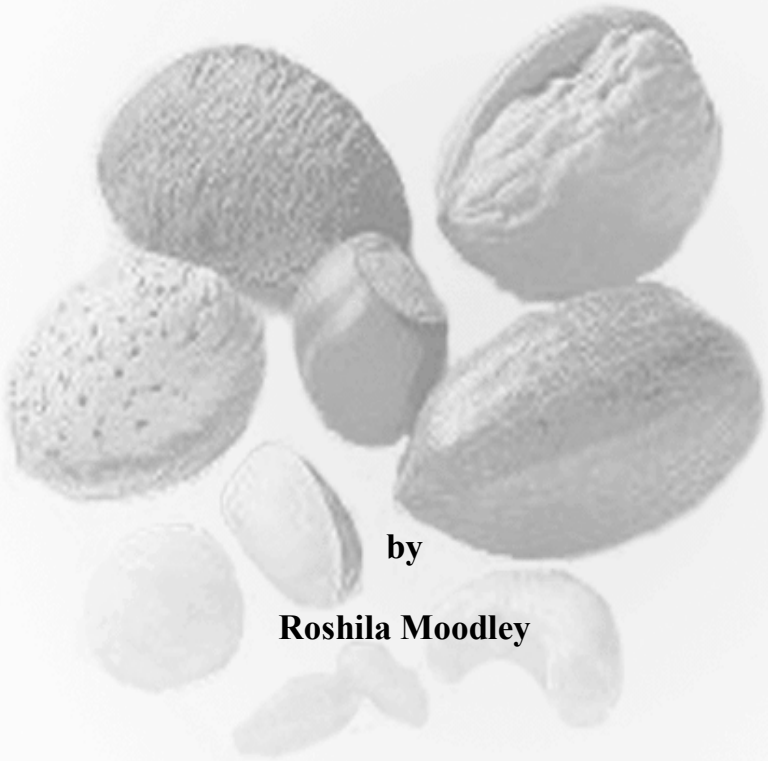
Elemental Distribution in Selected Edible Nuts and the Impact of Soil Quality on the Chemical Characteristics of Macadamia (*Macadamia integrifolia*) Nuts



by

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Submitted in fulfillment of the academic requirements for the degree of Master of Science in the School of Chemistry, University of KwaZulu-Natal, Durban.

2007

DECLARATION

I hereby declare that this dissertation is my own work, except where specifically acknowledged in the text. Neither the present dissertation nor any part thereof has been submitted by any other university for a degree.

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ACKNOWLEDGEMENTS

My sincere thanks and gratitude are to my supervisor, Prof. S.B. Jonnalagadda, who suggested the topic, helped me conceptualize it and who inspired me to reach the successful completion of this dissertation. I am grateful to him for sharing his expert knowledge and immense experience in research as well as assisting and guiding me with constructive criticism and invaluable suggestions. I also like to express my appreciation to Prof. A. Kindness (HOS) for affording me the opportunity to conduct this research and for his assistance whenever it was required.

I would like to acknowledge Kishore Singh (UKZN), Julian Subramoney (Welfare) and Indrani Moonsamy (Unilever) for providing the homegrown Macadamia nut samples. I would especially like to acknowledge Mr. Barry Moses from Ocean Agriculture, Port Shepstone, for obtaining the Macadamia nut and soil samples from the plantations in KwaZulu-Natal.

I would also like to acknowledge with thanks:

- My sister, Anisa, for establishing the right contacts in the agricultural sector.
- My friend, Shakira Shaik, and Dr Nisha Singh for sharing their research laboratory.
- The technical team in the School of Chemistry at UKZN (Westville) for their assistance, in particular Mrs Anita Naidoo.
- My colleagues in the School of Chemistry and CSA for their moral support.

The journey of my Masters degree would not have begun and surely not have ended without the continued support and encouragement of my husband, Riza and my mother who have been strong forces in all of my accomplishments. Last but not least, my endless love to my children, Zia and Zubair, who were patient and understanding to give up quality time with their mother.

ABSTRACT

Environmental and nutritional imperatives make it necessary to carry out regular and reliable monitoring of essential and toxic element levels in edible plants. This quantification is useful for evaluating the contribution of specific plants to a persons Recommended Dietary Allowance and for identifying deficiencies and toxicities in food. The growing popularity and increased consumption of tree nuts necessitates the determination of the elemental concentrations and nutritional properties of tree nuts. This study addressed the need for analytical information on the elemental concentrations in edible tree nuts consumed in South Africa. Focus was placed on Macadamia nuts to determine the effect of soil quality parameters on the elemental concentrations in nuts as South Africa is a major producer of Macadamia nuts.

The concentrations of elements and proximate chemical composition of five different edible tree nuts namely almond (*Prunus dulcus*), Brazil (*Bertholletia excelsa*), pecan (*Carya pecan*), Macadamia (*Macadamia integrifolia*) and walnut (*Juglans nigra*) that are commonly purchased by South Africans were investigated. In addition, three physicochemical properties of the extracted nut oils namely acid value, iodine value and saponification value were evaluated. The results showed a high concentration of Se (36.1 ± 0.4 μg g^{-1}) in the Brazil nuts only. Generally, the order of the concentrations of the elements in all the nuts is found to be Mg>Ca>Fe>Cu>Cr>As>Se. The concentrations of Mn and Zn showed greater variation amongst the different types of nuts.

The concentration and distribution of eight selected elements (As, Ca, Cr, Cu, Fe, Mg, Mn and Zn) in edible Macadamia nuts from eight sampling sites in the south-east coast region of South Africa were investigated The levels of the elements in all the Macadamia nuts is found to be in

the decreasing order of Mg>Ca>Fe>Zn>Cu>Cr>As. The omission of Mn from this order is due to it exhibiting a large range of variability with concentrations being lower at some sites and higher at others. The soil samples were also characterized for total and bioavailable metals. The influences of these concentrations combined with the competition effects amongst the different elements in the soil on the elemental uptake by the Macadamia nuts were investigated. The impact of soil quality parameters namely pH, cation exchange capacity and organic matter on the availability of the selected elements was also studied.

The data showed that when the soil concentrations (total and bioavailable) of an element essential for plant growth was below the plants physiological requirement level, the plant tended to accumulate the element and when the soil concentrations of essential elements exceeded the plants physiological requirement level, the plant partially excluded the element. The data also showed that total soil Mn and Zn were significant predictors of available Mn and Zn, respectively; total soil Ca and Cu were moderate predictors of available Ca and Cu, respectively and total soil Cr and Fe could not predict available Cr and Fe, respectively. The correlation analysis confirmed that antagonistic and synergistic relationships existed between the elements in soil which influenced the availability and uptake of these elements.

This study confirms that interactions between the elements in the soil influence the availability and uptake by the plant which subsequently affects the elemental concentrations in the Macadamia nuts. However, uptake and distribution of elements in the nuts are primarily dependent on the plants inherent controls that maintain the physiological requirement levels of the plant.

LIST OF PUBLICATIONS

Title: Elemental composition and chemical characteristics of five edible nuts (almond, Brazil, pecan, Macadamia and walnut) consumed in Southern Africa.

Authors: Moodley, Roshila; Kindness, Andrew and Jonnalagadda, Sreekanth B.

Journal: Journal of Environmental Science and Health, Part B, **2007**, *42*(5), 585 - 591.

(Appendix 1)

Title: Chemical composition of edible Macadamia nuts (*Macadamia integrifolia*) and impact of soil quality.

Authors: Moodley, Roshila; Kindness, Andrew and Jonnalagadda, Sreekanth B.

Journal: Journal of Environmental Science and Health, Part A. **2007**, *42*(14), 2097-2104.

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ABBREVIATIONS

AAS- Atomic Absorption Spectrometry

BA - Bioavailable

BAF- Bioaccumulation factor

CEC- Cation exchange capacity

CHD- Coronary heart disease

CRDL- Contract Required Detection Limit

CRM- Certified Reference Material

CVD- Cardiovascular disease

DM- Dry mass

DRI- Dietary Reference Intake

EDTA- Ethylenediamine tetraacetic acid

EIE- Easily ionized element

FAAS- Flame Atomic Absorption Spectrometry

FAO- Food and Agriculture Organization of the United Nations

FDA- Food and Drug Administration

HDL-C- High density lipoprotein cholesterol

ICP- Inductively Coupled Plasma

ICP-MS- Inductively Coupled Plasma-Mass Spectrometry

ICP-OES- Inductively Coupled Plasma-Optical Emission Spectrometry

IR- Infrared

LDL-C- Low density lipoprotein cholesterol

LDR- Linear dynamic range

MUFA- Mono-unsaturated Fatty Acid

NAZ- Normal analytical zone

PMT- Photo-multiplier tube

ppb- Parts per billion

ppm- Parts per million

PTFE-TFM- Polytetrafluoroethylene-tetrafluoromethaxil

PUFA- Polyunsaturated Fatty Acid

QTA- Quartz tube atomizer

r- Correlation coefficient

RDA- Recommended Dietary Allowance

SOM- Soil organic matter

UL- Tolerable Upper Intake Level

WIS- Wet-in-shell

WRB- World Reference Base

CHAPTER 1

1.1 Introduction

The world consumption of low-fat and healthy foods is increasing as more people are recognizing the need for a healthy lifestyle. The search for foods that contribute to a healthy diet and that aids in disease prevention has led to the identification of plant foods that have important phytonutrients, micronutrients, proteins, fiber and sterols. These identified foods need to come together so that their different nutritional factors can interact with one another to produce health benefits. Edible tree nuts are found to be one of the foods that exhibit health benefits associated with regular consumption since they contain nutrients that work independently or synergistically with one another or in conjunction with other foods. The popularity of edible tree nuts is growing as people are increasingly becoming aware of its nutritional properties and beneficial effects. There is also increasing evidence that the consumption of whole foods due to the effects of their phytonutrients is better than the consumption of nutrients taken as dietary supplements.[1] People are therefore looking to plants instead of dietary supplements to obtain their dietary requirements for a healthy existence.

Scientific evidence has demonstrated significant and consistent results which indicate that nuts have a protective effect on human health.[2] These studies further emphasize the importance of nuts in the diet. Most clinical studies on nuts have been devoted to the relationship between nuts and coronary heart disease (CHD), while epidemiological investigations have reported on correlations between nuts and cancer.[3] Direct experimental evidence also shows that nuts help reduce low density lipoprotein cholesterol (LDL-C) without changing the high density lipoprotein cholesterol (HDL-C).[4] Due to the protective role of nuts in the diet, nuts have been

included as part of a dietary plan clinically proven to significantly reduce blood pressure which is supported by the National Heart, Lung and Blood Institute.[5]

Essential elements are vital for various metabolic processes and toxic elements if present in relatively high quantities adversely affect these processes.[6] Some of the essential elements that serve as cofactors for many physiological and metabolic functions are Ca, Cr, Cu, Fe, Mg, Mn, Se and Zn. Although most of the essential elements are only required in trace amounts their deficiencies in human population are widespread, affecting up to two billion people.[7] The determination of the elemental distribution in food is therefore necessary to evaluate the total dietary intake of the essential elements which is required for any deficiencies to be detected. This information is also useful in detecting heavy metal contamination in food so that the health risks caused by exposure to toxic elements may be averted or the appropriate curative action may be taken after exposure.

Plant nutrition depends on many factors that include the ability of the soil to supply these nutrients, the rate of absorption of nutrients by the plant, the distribution of nutrients to functional sites and the nutrient mobility within the plant.[8] The uptake of elements by plants depends on the interactions amongst the different elements in the soil which are bound to soil particles that, in turn, varies according to organic matter content, pH, cation exchange capacity (CEC) and clay content of the soil matrix.[9] Since the availability of micronutrients in the soil changes with soil conditions, making generalizations on uptake of elements by plants is particularly difficult. An analysis of the soil and plant tissue would have to be done to obtain the levels of the nutrients potentially available to the plant roots and the amount of nutrients actually absorbed by the plant.

Given the importance of a healthy lifestyle and the contribution of tree nuts to assist in maintaining this lifestyle this research is aimed at expanding the knowledge on the chemical

characteristics of various edible tree nuts consumed in South African households. The concentrations of the essential elements namely Ca, Cu, Cr, Fe, Mg, Mn, Se, Zn and the toxic element As are determined. Furthermore, the proximate chemical compositions of the nuts as well as the physicochemical properties of the extracted nut oils are evaluated. This is done to provide a comparative study on various aspects of the different edible tree nuts. The elemental concentrations in the nut samples could be influenced by the type of nut as well as factors such as the availability of the element in the soil as determined by soil pH, CEC and soil organic matter (SOM) and the total elemental content of the soil as determined geochemically or by environmental contamination. To this end, further research on the impact of soil quality on the chemical characteristics of Macadamia nuts grown in South African soils is undertaken.

1.2 Statement of the Problem

Tree nuts are economically important crops for many countries as they have applications in a variety of food products. Almonds, Brazil nuts, pecan nuts, Macadamia nuts and walnuts are commercially available and are some of the tree nuts consumed in South African households. Despite this fact, there is limited information on the chemical composition and nutritional quality of these nuts. This knowledge can contribute to the understanding of the nutritional value of nuts and can also be of potential use to food consumption tables, especially in calculating the DRI's of these nutrients. A comparative study on the chemical characteristics of the edible tree nuts consumed in South Africa has also not been reported. To overcome this paucity in information the chemical characteristics of various tree nuts sold in South African markets needs to be evaluated.

The Macadamia nut industry in South Africa is rapidly expanding, especially in KwaZulu-Natal, with numerous plantations stretching across the South Coast and further inland. Growing Macadamia nut trees is also becoming ever more popular among home growers in South Africa.

