45.7	1.8	3.9
C		683(7)	237(6)	998(142)	34.7	1.5	4.2
D		500(46)	211(4)	715(112)	42.2	1.4	3.4
E		509(42)	108(5)	497(13)	21.2	1.0	4.6
F		581(45)	28(6)	459(29)	4.8	0.8	16.4
G		599(97)	32(2)	406(23)	5.3	0.7	12.7
H		591(86)	40(10)	342(15)	6.8	0.6	8.6
I		64(4)	45(5)	325(42)	70.3	5.1	7.2
J		406(47)	54(10)	257(36)	13.3	0.6	4.8
A	V	2.75(0.23)	0.13(0)	0.17(0)	4.7	0.1	1.3
B		0.68(0.01)	0.09(0)	0.1(0)	13.2	0.1	1.1
C		0.56(0.01)	0.05(0.01)	0.14(0)	8.9	0.3	2.8
D		1.37(0.13)	0.11(0.01)	0.11(0.04)	8.0	0.1	1.0
E		0.28(0.01)	0.12(0)	0.03(0.01)	42.9	0.1	0.3
F		0.28(0.03)	0.1(0)	0.18(0.05)	35.7	0.6	1.8
G		1.37(0.04)	0.08(0.01)	0.12(0.06)	5.8	0.1	1.5
H		0.22(0.02)	0.06(0.04)	0.1(0.06)	27.3	0.5	1.7
I		0.31(0.07)	0.06(0)	0.08(0.01)	19.4	0.3	1.3
J		0.23(0.06)	0.07(0)	0.01(0.01)	30.4	0.0	0.1
A	Zn	121.5(0.4)	18.4(1.2)	18.3(0.1)	15.1	0.2	1.0
B		130.7(0.4)	23(2)	41.8(0.2)	17.6	0.3	1.8
C		68.6(0.1)	15.1(3.5)	27.5(0.1)	22.0	0.4	1.8
D		70.9(0.1)	20.9(0.3)	34.6(0.1)	29.5	0.5	1.7
E		52.1(0.1)	7.2(0.6)	26.3(0.1)	13.8	0.5	3.7
F		114.6(0.2)	13.8(5.3)	24.3(0.1)	12.0	0.2	1.8
G		49.2(0.1)	10.9(2.3)	38.5(0.3)	22.2	0.8	3.5
H		50.6(0.4)	6.7(1.4)	13.4(0.1)	13.2	0.3	2.0
I		67(0.2)	36.6(5)	20.5(0.1)	54.6	0.3	0.6
J		67.5(0.1)	26.7(2)	41.7(0.4)	39.6	0.6	1.6

Site A – Umhlanga, Site B – Durban, Site C – Isipingo Beach, Site D – Amanzimtoti, Site E – Warner Beach, Site F – Winlkelspruit, Site G – Umgababa, Site H – Ilfracombe, Site I – Scottsburgh and Site J – Margate.

*Soil(T)- Soil Total, Soil (Ex)-Soil exchangeable, BAF-Bioaccumulation factor.

COMMISSION OF THE EUROPEAN COMMUNITIES

COMMUNITY BUREAU OF REFERENCE - BCR

CERTIFIED REFERENCE MATERIAL

CERTIFICATE OF ANALYSIS

304

BCR No 191

TRACE ELEMENTS IN LYOPHILISED BROWN BREAD

Element	Mass fraction (based on dry mass)		Number of accepted sets of results p
	Certified Value (1)	Uncertainty (2)	
Cd	28.4 $ng.g^{-1}$	± 1.4 $ng.g^{-1}$	12
Pb	187 $ng.g^{-1}$	± 14 $ng.g^{-1}$	12
Cu	2.6 $\mu g.g^{-1}$	± 0.1 $\mu g.g^{-1}$	8
Zn	19.5 $\mu g.g^{-1}$	± 0.5 $\mu g.g^{-1}$	13
Fe	40.7 $\mu g.g^{-1}$	± 2.3 $\mu g.g^{-1}$	12
Mn	20.3 $\mu g.g^{-1}$	± 0.7 $\mu g.g^{-1}$	11

(1) *This value is the unweighted mean of p values, each value being the mean of a set of results as obtained by different laboratories and methods.*

(2) *The uncertainty is taken as the 95% confidence interval of the mean value (1) and is applicable when the reference material is used for calibration purposes. When the reference material is used to assess the performance of a method, the user should refer to the recommendations laid down in the last chapter (Instructions for use) of the certification report.*

DESCRIPTION OF THE SAMPLE

The sample is a homogeneous powder consisting of particles that have passed through a 125 µm sieve. It is provided in screw-cap, dark glass bottles in units of approximately 40 g.

INSTRUCTIONS FOR USE

The portion for analysis should be taken after mixing the contents of the bottle. The moisture content is to be determined by drying another portion of the sample at 103 ± 2°C as described in the certification report (Chapter 11, Instructions for use). The recommended minimum sample intake is 200 mg.

All care must be taken to avoid contamination during opening of the bottle and handling of the material. The bottle should be stored in a dark and cool place.

Brussels, December 1986

BCR
for certified true copy