The need for assessing the quality of the nuts produced in South Africa is crucial in the face of the escalating market. In particular, the elemental accumulation in the nuts as a function of their location and soil quality parameters needs to be evaluated. Such information would make valuable contributions to food science and agriculture yet there is a general lack of this information. A study to determine the chemical characteristics of the Macadamia nuts and impact of soil quality parameters on these characteristics needs to be undertaken. Consequently, this particular gap in information will be attenuated.

1.3 Hypothesis

Null hypothesis (H_0): If the elemental uptake of plants is related to the total concentrations of elements in soil, the availability of elements in soil (that are influenced by cation exchange capacity, organic matter and pH) and competition effects amongst elements in soil then the elemental concentrations in nuts should also be related to these soil parameters and should also be affected by elemental interactions in soil.

Alternate hypothesis (H_a): The concentrations of elements in the nuts are independent of soil parameters and elemental interactions in soil and are dependent primarily on the physiological characteristics of the plant.

1.4 Objectives of the Study

The first objective of this study was to do a comparison on the chemical characteristics of various edible tree nuts consumed in South African households. The selected tree nuts were almond (*Prunus dulcus*), Brazil (*Bertholletia excelsa*), pecan (*Carya pecan*), Macadamia

(*Macadamia integrifolia*) and walnut (*Juglans nigra*). The following chemical characteristics were investigated:

- Concentrations of the essential elements viz. Ca, Cu, Cr, Fe, Mg, Mn, Se, Zn and As in the nuts.
- Proximate chemical composition namely %oil, %ash, %protein and %carbohydrate in the nuts.
- Physicochemical properties namely acid value, iodine value and saponification value of the extracted nut oils.

The second objective of the study was to determine the impact of soil quality parameters on the chemical characteristics of tree nuts consumed in South African households. KwaZulu-Natal is a major producer of the Macadamia nut in South Africa consequently this nut was selected for the analysis. The nut and soil samples were obtained from eight different sites in KwaZulu-Natal. The following analyses were performed on these samples:

- Concentrations of the essential elements viz. Ca, Cu, Cr, Fe, Mg, Mn, Zn and As in the nuts.
- Proximate chemical composition namely %oil, %ash, %protein and %carbohydrate in the nuts.
- Total concentrations of the elements Ca, Cu, Cr, Fe, Mg, Mn, Zn and As in the soil.
- Bioavailability of the elements Ca, Cu, Cr, Fe, Mg, Mn, Zn and As in the soil.
- pH, soil organic matter and cation exchange capacity of soil.
- Statistical analysis to evaluate the impact of soil parameters on the chemical composition of the nut.

CHAPTER 2

LITERATURE REVIEW

2.16 Soil

Soil is defined as the unconsolidated mineral or organic material on the immediate surface of the earth that serves as a natural medium for the growth of land plants.[11] Soils get their structure and minerals from their parent material. The parent material is the underlying geological material that contains consolidated or unconsolidated minerals that have undergone physical or chemical weathering influenced especially by climatic conditions. Physical weathering is usually due to temperature changes that cause rocks to disintegrate thus forming solid particles. Chemical weathering is usually caused by acidic solutions produced by the reaction of rainwater and CO_2. This acidic solution causes parent material to decompose releasing minerals and cations into the soil.

Soil, made up of finely ground rock and mineral particles, is known as inorganic soil. Inorganic soil is characterized by particle size and comprises sand, silt and clay. The 0.0625 to 2 mm particle size determines the aeration and drainage of the soil and is made up of sand.[10] The 0.004 to 0.0625 mm particle size is silt and the particle size less than 0.004 mm comprises clay.[10] The clay particles are chemically active and bind with water and plant nutrients.

Soil horizons describe the distinctive layers present in soil. Horizons are described by physical features like colour and texture. Most soils conform to a similar general pattern of horizons. The O-A-B-C-R sequence is the universal classification for the horizons.[10] The O horizon is the topmost layer containing organic matter, usually plant residues not fully decomposed. The A horizon is the surface soil that eluviates (is depleted of) Fe, Al, clay, organic compounds and other soluble constituents. Soil organisms such as worms, fungi and bacteria exist here, often in

close association with plant roots. The B horizon is the subsoil layer below the A horizon. This layer accumulates Fe, Al, clay and other organic compounds, a process known as illuviation. The C horizon is a layer of unconsolidated parent material. This layer is affected by soil forming processes and usually accumulates soluble compounds that bypass the B horizon.

2.17 Soil Organic Matter (SOM)

Soil is a complex, multi-component system of interacting materials, and the properties of soil result from the net effect of all these interactions. SOM is defined as the summation of plant and animal residues at various stages of microbial decomposition, cells and tissues of soil organisms and well-decomposed substances.[30] Representing approximately 35-50% of total SOM, humus is a dark, complex mixture of organic substances modified from original organic tissue, synthesized by various soil organisms and resistant to further microbial decomposition.[31] Due to its chemical make-up and reactivity, humus is a large contributor to soils ability to retain nutrients on exchange sites.[32] As the organic matter content of soil increases, the availability and therefore the uptake of Cu, Fe, Mn and Zn decreases.[65]

Organic matter is an essential component of soil because[33]

- it increases the nutrient holding capacity of soil.
- it is a pool of nutrients for plants.
- it chelates nutrients, preventing them from becoming permanently unavailable to plants.
- it is food for soil organisms from bacteria to worms that hold on to nutrients and release them in forms available to plants.
- it encourages root development.
- it improves aggregation thereby preventing erosion and reduces the negative environmental effects of pesticides and many other pollutants.

2.3 Cation Exchange Capacity (CEC)

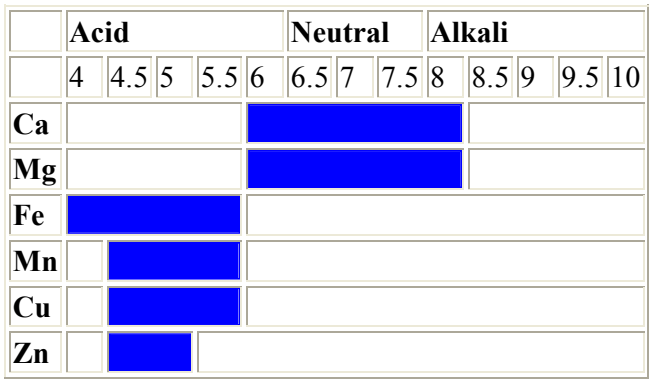
Mineral cations adsorb to the negative surface charges of inorganic and organic soil particles. The negative surface charges of organic particles result from the dissociation of hydrogen ions present in the carboxylic acid and phenolic groups, present in the organic structures in the soil.[12] These adsorbed cations are not easily leached by water and they provide a nutrient base available to plant roots. Adsorbed cations can be replaced by other cations in the soil solution in a process known as cation exchange and the released cations thus become available for plant uptake. CEC is the ability of the soil to hold onto nutrients and prevent them from leaching beyond the roots.[34] It is simply a measure of the quantity of sites on soil surfaces that can retain positively charged ions by electrostatic forces.[35] CEC is usually a good marker of soil fertility and quality. Normal CEC ranges in soils would be from less than 1 meq / 100 g, for sandy soils low in organic matter, to greater than 25 meq / 100 g for soils high in certain types of clay or organic matter.[36] An increase of organic matter in soil would increase a soil's CEC.

2.4 pH of Soil

Soil pH is a measure of the soil solutions acidity and alkalinity. Technically, pH refers to the hydrogen ion concentration in soil. The pH scale is not linear but logarithmic in scope. A soil with a pH of 6 is 10 times more acid than one with a pH of 7. Soil pH can impact plant growth based on its influence on the availability of essential plant nutrients and on the concentration of elements toxic to plants.[32] Acidity promotes the weathering of rocks that release K^+, Mg^{2+}, Ca^{2+} and Mn^{2+} and increases the solubility of carbonates, sulphates and phosphates.[12] pH is one of the major aspects controlling the availability of heavy metals in soil.[37] Macronutrients like Ca and Mg are more available within a pH range of 6 to 8, while the majority of micronutrients like Cu, Fe, Mn and Zn are more available within a pH range of 4 to 6.[32]

Outside of these optimal ranges the nutrients are available to plants at lesser amounts. Micronutrients are very tightly bound to the soil at high pH and are therefore more available at low pH levels than high pH levels.[32] This can cause potential metal toxicities for crops in acid soils. Table 1 below gives a guide to the availability of several nutrients at various pH values.

Table 1. Nutrient availability in relation to soil pH.[10]



2.5 Total Metal Concentrations in Soil

Total metal concentration is an operationally defined metal concentration representing the total amount of metal determined in soil after digestion in a strong acid. These analysis techniques are designed to dissolve as much of the metal in the soil as possible and make it available for dissolutional chemical analysis. Other methods that do not use wet-chemistry procedures to determine the concentrations of total metals in soil can also be used to derive these values.

Nitric acid is usually the acid of choice for total metal determination in soil due to its availability, chemical compatibility, oxidizing ability, purity and low cost. However, much like other acids, HNO_3 will not digest silicate material but it will digest metals present in soil. If the dissolution of silicate material is required then digestion is obtained using HF. Aqua regia

(a combination of HCl and HNO_3) is considered to be a more effective digestion medium. However, the aqua regia digestion, as affirmed by Beckett, has no advantage over the HNO_3 digestion in spite of hydrogen peroxide which oxidizes organic matter.[26]

2.6 Bioavailability of Metals in Soil

Bioaccessibility refers to the ability of a metal to interact with other environmental matrices and undergo fate and transport processes.[14] Environmental availability is specific to the existing environmental conditions and is a dynamic property, changing with environmental conditions. Bioaccessible metals are not sequestered in an environmental matrix, and represent the total pool of metals in a system that is potentially bioavailable to an organism.[186]

The concept of metal bioavailability includes metal species that are bioaccessible and are absorbed or adsorbed by an organism with the potential for distribution, metabolism, elimination and bioaccumulation.[14] Metal bioavailability is specific to the metal salt and particulate size, the receptor and its specific pathophysiological characteristics. Bioavailability of metals in soils can be examined using chemical extraction and bioassay tests.[15]

2.6.1 Factors that influence bioavailability of metals in soil

In order to understand bioavailability, plant materials and selective chemical leaching of soil must be analyzed and the results compared.[19] Plant uptake of trace elements is generally the first step of their entry into the agricultural food chain.[19] The limiting step for elemental entry to the food chain is usually from the soil to the root.[18] The elemental concentrations in soil pore solutions and climatic conditions are the two factors that largely control element mobility and availability.[19] Water is essential for the solid-liquid partitioning in soil before uptake by organisms.[20] The solubility of trace elements in the liquid determines its mobility and

bioavailability. For any trace element, only some of the total concentration of the element will be in soil solution whilst the rest would be adsorbed or bound to the soil matrix. The relative mobility of trace elements associated with different fractions are shown in Table 2.

Table 2. Chemical forms of metals in solid phases. Modified from Gunn.[17]

	FRACTIONS	MOBILITY
TOTAL	DISSOLVED - IN PORE WATER	HIGH
	EXCHANGEABLE - WEAKLY ADSORBED	HIGH
	ASSOCIATED WITH CARBONATE	HIGH
	ASSOCIATED WITH Fe, Mn OXIDES	MEDIUM
	COMPLEXED BY ORGANICS	MEDIUM
	ASSOCIATED WITH SULPHIDE	LOW
	CRYSTALLINE - IN THE MINERAL LATTICE	LOW

The availability of the element for uptake by biota is determined by the fraction to which the element is complexed. The dissolved fraction consists of metals in solution, including metal cation and anion complexes and hydrated ions.[21] Exchangeable fractions consist of metals bound to colloidal or particulate material. Carbonate fractions consist of carbonate minerals in sedimentary rocks and soil. The dissolved, exchangeable and carbonate fractions have high mobility and are therefore readily available to plants for uptake. The Fe-Mn oxide fraction consists of metals adsorbed to Fe-Mn oxide particles or coatings. The organic fraction consists of metals bound to various forms of organic matter. The Fe-Mn oxide and organic fractions have medium mobility and are therefore less available to plants for uptake. The sulphide fraction and the crystalline fraction (which consists of metals contained within the crystal

structure of minerals) are normally not available to biota. Environmental conditions and decomposition can increase the availability of these 2 fractions.

2.6.2 Extractants used in metal availability studies

The total metal concentration shows the metal quantity in the soil, but this may differ from plant-available forms [22] therefore, the available mobile forms should also be evaluated.[23] Availability of trace elements in soil have been measured using a wide variety of empirical extraction techniques that simulate the action of nutrient uptake by plants though chemical extractants cannot extract plant nutrients in the same manner as living plants. As discussed by Grimshaw, nutrient concentrations measured in extractants can only provide an estimate of potential nutrient availability for plant uptake or for cation exchange between soil and soil solutions.[24] However, good correlation between soil extractants and plant uptake has allowed use of the extractant to make reasonable prediction of plant available nutrients in soil and fertilizer recommendations.[25] Caution is recommended when using soil extractants because they only measure metal availability. Plant physiology, rhizosphere biochemistry and the number of competing ions in soil solution can alter the relationship between the extractant and plant.

Extraction agents are grouped according to their chemical properties into neutral salts, chelating agents, acids or bases. There is growing consensus that 1 M ammonium acetate is the most appropriate empirical soil extractant for various soil types, analysed for a range of nutrients and contaminants. Ammonium acetate (1 M) is perhaps the most preferred reagent for exchangeable metals because of its relatively high concentration and the metal complexing power of the acetate ion, which prevents readsorption or precipitation of released metal ions.[27] The relatively high concentration of the ammonium ion results in the displacement of more of the metal cations adsorbed onto soil and therefore provides a better indication of potential metal

availability. 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.[28]

Ethylenediamine tetraacetic acid (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 forms strong complexes with numerous heavy metals and is widely used to sequester di- and trivalent metal ions. It releases heavy metals from soil exchangeable [29] and organically complexed 'pools'.[27] It also has an influence on heavy metals bound in carbonates and hydroxides of iron.[26]

2.7 Bioaccumulation

Bioaccumulation is an important process since it results in chemicals affecting living organisms. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemicals concentration in the environment.[16] Compounds accumulate in an organism if they are taken up and stored faster than they are metabolized or excreted.[16] Bioaccumulation is a normal and essential process for the growth and nuturing of organisms. Metal bioaccumulation can apply to the entire organism, including both metal adsorbed to surfaces or absorbed by the organism.[186] The mobility, solubility and bioaccumulation of trace elements depend on a plethora of soil, microbial and plant factors as well as the properties of the trace element.

2.7.1 Bioaccumulation factors

The relative accumulation of metals taken up by plants can be calculated by dividing the concentration of the metal in the plant by the concentration in the soil.[185] This relative accumulation is known as the bioaccumulation factor (BAF).

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

The BAF can be obtained for both total and bioavailable amounts of metals found in soil.

2.8 Competition Effects amongst Metals in Soil

The competition between the ions present in the soil influences metal mobility and uptake by the plant. Synergism usually occurs when two metals compete for the same adsorption site in the soil. When the concentration of one metal increases in the soil, the soil retention capacity of the other metal is reduced.[159] This increases the bioavailability of the released metal. Thus, when both elements are present in the soil solution they exhibit enhanced bioavailability and reduced retention.

Antagonism usually occurs when two metals are taken up by the plant by the same mechanism. Antagonism is more commonly associated with plant uptake as plant roots favour the uptake of the metal found in higher concentrations in the soil.[159] A well recognized competition in plant uptake occurs between nutrients such as Ca and heavy metals. Plants favour uptake of the nutrients over heavy metals eg. plants favour the uptake of Ca over Pb. This is because Pb mimics the physiological behaviour of Ca thus it may inhibit several enzymes in plants when Ca is not readily available and the plant takes up Pb rather than Ca.[159]

2.9 Dominant Soils of the World

The World Reference Base (WRB) was an initiative of the Food and Agriculture Organization of the United Nations (FAO), International Society for Soil Science (ISSS) and The International Soil Reference and Information Centre (ISRIC).[155] The objective for the development of the WRB was to reach international consensus on the major soil groups at a global level to facilitate the exchange of information, to provide a common scientific language and to strengthen the applications of soil science. The FAO Revised Legend published in 1988 [156] was adopted as the Framework for the WRB and developed for improved depth and validity. The final text for the WRB was adopted in 1997 and was consequently presented at the World Congress of Soil Science in August 1998.[157]

A map of the Dominant Soils of the World (Fig.1) from the WRB shows that Luvisols and Cambisols are the predominant soil types in the KwaZulu-Natal East Coast.

2.9.1 Luvisols

The Reference Soil Group of the Luvisols contains soils whose surface horizon is depleted of clay with an accumulation of clay in a subsurface horizon.[158] The parent material comprises a wide variety of unconsolidated materials including glacial till and aeolian, alluvial and colluvial deposits.[158] Luvisols extend over 650 million hectares worldwide, for the greater part in temperate regions such as Russia, the USA and the Mediterranean.[158] Smaller areas occur in Australia and the southeastern parts of the Republic of South Africa.[158] The chemical properties of Luvisols vary with parent material and pedogenetic history. Surface soils are normally completely or partly decalcified and slightly acid in reaction with a small amount of organic matter. Luvisols are potentially suitable for a wide range of agricultural uses. Luvisols are widely grown to small grains, wheat and sugar beet or planted to tree crops.[158]

2.9.2 Cambisols

The Reference Soil Group of the Cambisols holds soils with incipient soil formation. The parent material comprises medium to fine-textured materials derived from a wide range of rocks.[158] Cambisols are characterized by an absence of illuviated clay, organic matter, Al or Fe compounds.[158]

Cambisols cover an estimated 1.5 billion hectares worldwide.[158] Cambisols occur in widely differing environments, in level to mountainous terrain, in all climates and under a wide range of vegetation types. The soil texture is loamy to clayey. They have a neutral to weakly acid soil reaction, a satisfactory chemical fertility and an active soil fauna. Cambisols make good agricultural land and are intensively used. Some Cambisols are kept under forest while some are used for production of food and oil crops.[158]

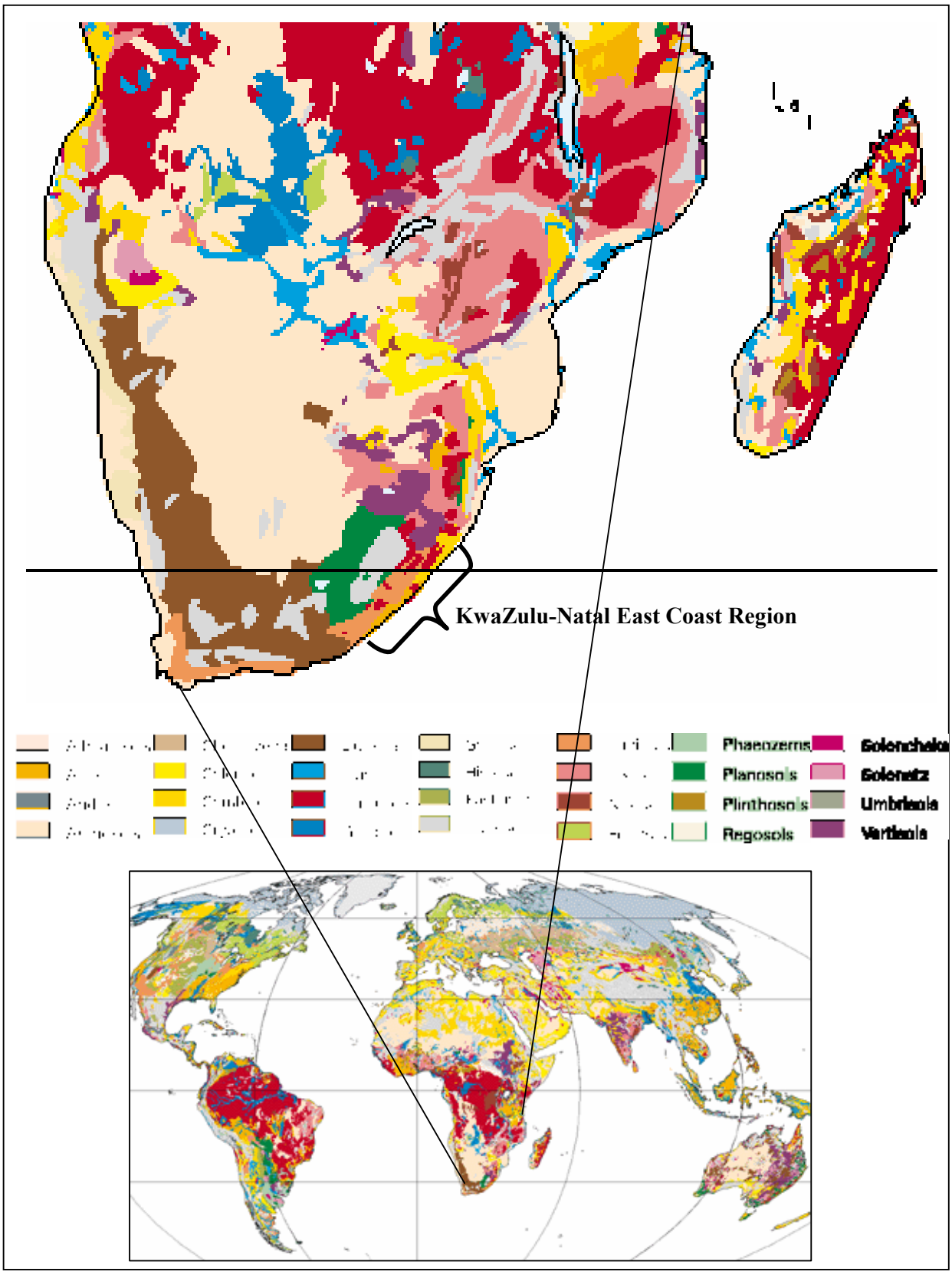


Fig.1 Dominant Soils of the World.[155]

2.10 Essential Elements in Plants

A precise set of criteria were established by Arnon and Stout in 1939,[58] for essential elements in plants. To satisfy these criteria the element must be required for the completion of the life cycle of the plant, must not be replaceable by another element, must be directly involved in plant metabolism and the element must be required by a substantial number of plant species, not just a single species or two. The table below gives the typical concentrations of essential nutrients sufficient for plant growth. However, plant concentrations of essential elements may exceed these typical concentrations and may vary somewhat from species to species.

Table 3. Typical concentrations of essential nutrients sufficient for plant growth[59] and typical range of soil content.[60]

Element	**Typical Concentrations Sufficient for Plants ($\mu g\ g^{-1}$)**	**Typical Range of Soil Content**
Ca	5 000	200-8,000 ppm*
Cu	6	2.5 - 60 mg/kg
Mg	2 000	50-1,000 ppm*
Mn	50	<1 - 18300 mg/kg
Fe	100	0.01 - 21%
Zn	20 to 50	1.5 - 2000 mg/kg

* Optimum Soil Test Values for Most Crops.

There is an increasing awareness of the need to assess plant micronutrient requirements and their availabilities in soil.[62] The phytoavailability of micronutrients has gained more interest due to their importance in plant nutrition and since imbalanced acquisition of micronutrients can lead to both reduced yield and unfavourable quality of plant products.[61] The quantity of a micronutrient that is available for plant uptake is invariably very much less than the total amount in soil and the availability of each micronutrient depends on the form in which it can be taken up by plant roots.[62] The uptake of elements by plants also depends on their availability in the rhizosphere or in the surrounding medium.[63] The various elements are bound to the soil particles, which in turn varies depending on organic matter content, pH, CEC and clay content of the soil matrix.[64] Thus the availability of micronutrients changes with soil conditions and this makes generalizations on the uptake of elements by plants extremely difficult.

2.10.1 Accumulators and excluders

There are some plants, called excluders, which have low uptake of the element at quite high external concentrations of the element.[63] Other plants, called accumulators have high accumulation of elements at very low external element concentrations.[63] These plants either have some kind of barrier to avoid uptake or certain detoxification mechanisms which allows for accumulation of such high amounts of metals.[67] However, at high external concentrations, the accumulators do not increase their uptake, probably due to competition between elements at the uptake site. Different plant species and even different genotypes of species also have different efficiencies in taking up specific elements.[68]

2.10.2 Relationships found in plants and soil for different elements

Interactions or the balance of the elements within the plant and the effect of the soil parameters on the elemental uptake have been studied considerably and are detailed below.

Soil Ca and plant Ca are usually positively related, but soil pH, fertilizer treatments and climatic factors can have some effect on this relationship.[65] Ca deficiencies are usually associated with low pH soils and soils with low CECs.[69] Barreto et al. showed that soil pH exhibits a significant positive correlation with Ca and Mg and a significant negative correlation with Fe and Al.[70]

In plants, Cr(III) has been reported to affect photosynthesis, lower the biomass weight and decrease the concentrations of most nutrients such as Cu, Mg, Mn, Fe, P and K.[71] Furthermore, toxic concentrations of Cr(III) usually inhibit photosynthesis and disturb the mineral nutrition of the plant which eventually leads to a decrease in plant growth. Banerjee et al. found that a small amount of Cr accumulated in roots if the soil concentration was high and a very low percentage of this Cr was transferred from the roots to the shoot and leaves.[195] The mobility of Cr in soil depends on its oxidation state. The reduction of Cr in soils is accelerated by the presence of organic matter and divalent Fe.[145] Alkaline materials like $CaCO_3$ that increase soil pH favor the oxidation of Cr(III)to Cr(VI)[177].This can cause a higher Cr mobility and uptake by vegetation.[179]

Cu deficiencies occur primarily on high organic matter soils and sandy soils which contain low amounts of indigenous Cu and which have pH values approaching 7.0.[72] Excessive Cu plant levels could occur where large quantities of some animal manures, particularly poultry litter, have been applied over a prolonged period.[73] McBride et al.[85] found that for 31 metal contaminated soils from different parts of Europe, measured soluble Cu was best related to total soil Cu content and other soil properties such as organic matter and pH were not significant.

However, for another group of soils, these same authors found that, along with total Cu, both pH and organic matter content were also significant predictors of soluble Cu.

Many soil and plant factors influence the availability and uptake of Fe in the plant. Deficiency may occur when the soil-water pH is near neutral and the soil is high in organic matter.[74] Fe deficiency has been observed in pecan trees. In pecans, high Zn in the trees is thought to be a contributing factor in inducing Fe deficiency.[75] Excessive Zn interferes with the normal function of Fe in plants giving rise to symptoms similar to Fe deficiency. Fe competes with Zn and Cu in their ionic forms.[76]

Plant Mg can be affected by several factors. Both Ca and K interfere with Mg absorption in plants.[76] A decreasing soil pH can markedly reduce the uptake of Mg irrespective of the Mg soil level. The uptake of Mg decreases sharply when the soil-water pH drops below 5.4. The correlation between soil Mg and plant Mg decreases as the soil-water pH increases.[74] The usual cause for Mg deficiency is generally low soil pH or low soil Mg.

Mn availability is markedly influenced by soil-water pH, probably more so than any other micronutrient.[77] The Mn content of plants is frequently more closely related to soil pH than to the concentration of Mn in the soil.[74] Fe interferes with Mn uptake.[76] Mn levels in pecan nuts grown in Arizona were found to be considerably higher than those grown in Las Cruces and those in Las Cruces, in turn, were found to be higher than those grown in Texas or in eastern New Mexico.[78] The reason for this, according to Walworth et al.,[78] is not well understood however their observations suggest that Mn levels could reach approximately 2500 $\mu g\ g^{-1}$ before the crop is adversely affected.

The Se content of most soils lies between 0.1 and 2 $\mu g\ g^{-1}$.[79] Levels of Se in plant foods are strongly related to the content of the element in soils.[80] However, in the case of Se, uptake into the edible portion of plant tissues, is generally not sufficient to cause plant toxicities but has

lead to toxic effects of animals consuming enriched plant tissue.[66] In the central region of Brazil, where the soils are rich in Se, concentrations above 500 μg g^{-1} have been reported in Brazil nuts whereas nuts from western Acre, where the Se content in soil is lower, concentrations of only 30 μg g^{-1} have been reported.[80]

Plants are quite sensitive to Zn, with phytotoxicity of Zn being one of the primary concerns of excess Zn in soils.[81] Because Zn kills plants at concentrations lower than those generally associated with adverse health effects in animals, phytotoxicity prevents Zn transfer from soil at toxic levels through the food chain.[82] Concentrations of Zn in plant tissue associated with phytotoxicity vary greatly both within and across species. Twenty varieties of soybean grown on the same high Zn soil were found to have different uptake as well as yield responses.[83] Four barley cultivars grown under identical conditions had Zn concentrations ranging from 52 to 126 mg kg^{-1}.[84] McBride et al.[85] analysed 31 metal contaminated soils from different parts of Europe and showed that soil pH and total soil Zn were highly significant contributors to Zn solubility while organic matter was not. Conversely, Zhao et al.[86] found that organic matter in addition to soil pH and total soil Zn were significant predictors of Zn solubility. Anderson and Christensen [87] reported that pH is more important than any other single property in predicting Zn mobility and that organic matter did not have much effect.

2.11 Micronutrients and Macronutrients needed for Humans

A nutrient is either a chemical element or compound used in an organism's metabolism or physiology. A nutrient is essential to an organism if it cannot be synthesized in the organism and must be obtained from a food source. Six nutrient groups exist and are broadly classified into those providing energy and those used as components in the body or cellular structures.[38] The nutrients known to be essential are proteins, carbohydrates, fats, minerals, vitamins and

water. Macronutrients are nutrients needed in large amounts to provide calories or energy.[39] These nutrients are carbohydrates, proteins, water and fats.[39] Micronutrients describe vitamins and minerals that are essential but only in very small amounts.

2.11.1 Micronutrients needed for humans

Table 4. Dietary Reference Intakes (DRIs)- Recommended Intakes for Individuals. Food and Nutrition Board, Institute of Medicine, National Academies.[40]

Life Stage	**Ca**	**Cr**	**Cu**	**Fe**	**Mg**	**Mn**	**Se**	**Zn**
Males	(mg/d)	(µg/d)	(µg/d)	(mg/d)	(mg/d)	(mg/d)	(µg/d)	(mg/d)
14– 18 y	1,300	35	890	11	410	2.2	55	11
19– 50 y	1,000	35	900	8	400	2.3	55	11
>51 y	1,200	30	900	8	420	2.3	55	11
Females								
14– 18 y	1,300	24	890	15	360	1.6	55	9
19– 50 y	1,000	25	900	18	310	1.8	55	8
> 51 y	1,200	20	900	8	320	1.8	55	8

This table presents the Recommended Dietary Allowances (RDAs) set to meet the needs of 97 to 98% of the individuals in a group.

Table 5. Dietary Reference Intakes (DRIs): Tolerable Upper Intake Levels (UL*). Food and Nutrition Board, Institute of Medicine, National Academies.[40]

Males/Females	**As**	**Ca**	**Cr**	**Cu**	**Fe**	**Mg**	**Mn**	**Se**	**Zn**
(Life Stage)		(g/d)		(ug/d)	(mg/d)	(mg/d)**	(mg/d)	(ug/d)	(mg/d)
9 - 13 y	ND	2.5	ND	5,000	40	350	6	280	23
14 - 18 y	ND	2.5	ND	8,000	45	350	9	400	34
19 - 70 y	ND	2.5	ND	10,000	45	350	11	400	40
>70 y	ND	2.5	ND	10,000	45	350	11	400	40

* UL = Maximum level of daily nutrient intake that is likely to pose no risk of adverse effects.

** Represent intake from a pharmacological agent only. ND = Not determinable.

Some of the essential elements that serve as cofactors for many physiological and metabolic functions are Ca, Cr, Cu, Fe, Mg, Mn, Se and Zn. Ca is needed for developing and maintaining the rigidity of bones and for the maintenance of healthy teeth. The DRI for Ca, for most adults, is 1000 mg / day which is the highest requirement of all the essential micronutrients. Long-term Ca deficiency can lead to osteoporosis, in which the bone deteriorates and there is an increased risk of fractures. Milk and milk products, nuts [187], broccoli and cereals are rich sources of Ca.

Mg is a key substance in the proper functioning of nerves and muscles. It is also needed for healthy maintenance of bones therefore it is often coupled with Ca in supplements because of its synergistic effects. Inadequate Mg intake frequently causes muscle spasms, and has been associated with cardiovascular disease, diabetes, high blood pressure, anxiety disorders and osteoporosis.[10] The most common symptom of excess oral Mg intake is diarrhoea. Tree nuts, soy foods, spinach and cereals have high levels of Mg. [189]

Cr works with insulin to regulate the body's use of sugar and is essential for fatty acid metabolism. Cr deficiency results in disturbances in glucose, lipid, and protein metabolism.[41] Supplemental Cr may be used to relieve symptoms of its deficiency however, an excessive intake of Cr can be toxic. Foods rich in Cr are egg yolks, meat, whole grains and cheese.

Some of the many functions of Cu include helping to form hemoglobin in the blood, regulating blood pressure and promoting fertility. Symptoms of Cu deficiency include skeletal defects, high blood pressure, infertility and anemia.[42] Acute Cu poisoning is rare in higher mammals owing to the potent emetic action of Cu. In humans, acute Cu toxicity has usually been associated with accidental consumption; symptoms include a metallic taste in the mouth, nausea and vomiting.[43] Shellfish, organ meats, tree nuts[188], yeast, black pepper and thyme are rich sources of Cu.

Fe is found in hemoglobin. Its deficiency causes anemia and increases susceptibility to infections whilst an excess of iron may increase your risk of developing cancer, cirrhosis or heart attacks.[44] Mn is an important cofactor in the key enzymes of glucose metabolism. It is also essential for the proper formation of bone, cartilage and connective tissue. Red meat, seafood, fish, beef, beans and cereals are rich in Fe whilst tea, nuts,[190] and dried fruit are rich in Mn.

Se is an antioxidant, protecting cells and tissues from damage wrought by free radicals. Although its full therapeutic value is unknown, adequate Se levels may help resist arthritis, prevent heart disease and inhibit cancer, particularly of the prostrate and breast.[45] However, an excess can produce toxicity with symptoms of depression, gastrointestinal disturbances and excessive tooth decay.[46] The major source of dietary Se is food, like the Brazil nut,[104] with those from areas high in soil Se having the highest concentrations.

Zn is integral to the synthesis of Ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). Zn deficiency is a common problem with some of its symptoms being hair loss or discoloration, white streaks on the nails and poor wound healing. Zn appears to have a protective effect against the toxicities of both Cd and Pb.[47] Significant dietary intake of Zn has also recently been shown to impede the onset of flu.[10] A 1994 randomized, double-blind, placebo-controlled trial showed that Zn (14 mg per day) doubled the rate of body mass increase in the treatment of anorexia nervosa.[48] Foods rich in Zn are meat, seafood, eggs, poultry and whole grains.

2.11.2 Macronutrients needed for humans

Table 6. Dietary Reference Intakes (DRIs): Acceptable Macronutrient Distribution Ranges.Food and Nutrition Board, Institute of Medicine, National Academies.[40]

Macronutrient	Children, 1–3 y %	Children, 4–18 y %	Adults %
Fat	30–40	25–35	20–35
n-6 PUFA (linolenic acid)	5–10	5–10	5–10
n-3 PUFA (α-linolenic acid)	0.6–1.2	0.6–1.2	0.6–1.2
Carbohydrate	45–65	45–65	45–65
Protein	5–20	10–30	10–35

Carbohydrates are the macronutrients that we need in the largest amounts. According to the DRIs 45% - 65% of calories for adults should come from carbohydrate. We need this amount of carbohydrate because they are the body's main source of fuel, are easily used by the body for energy, are needed for the central nervous system, the kidneys, the brain, the muscles (including the heart) to function properly and are also important in intestinal health and waste elimination.[39] Carbohydrates are mainly found in starchy foods, fruits, milk and yogurt. Other foods like vegetables, beans, nuts and seeds contain carbohydrates, but in lesser amounts.

According to the DRIs 10% - 35% of calories for adults should come from protein. We need protein for growth (especially important for children, teenagers and pregnant women), tissue repair, immune function, making essential hormones and enzymes, energy when carbohydrate is not available and preserving lean muscle mass.[39] The richest sources of protein are animal foods such as chicken, meat, fish, cheese and eggs.[49] However, plant proteins are believed to be healthier and are found in beans (especially soy beans), lentils, nuts and seeds.[49]

Although fats have received a bad reputation for causing weight gain, some fat is essential for survival. According to the DRIs 20% - 35% of calories for adults should come from fat. We need this amount of fat for normal growth and development, energy (fat is the most concentrated source of energy), absorbing certain vitamins, providing cushioning for the organs, maintaining cell membranes and providing taste and stability to foods.[39]

2.11.3 Fatty acids

The fatty acid profiles have been widely studied and are presented below.

Saturated fatty acids (SFAs) are the biggest dietary cause of high LDL-C levels ("bad cholesterol").[50] Saturated fats are found in animal products such as butter, cheese, whole milk, ice cream, cream and fatty meats.

Unsaturated fatty acids are fats that help to lower blood cholesterol if used in place of saturated fats. [50] There are two types: mono-unsaturated and polyunsaturated fats.[10]

Research has indicated that the consumption of a diet with oils high in monounsaturated fatty acids (MUFAs) appears to lower serum cholesterol levels.[51] Examples include Macadamia oil (80% MUFA), olive oil (76% MUFA) and canola oil (61% MUFA).[52]

Omega-3 and omega-6 fatty acids are polyunsaturated fatty acids (PUFAs) and are considered essential fatty acids because they cannot be synthesized by humans.[10] The foods which are richest in omega-3 fatty acids are blue fish and tree nuts, particularly walnuts.[53] Recently, it has been proposed that essential fatty acid deficiency may play a role in the pathology of protein energy malnutrition.[54] The results of epidemiological studies[55] and randomized

controlled trials[56] suggest that replacing dietary saturated fatty acids with omega-6 and omega-3 PUFAs lowers LDL-C and decreases cardiovascular disease (CVD) risk.

Trans fatty acids are fats found in fried foods, commercial baked goods, processed foods and margarines formed when vegetable oils harden and can raise LDL-C levels as well as lower HDL-C levels ("good cholesterol").[10]

Table 7. Fatty acid content of 100 g of nuts - *USDA Nutrient Database.* [57]

	Cholesterol	**SFA**	**MUFA**	**PUFA**	**Linoleic acid**	**α-Linolenic acid**
	mg	g	g	g	g	g
Almond	0	4	32	12	12	0
Brazil	0	16	23	24	24	0
Macadamia	0	12	59	1	1	0
Pecan	0	6	41	22	21	1
Walnut	0	6	9	47	38	9

SFA -Saturated fatty acid. MUFA -monounsaturated fatty acid.

PUFA -polyunsaturated fatty acid

2.12. Acid Value, Saponification Value and Iodine Value

The suitability of oil for consumption, its stability and deterioration during storage can be evaluated by determining the acid value, saponification value and iodine value of the oil.

The acid value is the mass of potassium hydroxide (KOH) in milligrams that is required to neutralize one gram of the sample.[10] The acid value is used to quantify the amount of carboxylic acid groups present in a chemical compound such as a fatty acid.[10] A low acid value

suggests a good quality oil, suitable for human consumption whereas a high acid value indicates that the oil would need further refining to make it suitable for edible purposes.[191] The acceptable limit for acid value is reported to be 7 to 8 mg KOH/g oil.[192]

The saponification value is the number of milligrams of potassium hydroxide (KOH) saponifying the esters in one gram of the sample and neutralizing the free acids in one gram of the sample under the conditions specified.[10] It is a measure of the average molecular weight (or chain length) of all the fatty acids present.[13] The higher the saponification value the smaller the chain lengths. Soapmakers usually use the NaOH saponification value while laboratories measure the KOH saponification value.[13] Because there is an inverse relationship between saponification value and weight of fatty acids in the oil, oils with high saponification values contain a great number of fatty acids of low molecular weight.[193]

The iodine value is a measure of the degree of unsaturation of fats and oils and is expressed as the mass of iodine in grams that is consumed by 100 grams of a sample (% iodine absorbed).[13] A low iodine value indicates the presence of few unsaturated bonds which means low susceptibility to oxidative rancidity and greater stability.[193] Oils with high iodine values would contain more unsaturated bonds, a low resistance to oxidation and shorter shelf life. Although oils with unsaturated bonds are known to be good for health these oils are more unstable than those with saturated bonds and oxidation of the unsaturated bonds compromises the quality of the oils.

2.13 Arsenic

Arsenic is widely and evenly distributed in solids and water in low concentrations. Generally, the earth's crust contains an average of 2 μg g^{-1} or less of As, with most soils containing less than 10 μg g^{-1}.[88] Both As(III) and As(V) are strongly adsorbed to hydrous oxides of Fe, Mn

and Al.[89] Most of the As in water occurs naturally from the erosion of rock surfaces with the current South African standard for arsenic in drinking water being set at 10 μg g^{-1} for Class 0 water (ideal) and at 50 μg g^{-1} for Class 1 water (acceptable).[194] Where As concentrations are abnormally high, the source is usually industrial. The primary anthropogenic sources include releases from mining and smelting operations; agricultural uses of As, such as pesticides and insecticides; livestock-related uses, such as cattle and sheep dips; releases from coal burning and uses as wood preservatives.[90] The Food and Drug Administration (FDA) limit for As in fruit and vegetable is set at 1.4 μg g^{-1} and most foods contain less than 1 μg g^{-1} although crustaceans and other shellfish concentrate As and may contain up to 170 ppm.[91] Chronic As poisoning from the ingestion of contaminated foods and water have been reported. Symptoms are varied and include pigmented skin lesions, gangrene of the lower extremities, along with paralysis, anemia and disturbances of the liver and circulatory system.[92,93] Skin cancer is believed to be related to exposure to As from contaminated water, foods and medicines. High incidences of skin cancer have been reported in areas with high concentrations of As in drinking water.[92,93]

2.14 History of Tree Nuts chosen in this Study

The first historic mention of almonds and walnuts is reported to be in the bible.[94] In Egypt, almonds were found in the tomb of Tutankhamen. Almonds and walnuts were also commonly consumed in ancient Greece.[94] Walnuts are indigenous to Southeastern Europe, Eastern Asia and Northern America. Today, the leading commercial producers of walnuts are the USA, Turkey, China, Iran, France and Romania.[95] The most important producers of almonds for the European market are Spain and Italy.[96] Californian almonds are also of increasing importance. The pecan nut is one of the oldest tree nuts that grow naturally in North America. The USA is

the world's largest pecan nut producer. Other countries producing pecan nuts include Australia, Brazil, Israel, Mexico, Peru and South Africa.[97] The Brazil nut is an Amazonian plant whose native range is Guiana, Colombia, Venezuela, Peru, Bolivia and Brazil.[98] Minor plantations have been established in Kuala Lampur and Ghana.

The first European to discover the Macadamia nut is now known to be the explorer Allan Cunningham in 1828.[99] However, it was not until 1858 that German botanist Ferdinand von Mueller gave the scientific name *Macadamia intergrifolia* to the tree - named after the noted scientist Dr. John MacAdam.[99] For thousands of years before European settlement the aborigines of eastern Australia feasted on the native nuts which grew in the rainforests. This nut was known as jindilli or boombera and is now know as the Macadamia nut.[99] The high oil content of these nuts was a coveted addition to their indigenous diet.

Macadamia nut trees have a shallow root system. They prefer fertile, well-drained soils, rainfall of 1 000–2 000 mm and temperatures above 10 °C, optimally 25 °C. The climatic conditions and rainfall patterns in Australia and South Africa are similar and suitable to Macadamia nut production. Australia is the largest producer of Macadamia nuts, accounting for 43% of global production while Hawaii produces about 24%. South Africa, Malawi and Kenya account for about 26% of supply and the remaining 7% are grown in Latin America.[100] South Africa's 2006 Macadamia nut production was 19 500 MT wet-in-shell (WIS) and is projected to reach about 44 000 MT WIS in 2010. The production is expected to further double by 2016, when all new plantations would have reached full production.[101]

2.15 The Health Benefits of Tree Nuts

There is sufficient sound, relevant scientific evidence that shows consistency across different studies and among different researchers to support the health benefit claims of tree nuts. It is undisputed that dietary habits affect the risk of CHD. In the search for bioactive components in foods that favorably affect CVD risk, nuts have begun to attract much attention.[102] There is substantial evidence that nuts have favorable effects on CHD. The possible mechanisms whereby nuts may improve lipid profiles do not rely exclusively on the beneficial action of unsaturated fatty acids (PUFA and MUFA) but may include the effects of fiber, essential elements like Cu, Mg and Se, plant protein, plant sterols and phenolic compounds.[103]

Brazil nuts provide the highest natural source of Se.[104] Anyone using it 'therapeutically' employs the nuts for their high content of Se. Research has shown that introducing Brazil nuts to the diet of laboratory rats results in the protection against tumor formation.[105] Evidence for the possible anticancer benefits of Se comes from large-scale Chinese studies showing that giving Se supplements to people who live in Se-deficient areas reduces the incidence of cancer.[106,107] Se compounds have also been shown to have antitumorigenic activities in animal models when the drug is administered at levels greater than those associated with nutritional needs.[108] Brazil nut oils are often used in soaps, shampoos and hair repair products in South America. In the Brazilian Amazon, the nuts are grated with the roots of *Socratea* palms. This food provides a valuable source of calories, fat and protein for much of the Amazon's rural and tribal people.[104]

Mg intake and the risk of type 2 diabetes in men and women was investigated by Lopez-Ridaura et al.[109] in 1980. The findings suggested a significant inverse association between Mg intake and diabetes risk. The study supports the dietary recommendation to increase consumption of major food sources of Mg such as nuts, whole grains and legumes. In another

study, Ros et al.[110] reported that substituting walnuts for monounsaturated fat in a Mediterranean-type diet improved hypothelial function in hypercholesterolemic subjects. The investigators explained that nuts contain sizable amounts of antioxidants a well as L-arginine and α-linolenic acid which could confer antiatherogenic properties. A walnut enriched diet also contains high Mg levels and according to Schechter et al.[111], Mg is also an antioxidant and therefore may additively alter lipid profile and improve endothelial function.

Sabaté et al.[112] reported the effects of walnuts on serum lipid levels and blood pressure. They reported that replacing a portion of the fat in a cholesterol lowering diet with walnuts further lowered serum cholesterol levels and produced a more favorable lipoprotein profile. They also suggested that incorporating walnuts into the diet as a snack or in desserts, breads or entrees would be acceptable as part of a cholesterol lowering diet.

The Macadamia nut contains approximately 75% fat and no cholesterol and greater than 85% of its energy comes from the fat. Macadamia nuts contain higher levels of MUFA than any other food source known to date (greater than 60 g / 100 g of nuts).[113] Diets containing high MUFA -rich foods have been shown to reduce plasma LDL-C without any detrimental effect on HDL-C.[114] The research conducted by Garg et al. [115] demonstrated that Macadamia nut consumption as part of a healthy diet favorably modifies the plasma lipid profile in hypercholesterolemic men despite the diet being high in fat. These results in association with previously published reports on the beneficial effects of tree nuts on biomarkers of CAD, allow a prudent recommendation for the inclusion of Macadamia nuts as part of a heart healthy diet.

Gallbladder disease is common among adults in the USA and Western countries and is a major source of abdominal morbidity.[116] Approximately 80-90% of all gallstones are cholesterol gallstones which form when the liver begins secreting bile that is abnormally saturated with cholesterol.[117] The excess cholesterol crystallizes and then forms stones which are stored in the

gallbladder. Earlier research has shown that nuts are rich in several compounds that have beneficial effects on blood cholesterol and lipoprotein profiles and they are also rich sources of protein and dietary fiber. The relationship between nut consumption and the risk of cholecystitis (inflammation of the gallbladder) was prospectively examined in a cohort of women in the USA by Chung-Jyi et al.[118] This was done over a 20 year period, from 1980 to 2000, and the findings revealed a significant inverse association between nut consumption and the risk of cholecystitis.

Obesity is a major public health problem in many countries and a risk factor for CAD. Part of the overall efforts of researchers to help reduce obesity is to look carefully at the physiological changes that take place during weight loss. A human study headed by scientists and ARS's Grand Forks Human Nutrition Research Centre has shown specific findings about the importance of adequate amounts of Cu during weight-loss.[119] When obese people go on weight-loss diets, they often lose Ca from the bones. The study showed that women with a higher Cu intake retained more Ca in their bones. This suggests that during weight loss, the DRI for Cu may not be adequate. The Zn and Cu content of 74 foods was determined by Freeland-Graves et al.[120] Nuts, legumes and whole grains were found to be excellent sources of both Zn and Cu. Fruit and vegetables were found to be poor sources of trace elements with the exception of bean sprouts. Therefore, introducing nuts into the weight-loss diet can help prevent Ca deficiency in the bones due to its high Cu content. Mg is also essential for Ca absorption. Without Mg, Ca collects in the soft tissues and causes arthritis. Increasing the intake of Mg can help correct Ca deficiency diseases like arthritis and osteoporosis. A Mg rich diet also consists of nuts, legumes whole grains and beans.[121]

Nuts contain high concentrations of lipids and as such may lead to body weight gain and obesity so advocating an increased consumption of nuts might not be good clinical advice. This is not true since ecological data does not relate obesity to long term nut consumption. For

instance, per capita nut consumption in Mediterranean populations is about double that of the USA yet these populations' obesity rates are much lower.[122] An inverse or no relationship between frequency of nut intake and body mass index has also been observed in many studies. Fraser et al.[123] reported a statistically significant negative association between consumption of nuts and body mass index in his Californian subjects, showing that those who ate nuts more frequently were leaner than the infrequent nut eaters. Similar trends were seen in the Nurses Health Study [124] and the Physicians Health Study.[125] Suggested reasons for the disassociation between nut consumption and weight gain are increased resting metabolic rate, enhanced satiety and incomplete absorption of energy from nuts.[126] Nuts may increase resting energy expenditure because of their high protein and unsaturated fat content and this may result in less fat absorption. Nuts are energy dense and good sources of fiber and protein, dietary factors that increase satiety ratings.[127] Haddad et al.[128] showed that the pecan nut diet increased stool fat excretion. This could explain, in part, why the added fat or calories in a nut-based diet do not lead to weight gain. The pecan nut fat may not have been well absorbed because of the structure of the lipid storing granules or the fiber composition of the nuts.

2.16 Methods

The following methods used for sample analysis are comprehensively presented in this section:

- *Characterization of the Extracted Oils*
  - *Determination of Acid Value, Saponification Value and Iodine Value*
- *Walkley-Black Method for the Determination of SOM*
- *Determination of CEC at pH 7 with Ammonium Acetate by Chapman*
- *The Kjeldahl Method for the Determination of Protein in Nuts*
- *Determination of Arsenic using HG-AAS*
- *Extraction of Bioavailable Metals*

2.16.1 Characterization of the extracted oils

2.16.1.1 Determination of acid value

In a typical procedure, a known amount of sample dissolved in organic solvent is titrated with a solution of NaOH with known concentration and with phenolphthalein as a color indicator.[10]

Procedure [148]

1. Mix 25 mL of diethyl ether and 25 mL of ethanol in a 250 mL conical flask.
2. Add the resulting mixture to 10 g of oil in a 250 mL conical flask (warming may be necessary in some cases) and add a few drops of phenolphthalein indicator to the mixture.
3. Titrate the mixture with 0.1 M NaOH to the end point where a dark pink colour should persist for 30 seconds.

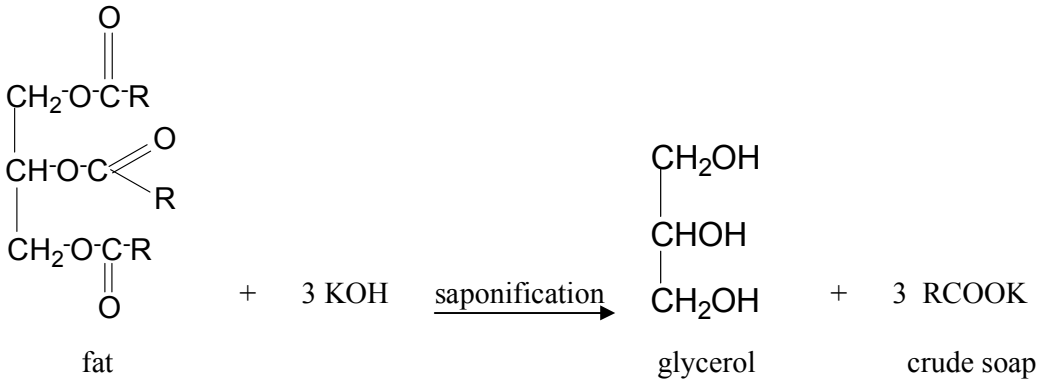
Calculation

The acid value, mg KOH/g of sample = [(S - B) x M x 56.1]/ [grams of sample]

Where: B = Titration of blank; S = Titration of sample; M = Molarity of NaOH solution.

2.16.1.2 Determination of saponification value

Reaction of vegetable oils with an alkali breaks the ester bond to form the salt of a carboxylic acid and glycerol.[10]



Procedure(ISO 3657 (2002))[148]

1. Add 25 mL of a 0.1 M ethanolic KOH solution to 2 g of oil in a conical flask. Stir the mixture then boil gently for 60 min. under reflux.

2. Add a few drops of phenolphthalein indicator to the resulting hot solution and titrate with 0.5 M HCl to the end point where the pink colour of the indicator just disappears.

Calculation

Saponification value = [(B - S) x M x 56.1]/ [grams of sample]

Where: B = Titration of blank; S = Titration of sample; M = Molarity of HCl solution.

2.16.1.3 Determination of iodine value

In a typical procedure, the fatty acid (contains double bonds that react with iodine compounds) is treated with an excess of Hanus reagent (a solution of iodobromine (BrI)). The unreacted iodobromine is then reacted with potassium iodide (KI) which converts it to iodine. This iodine concentration is determined by titration with sodium thiosulfate ($Na_2S_2O_3$) and used to calculate the iodine value. A low iodine value implies a high degree of saturation.[149]

$$BrI + KI \longrightarrow I_2 + KBr$$

Hanus reagent

$$2S_2O_3^{2-} + I_2 \longrightarrow 2I^- + (S_2O_6S_2)^{2-}$$

Procedure(ISO 3961 (1996))

1. Add 20 mL of carbon tetrachloride (CCl_4) to 0.4 g of oil in a flask to dissolve the oil. Add 25 mL of Hanus reagent to the flask under the fume hood. Seal the flask, swirl the contents vigorously and place in the dark for 2 hrs 30 min.

2. At the end of this period, add 20 mL of 10% aqueous KI and 125 mL of freshly boiled and cooled water to the flask using a measuring cylinder.

3. Titrate the resulting solution with 0.1 M $Na_2S_2O_3$ until the yellow colour almost disappears. Add a few drops of 1% starch indicator to this solution and continue the titration until the blue coloration disappears after vigorous shaking.

Calculation

The iodine value = [(B - S) x M x 12.69] / [grams of sample]

Where: B = Titration of blank; S = Titration of sample; M = Molarity of $Na_2S_2O_3$ solution.

2.16.2 Walkley-Black method for the determination of SOM [150]

In this reaction carbon is oxidized by the dichromate ion. Excess dichromate ion is then back titrated with ferrous ion.

a. Dichromate ion reacts with carbon as follows:

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

b. Ferrous ion reacts with dichromate as follows:

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

Solutions

1. *Potassium Dichromate (1 M)*: Weigh 49.04 g potassium dichromate ($K_2Cr_2O_7$) into a 1 L volumetric flask. Dissolve and dilute to volume with deionized water and mix well.

2. *Ferrous Ammonium Sulfate (0.5 M):* Slowly add 20 mL H_2SO_4 to a 1 L volumetric flask containing 800 L of deionized water. Thereafter add 196.1 g of ferrous ammonium sulfate ($Fe(NH_4)_2(SO_4)_2.6H_2O$). Dissolve and dilute to volume with deionized water.

3. *Diphenylamine Indicator*: Dissolve 0.500 g of diphenylamine ($C_6H_5NHC_6H_5$) in 20 mL deionized water. Slowly add 100 mL of concentrated H_2SO_4. Carefully mix with a glass stirring rod as the solution is corrosive and can cause severe burns.

Procedure

1. Weigh 1.00 g of soil (passed through a 0.5 mm mesh sieve) into a 500 mL Erlenmeyer flask.
2. Add 10 mL of 1 M potassium dichromate solution.

3. Add 20 mL of concentrated H_2SO_4 and mix by gentle rotation for 1 min. taking care not to throw soil up onto the sides of the flask. Allow to stand for 30 min. thereafter dilute to 200 mL with deionized water.

4. Add 10 mL of concentrated phosphoric acid (H_3PO_4), 0.2 g sodium fluoride (NaF) and 10 drops diphenylamine indicator.

5. Titrate with 0.5 M ferrous ammonium sulfate solution until the color changes from dull green to a turbid blue. Add the titrating solution drop wise until the end point is reached when the color shifts to a brilliant green.

6. Prepare and titrate a blank in the same manner.

Calculation

% Organic Matter = 10[1(S÷B)] x 0.67

Where: B = Titration of blank; S = Titration of sample.

2.16.3 Determination of CEC at pH 7 with ammonium acetate by Chapman method [151]

Advantages of pH 7 Ammonium Acetate CEC: This method has been widely used in the U.S.A for decades. Consequently, a large data base exists for soil CEC by this method.

Solutions

1. *Ammonium acetate (CH_3COONH_4) saturating solution (1 M)*: Dilute 57 mL glacial acetic acid (99.5%) with ~800 mL of distilled H_2O in a 1 L volumetric flask. Add 68 mL of concentrated NH_4OH, mix and cool. Adjust pH to 7.0 with NH_4OH if needed and dilute to 1 L.

2. *KCl replacing solution (1 M)*: Completely dissolve 74.5 g KCl in distilled water and dilute to a final volume of 1 L.

Procedure

1. Add 25.0 g of soil to a 500 mL Erlenmeyer flask.

2. Add 125 mL of the 1 M CH_3COONH_4, shake thoroughly, and allow standing overnight.

3. Fit a 5.5 cm Buchner funnel with retentive filter paper, moisten the paper, apply light suction, and transfer the soil. If the filtrate is not clear, re-filter through the soil.

4. Gently wash the soil four times with 25 mL additions of the CH_3COONH_4, allowing each addition to filter through but not allowing the soil to crack or dry. Apply suction only as needed to ensure slow filtering. Discard the leachate.

5. Wash the soil with eight separate additions of 95% ethanol to remove excess saturating solution. Only add enough to cover the soil surface, and allow each addition to filter through before adding more. Discard the leachate and clean the receiving flask.

6. Extract the adsorbed NH_4 by leaching the soil with eight separate 25 mL additions of 1 M KCl, leaching slowly and completely as above. Discard the soil and transfer the leachate to a 250 mL volumetric flask. Dilute to volume with additional KCl.

7. Determine the concentration of NH_4-N in the KCl extract by distillation using the Kjeldahl Method (Section 2.4.4). Also determine NH_4-N in the original KCl extracting solution (blank) to adjust for possible NH_4-N contamination in this reagent.

Calculation

CEC (meq/100g) = [(B - S))x M] x 100 / [grams of sample]

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

2.16.4 The Kjeldahl method for the determination of protein in nuts [152]

This method is suitable for the determination of protein in blood meal, wheat flour, dry cereal and pet food. The organic sample is digested in hot H_2SO_4 which converts amine nitrogen in the sample to ammonium sulphate. An excess of concentrated NaOH is added to this solution and the liberated ammonia is distilled into a measured excess of standard HCl. The excess HCl is determined by back titration with standard NaOH.

Procedure

Digestion

1. Add 25 mL of conc. H_2SO_4, 10 g of powdered K_2SO_4 and a crystal of $CuSO_4$ (catalyst) to 0.5 g of sample in a Kjeldahl flask.
2. Heat the Kjeldahl flask in a heating mantle under the fume hood till the digestion is complete. Complete digestion occurs after 2 to 3 hours, when the solution is colourless or feint yellow.

Distillation of Ammonia

1. Transfer the sample to a 500 mL Kjeldahl flask and add enough distilled water to give a total volume of 250 mL.
2. Measure precisely 50.0 mL of standard 0.1 M HCl into the receiver flask. Clamp the flask so that the tip of the adapter extends just below the surface of the acid. Circulate water through the jacket of the condenser.
3. With the Kjeldahl flask tilted, slowly pour about 85 mL of concentrated NaOH solution, made by dissolving 45 g of NaOH in 75 mL of distilled water, down the side of the container to minimize mixing with the solution in the flask.

4. Add several pieces of granulated Zn and a small piece of litmus paper. Immediately connect the flak to the spray trap. Very cautiously mix the solution by gentle swirling. After mixing is complete the litmus paper should indicate that the solution is basic.

5. Immediately bring the solution to a boil and distill at a steady rate until one-third of the original solution remains. Control the rate of heating during this period to prevent the receiver acid from being drawn back into the distillation flask.

6. After the distillation is judged complete, lower the receiver flask until the tip of the adapter is well clear of the acid. Then discontinue heating, disconnect the apparatus and rinse the inside of the condenser with small portions of distilled water.

7. Disconnect the adapter, and rinse it thoroughly. Add 2 drops of bromocresol green and titrate the residual HCl with standard 0.1 M NaOH to the colour change of the indicator.

Calculation

$n = 0.1\ M \times [\ (50\ mL - B) / 1000\ ]$

$\%N = [\ (n \times 14.07\ g) / 0.5\ g\ ] \times 100$

% Protein = %N x conversion factor

Where: B = Titration of base; n = mols of NH_3; %N = % nitrogen.

2.16.5 Determination of arsenic using HG-AAS [153]

The following equations show the reaction of $NaBH_4$ in acidic solution and the reduction of the hydride-forming element:

$$BH_4^- + H_3O^+ + 2H_2O \rightarrow H_3BO_3 + 4H_2\uparrow$$

$$3BH_4^- + 3H^+ + 4H_3AsO_3 \rightarrow 4AsH_3\uparrow + 3H_2O + 3H_3BO_3$$

Solutions

1. *HCl* solution (1.5% v/v).

2. *NaOH* solution (1% v/v).

3. *$NaBH_4$ reductant solution* (3% v/v): Dissolve 3 g sodium tetrahydroborate(III) in 1% NaOH. Dilute to 100 mL with the 1% NaOH solution.

4. *Arsenic stock solution* (1000 mg L^{-1}): Dissolve 1.3203 g As_2O_3 in a minimum volume of 20% NaOH and neutralize with HNO_3. Dilute to 1 L to give 1000 mg L^{-1} As.

5. *Working solution (1 mg L^{-1})*: 1mg As stock solution diluted to 1 L with 1.5% HCl.

6. *Aliquots for calibration*: 10, 25, 50µL of working solution diluted to 10 mL with 1.5% HCl.

7. *Pre-reduction solution*: Dissolve 3 g KI and 5 g L(+)-ascorbic acid in 100 mL of H_2O.

Procedure

1. Add 1 mL of the pre-reduction solution per 10 mL sample and allow standing for 30 min.

2. Run the calibration solutions to establish a calibration plot of either Absorbance vs. mass or Absorbance vs. concentration.

3. Run the sample solutions, making sure to properly clean the sample reagent bottle with 1.5% HCl after each determination.

2.16.6 Extraction of bioavailable metals

Procedure

1. Prepare extractant solution by diluting 38.542 g $NH_4CO_2CH_3$ (0.5 M), 25 mL CH_3COOH (96%) and 37.225 g EDTA (0.1 M) to 1L.

2. Mix 5 g of dry soil sample with 50 mL of extractant solution in a 250 mL polyethylene bottle.

3. Shake this suspension in a laboratory shaker for 2 hrs.

4. Centrifuge for 10 min. at 600 rpm then filter through a Millipore filter membrane (pore diameter 0.45 μm, membrane type HVLP) to permit the determination of the extracted elements.

5. Store sample solution in polyethylene bottles.

2.17 Instrumentation

The basic techniques used for the preparation and analysis of the nuts and soil samples were:

- *Digestion*

  - the Anton Paar Microwave Digestion System was used for the digestion of all samples.

- *Atomic Spectrometry*

  - ICP-OES was used to determine the concentrations of Ca, Cr, Cu, Fe, Mg, Mn, Se and Zn in all samples.

  - HG-AAS was used to determine the concentration of As in the Macadamia nuts and soil samples.

2.17.1 Microwave digestion

Precision of the analytical results depend on the sample material being supplied as a quantifiable, matrix-free solution that is devoid of contamination or loss of analyte. This sample solution may be obtained by use of the closed vessel in a microwave oven, where the sample is digested. The use of laboratory microwave units has become increasingly popular because of the significant improvement in chemical reaction rates that are possible using microwave radiation.[130] During a short period of time, very high pressures (75 bar) and temperatures (260 °C) are reached and the solid sample is dissolved into solution, usually by use of a strong acid (HNO_3). The microwave sample preparation technique digests samples in less time than traditional methods, uses less acid, can easily digest even the toughest organic or inorganic sample matrix and retains even volatile elements.[131] Traditional methods require several hours to digest the sample since time is required to heat the vessel and heat transfer to the solution is by conduction. In contrast, heat transfer by microwave radiation is instantaneous and without heating the vessel. Less acid is used for microwave digestion since the closed vessel prevents evaporative losses.

The decrease in sample preparation time using microwave digestion can be attributed to the closed vessels and the rapid heating of the sample mixture.[132] The higher temperatures achieved in the closed system give microwave digestion a kinetic advantage over hot plate digestion, as described by the Arrhenius Equation:

$$\frac{d \ln k}{d T} = \frac{E_a}{RT^2}$$

Integration of this equation gives:

$$\ln \frac{k_2}{k_1} = \frac{E_a}{2.303R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)$$

In this expression k_1 and k_2 are rate constants for the reaction of interest at T_1 and T_2 respectively, E_a is the activation energy and R is the ideal gas constant. These equations show that the reaction rate increases exponentially with increasing temperature. This translates into approximately a 100-fold decrease in the time required to carry out a digestion at 175 °C when compared to a 95 °C digestion. In addition, because the acid converts the microwave energy into heat almost instantaneously, rapid heating of the sample is achieved, further decreasing the reaction times.

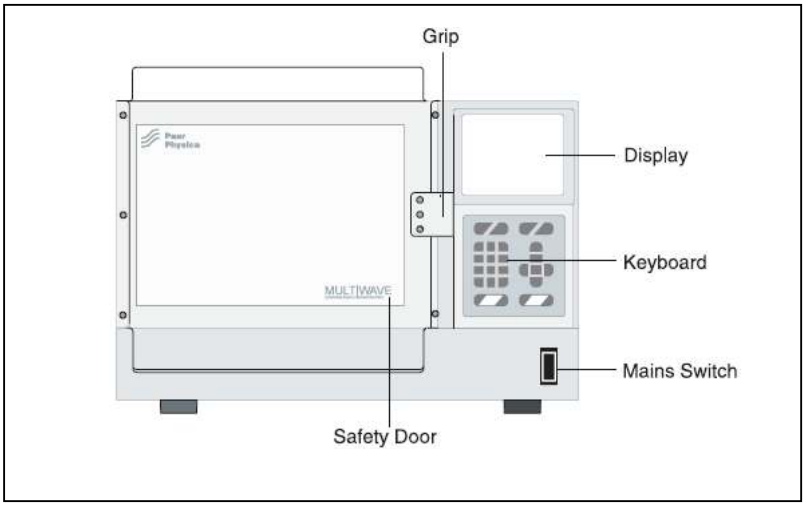


Fig.2 Diagrammatic representation of the Anton Paar Multiwave System.[129]

2.17.2 Inductively coupled plasma - optical emission spectrometry

This section provides more detail on the ICP-OES technique. Some of the general characteristics of the instrument are described to provide an overview of the operation and performance of the ICP. The features common to the ICP-OES methodology include sample preparation, sample introduction, instrument calibration and wavelength selection. This section also provides an overview of the ICP-OES methodology with a generalized discussion on interferences encountered in ICP-OES.

2.17.2.1 The inductively coupled plasma (ICP) discharge

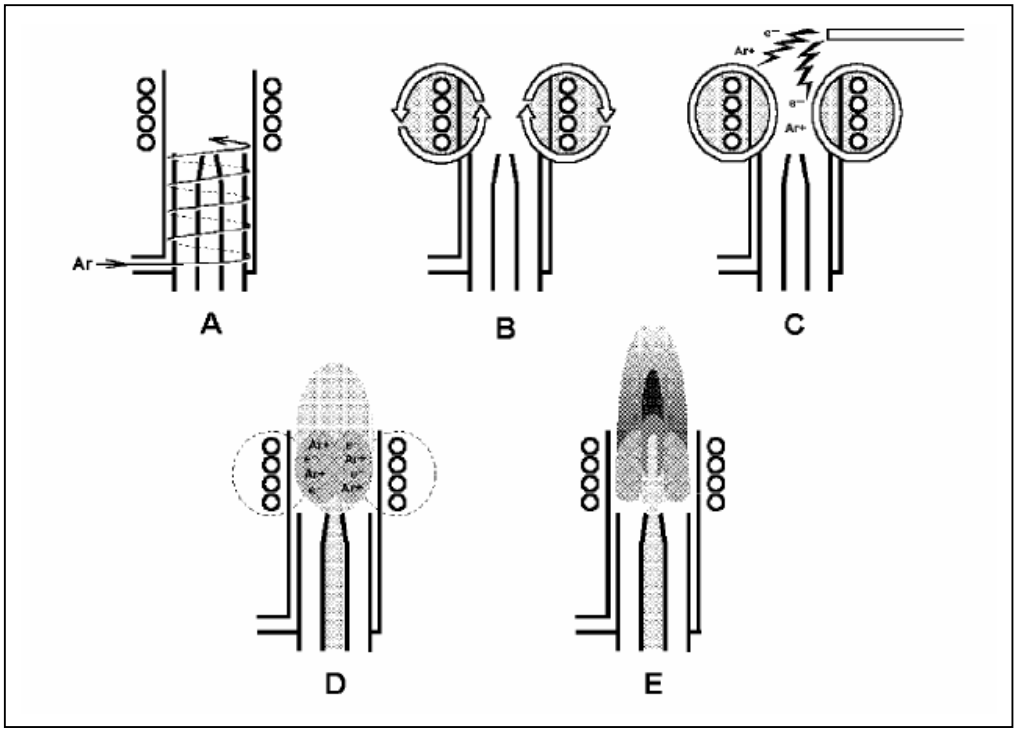


Fig.3 Cross section of an ICP torch and load coil depicting an ignition sequence.[135]

A - Argon gas is swirled through the torch. **B** - RF power is applied to the load coil. **C** - A spark produces some free electrons in the Ar. **D** - The free electrons are accelerated by the RF fields causing further ionization and forming a plasma. **E** - The sample aerosol-carrying nebulizer flow punches a hole in the plasma.

The ICP discharge appears as a very intense, brilliant white, teardrop-shaped discharge. Figure 3 shows a cross-sectional representation of the discharge in the different regions of the plasma as suggested by Koirtyohann et al.[136] Most samples begin as liquids that are nebulized into an aerosol, in order to be introduced into the ICP. The sample aerosol is then carried into the center of the plasma by the inner argon flow or nebulizer. The first function of the high temperature plasma (10 000 K) is to remove the solvent from the aerosol (desolvate), usually leaving the sample as microscopic salt particles.[135] The next steps involve decomposing the salt particles into a gas of individual molecules (vaporization) that are then dissociated into atoms (atomization).[135] Once the sample aerosol has been desolvated, vaporized and

atomized, excitation and ionization occurs. In order for an atom or ion to emit its characteristic radiation, one of its electrons must be promoted to a higher energy level through an excitation process. The excited atoms and ions emit their characteristic radiation which is collected by a device that sorts the radiation by wavelength. The radiation is detected and turned into electronic signals that are converted into concentration information for the analyst. The layout of a typical ICP-OES instrument is shown in Figure 4.

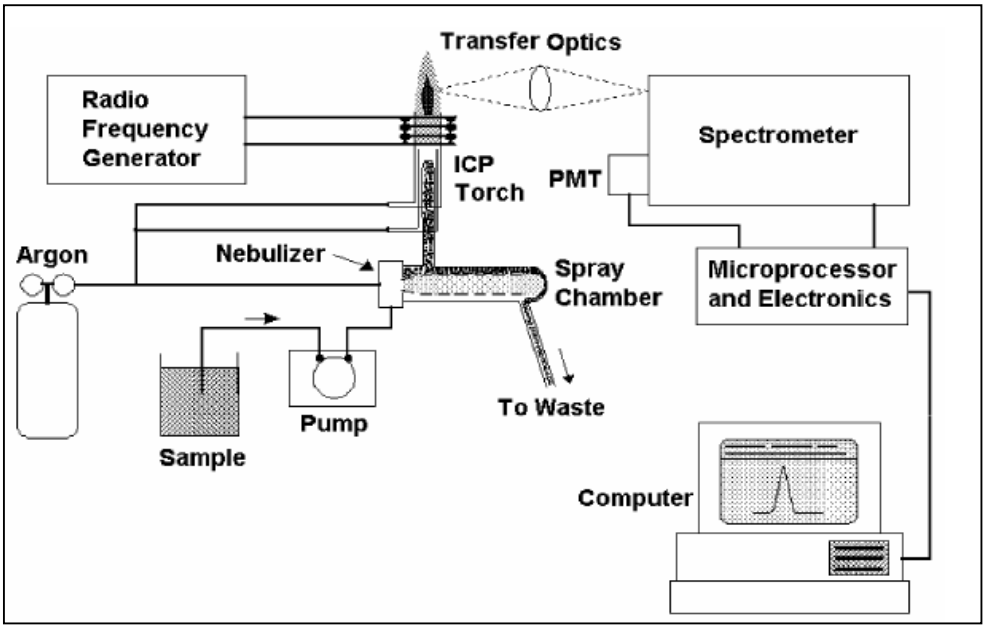


Fig.4 Major components and layout of a typical ICP-OES instrument.[135]

2.17.2.2 Detection of emission

Sample information, using ICP-OES, is obtained by measuring the light emitted by the excited atoms and ions in the plasma.[134] The emission from the plasma is polychromatic since these excited atoms and ions emit light at several different wavelengths. This polychromatic radiation must be separated into individual wavelengths to identify the emission from each excited species and to measure the intensity without interference from other wavelength emissions.[134] The separation of light according to wavelength is generally done using a monochromator or

polychromator, used to measure light one wavelength at a time or many different wavelengths at once, respectively. The detection of light that has been separated from other wavelengths is done using a photosensitive detector such as a photo-multiplier tube (PMT) or advanced detector techniques such as a charge-injection device (CID) or a charge-coupled device (CCD).

2.17.2.3 Extraction of information

Qualitative information can be obtained if an emission is present at the wavelengths characteristic of that element. Typically, at least three spectral lines of the element should be inspected to undeniably classify that emission as belonging to that element. Occasional spectral line interferences from other elements can lead to uncertainties about the presence of an element in the plasma however this can be overcome by choosing an alternative emission line. Fortunately, there are a relatively large number of emission lines to choose from for most elements.

Quantitative information can be obtained by plotting emission intensity versus concentration curves, known as calibration curves. This is done by introducing standard solutions into the ICP and measuring the intensity of the characteristic emission for each element. The measured emission intensity from an analyte is subsequently interpolated against the element's calibration curve to determine the concentration corresponding to that intensity.

2.17.2.4 Detection limits

The detection limits for the ICP-OES technique are generally in the $\mu g\ L^{-1}$ (ppb) range. The detection limit is considered to be the lowest concentration at which an element can be detected in a sample. Whilst most of the elements determined by ICP-OES have low detection limits certain elements are frequently not determined at trace levels. The following table shows the

detection limits reported by the Perkin Elmer Optima 5000 DV. The detection limits are corresponding to 3 times the standard deviation of the blank (3σ, n=10).

Table 8. Detection limits (DL) reported by the Perkin Elmer Optima 5000 DV.[138]

Element	***Wavelength (nm)***	***DL ($\mu g\ L^{-1}$)***
Ca	393.36	0.02
Cr	267.72	0.2
Cu	224.70	0.4
Fe	259.94	0.1
Mg	285.22	0.03
Mn	257.61	0.1
Se	196.03	3.0
Zn	206.20	0.2

2.17.2.5 Linearity

The upper limit of linear calibration for ICP-OES is usually 10^4 to 10^6 times the detection limit for a particular emission line.[135] For example, the maximum linear concentration for the Mn 257.610 nm emission line is about 50 mg L^{-1} or about 10^5 times its 0.0001 mg L^{-1} detection limit. The range of concentrations from the detection limit to this upper limit is known as the linear dynamic range (LDR) of the emission line. There are two major advantages of long LDRs. Firstly; it makes for easier instrument calibration. In ICP-OES, where linear calibration curves are the norm, only two solutions, the blank and a high standard, need to be analyzed to produce a calibration curve. The other advantage of long LDRs is that less sample dilution is required.

2.17.2.6 ICP-OES interferences

The ICP-OES technique is capable of determining a large number of elements over a wide range of concentrations in the same analytical run. This multi-element capability arises from the fact that all of the emission signals are emitted from the plasma at the same time. The ICP-OES technique experiences the least number of interferences of any of the commonly used analytical atomic spectrometry techniques.[135]

Matrix interferences are caused by the differences in surface tension, viscosity and dissolved solid content in the sample.[134] These differences affect the nebulizer uptake rate and transport of sample to the plasma. An important interference correction technique for matrix interferences is that of matrix matching. Matrix matching involves matching the solvents and concentrations of acids. Usually, the acid content of the standards and samples are matched. When preparing the blank solution to be used in the standardization process, the blank should also be matrix matched with the standard solutions used. For analysis of most common aqueous samples, this would usually involve adding a specified amount of acid to some deionized water. In cases where the standard and sample matrices are quite different or cannot be matched, resulting in interferences, the method of standard addition or an internal standard should be used.

Physical interferences refer to a change in spray efficiency and occur during nebulization and sample transport.[134] These processes determine the particle size and rate at which analytes are delivered to the plasma. These processes do not have a significant effect on ICP as the consumption rate is relatively small. Physical interferences are also minimized by the ICP's multi-element capability and matrix matching.

Chemical interferences are characterized by molecular compound formation, ionization effects and solute volatilization effects which result in a change in the measured atomic concentration.[134] These effects are not severe in ICP analysis and are minimized by matrix matching and careful selection of operating conditions. Chemical interferences are mainly eliminated by the high operating temperature of the argon plasma. This temperature is high enough to cause dissociation of most chemical bonds.[134]

Spectral interferences include unresolved overlap of molecular band spectra, direct spectral overlap and baseline shift.[134] Baseline shift and direct spectral overlap cause the most inaccuracies in environmental ICP analysis.[139] Baseline shift is caused mainly by high concentrations of Ca and Mg in the sample and stray light. Direct spectral overlap causes a false analyte concentration due to an emission line from an element in the sample that falls at or near the analyte wavelength. These effects can be minimized by careful selection of wavelengths since there is the flexibility to choose from many possible emission lines, use of high resolution spectrometers and advanced background correction techniques.[135]

A quality control procedure can be introduced to improve inaccurate results due to unexpected interferences with ICP. The most generally applicable quality control procedure is the analysis of samples of known composition, certified reference materials (CRMs), in conjunction with the analyte samples. These reference materials should match the sample matrix and the concentration range of the analyte elements for effective sample analysis.

2.17.2.7 Wavelength selection

The selection of wavelengths for measurement of the emission from the analyte elements is done using several criteria. Firstly, the wavelengths must be accessible with the instrument being used. Secondly, the wavelengths selected must be appropriate for the concentrations of

the elements to be determined. When the analyte concentration falls outside the working range of an emission line, a different emission line should be used. In this case, a less sensitive line would be more appropriate. When working at or near the detection limit for an element, the most sensitive line is usually the best choice. Lastly, the wavelengths must be free from spectral interferences.

2.17.3 Hydride generation - atomic absorption spectrometry (HG-AAS)

The hydride generation technique, which makes use of the separation of the analyte element from the matrix by conversion to its volatile hydride, offers a route to the trace analysis of several important elements which have specific problems when analysed by conventional methods.[141] HG-AAS is a measurement technique applied to the determination of Sb, As, Bi, Ge, Pb, Se, Te and Sn in a variety of matrices. Hydride generation is especially valuable for the determination of trace levels of As and Se because the useful resonance lines of these two elements are below 200 nm, a region where there is considerable spectral interferences from radicals in FAAS.[141] Other advantages include the elevated efficiency of analyte introduction to the atomiser, facile concentration of the analyte and the opportunity of speciation. The generation of the hydride by reaction with a freshly prepared solution of sodium tetrahydroborate(III),[142] introduced into analytical chemistry by Braman et al., [143] is now almost universally used for the synthesis of hydrides. Argon is normally used as the carrier gas. One of the attractions of the hydride generation method is the simplicity of the equipment which allows the method to be used with a conventional AAS.

2.17.3.1 The hydride generation technique

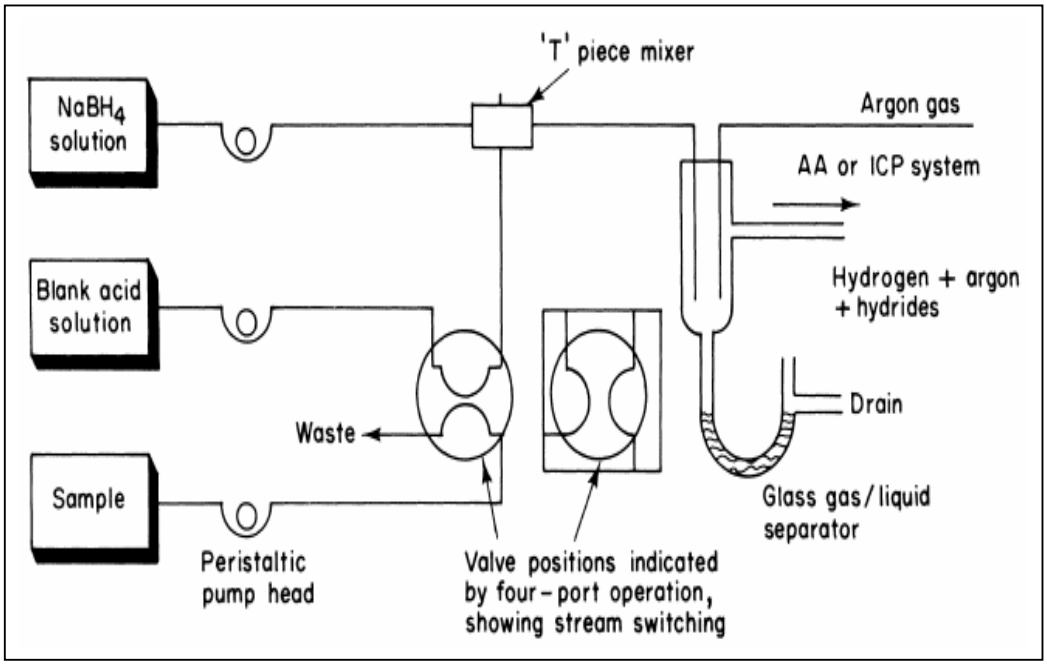


Fig.5 Flow diagram of the automatic hydride generator used for acid solutions.[146]

In HG-AAS the correct analyte oxidation state has to be produced prior to sample introduction and this usually involves the reduction of the element from a higher oxidation state to its lowest oxidation state, usually (II) or (III). Sodium tetrahydroborate(III) solution used as the reductant is dispensed into the acidic sample solution. The reaction of many metalloid oxyanions with sodium tetrahydroborate(III) and HCl produces a volatile hydride eg. H_3As, H_2Te, H_2Se etc. The time from reagent mixing to when the volatile hydride is separated from the liquid and sent to the optical cell is also important. After being mixed together the mixture flows through a tube of a specific length and is ultimately flowed into a gas/liquid separator where the hydride and some gaseous hydrogen are separated from the liquids. These gases are then purged by an inert gas (usually argon) into the optical cell via a gas transfer line. The cell is heated by a flame in order to atomize the element. The design and the operation principle of the atom cell may vary however; the quartz tube atomizer (QTA) technique is the predominant one.

The reaction of $NaBH_4$ in acidic solution and the simultaneous reduction of the hydride-forming element can be described as follows:

$$BH_4^- + H_3O^+ + 2H_2O \rightarrow H_3BO_3 + 4H_2\uparrow \quad \text{……..(1)}$$

$$3BH_4^- + 3H^+ + 4H_3AsO_3 \rightarrow 4AsH_3\uparrow + 3H_2O + 3H_3BO_3 \quad \text{……..(2)}$$

Equation 2 is representative of all the hydride-forming elements. Many of the main parts of the HG-AAS system are identical to that of AAS. These include a hollow cathode lamp, air/acetylene flame, optical cell and the relatively uncomplex hydride generation system. The nebulizer required in AAS is not used in HG-AAS. The hollow cathode lamp provides the analytical light line for the element of interest and also provides a constant yet intense beam of that analytical line. The hydride generation system mixes the acidic liquid sample with the sodium tetrahydroborate(III) solution thus creating a volatile hydride that is aspirated at a controlled rate into the optical cell or QTA. The hydride decomposes in the heated QTA, the analyte is atomized and its atomic absorption is measured. This is done by the monochromator isolating the analytical lines passing through the optical cell and removing scattered light of other wavelengths from the optical cell thereby allowing only a narrow spectral line to impinge on the PMT. The PMT, which is the detector, determines the intensity of photons of the analytical line exiting the monochromator.

CHAPTER 3

EXPERIMENTAL

There are 4 major steps in analytical chemistry. These steps are sample selection, sample preparation, sample analysis and interpretation of results. This chapter details the techniques employed in sampling and the methods and instruments used for the experimental analysis of the samples.

3.1 Sampling

Nuts samples that are commercially available in South Africa were purchased from a local supermarket (Fruit and Veg City - Umgeni Business Park) in KwaZulu-Natal, Durban. Due to the large variation of nuts available as a result of flavouring and processing, only raw, unflavoured nuts were bought to minimize the sample variability. The selected nuts were almond (*Prunus dulcus*), Brazil (*Bertholletia excelsa*), pecan (*Carya pecan*), Macadamia (*Macadamia integrifolia*) and walnuts (*Juglans nigra*). All hulls, husks and shells were removed to give the edible kernel. It could not be established if the nuts were grown locally or imported due to insufficient information.

Further, Macadamia nuts and soil samples were collected from the Macadamia nut plantations in KwaZulu-Natal (Fig.7c). This was done in May 2006 during the harvest period. The weather during the time of sampling was warm (25 °C) with no rain or wind prior to or during collection. The fallen Macadamia nuts, with husks, that were beneath the trees were picked and these represented the nuts samples. A soil sampling technique had to be adopted to obtain correct representation of the soil. This was achieved by systematically collecting samples from 6 points within the drip-line of the tree canopy with the use of a hand auger. The sampling depth which is usually the surface to tillage depth was 8 inches which also corresponds with the crop rooting depth. The representative soil samples were composited in a clean plastic bucket to achieve homogeneity and reduced to 500 g by quartering. This soil sample was then taken for drying and sieving.

3.2 Sampling Sites

The Macadamia nuts and soil samples were collected from eight different sites in KwaZulu-Natal, represented in Figure 8. The chosen sites were: Site 1 - Umhlanga, Site 2 - Chatsworth, Site 3 - Ifafa, Site 4 - Hibberdene, Site 5 - Paddock, Site 6 - Uvongo, Site 7 - Southbroom and Site 8 - Port Edward. The geographical coordinates (decimal degrees) for the 8 sampling sites are presented in Table 9.

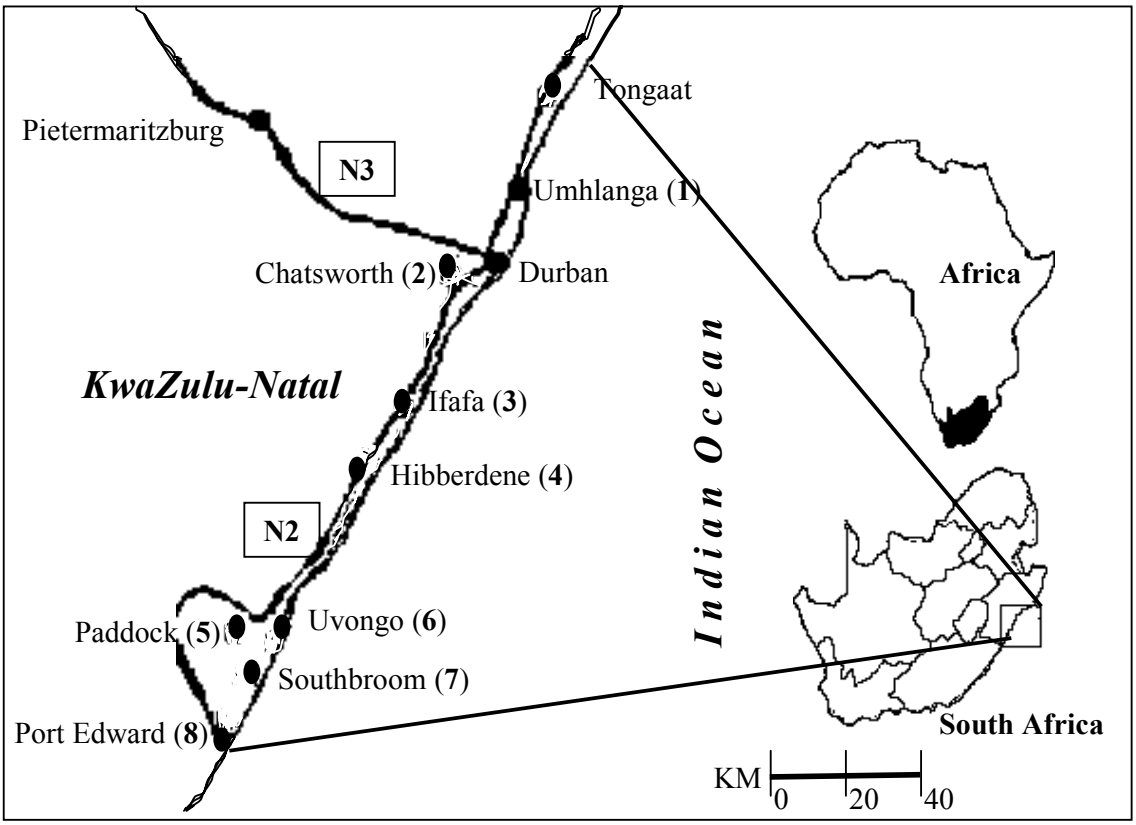


Fig.8 Map of the selected sampling sites in KwaZulu-Natal

Table 9. Geographical Coordinates, in Decimal Degrees, for the 8 chosen sites. [154]

Site	*Latitude*	*Longitude*
Umhlanga	-29.650	31.057
Chatsworth	-29.917	30.900
Ifafa	-30.417	30.583
Hibberdene	-30.600	30.550
Paddock	-29.650	31.057
Uvongo	-29.917	30.900
Southbroom	-30.417	30.583
Port Edward	-30.600	30.550

3.3 Sample Preparation and Analysis

Once the nuts and soil samples were obtained, the samples had to be prepared and analysed. Various sample preparation techniques, experimental methods and instruments had to be used to obtain the data for analysis.

3.3.1 Reagents and standards

All chemicals used were supplied by Merck and Sigma Chemical Companies and were of analytical-reagent grade (Appendix 5). Double distilled water was used throughout the experiments. All glassware and other equipment were cleaned with 6.0 M HNO_3 and rinsed off with double distilled water to prevent contamination.

3.3.2 Preparation of Macadamia nuts and soil samples

The nuts that were purchased were shelled, washed with double distilled water, dried in an oven at 40 °C overnight and stored in polyethylene bags. The nuts collected from the farms were dehusked then dried in an oven at 40 °C overnight. This drying also facilitated effortless cracking of the shells. Once the shells were cracked by use of a nut cracker and removed the nuts were stored in polyethylene bags for further study. The soil samples were frozen until drying to prevent nutrient transformations caused by microorganisms.[184] Oven drying above 48 °C is not recommended because the excess heat can change the availability of nutrients.[184] The soil samples were therefore dried in an oven at 40 °C overnight. The dried soil was passed through a 2 mm mesh sieve to remove any gravel that might be present and to obtain only the soil fraction ie. sand, silt and clay. The soil was then stored in polyethylene bags for analysis.[196]

3.3.3 Extraction of oils

The oil content was determined by grinding a known mass of the nuts (approximately 20 g without shells) in a commercial food processor and by extracting with 90 mL of a 2:1 chloroform-methanol mixture, after mixing together for 15 min.[147] The mixture was then filtered and the residue was dried, reground and extracted again. This was done three times thereafter the chloroform-methanol extracts were combined and the solvents were evaporated using a rotary evaporator. The collected oils were quantified gravimetrically and reported as percentage oil in sample. The defatted nut flour obtained by this process was sieved through a 500 μm mesh, packed in polyethylene bags and stored in a cool dry place for analysis. [14]

3.3.4 Proximate chemical composition of nuts

The lipid content was determined as per method described by Kannamkumarath et al. (Section 3.3.3).[147] The ash content was determined by incineration of known masses of the defatted nut samples in a muffle furnace at 600 °C for six hours. Nitrogen in the defatted nut samples was determined by the Kjeldahl Method (Section 2.16.4).[152] The nitrogen value obtained was multiplied by a conversion factor and reported as mass of protein in sample.[160,161] The conversion factor for walnuts and pecan nuts is 6.25, almonds 5.18, Brazil nuts 5.46 and Macadamia nuts, 5.3. The available carbohydrate was obtained by difference. This was done by subtracting the amount of oil, ash and protein from the total dry matter.[162]

3.3.5 Digestion of samples

The defatted nut flour samples were digested prior to analysis using the microwave-assisted closed vessel digestion technique. This method facilitates rapid dissolution of the sample matrix, requires low oxidizing reagent use and causes minimal contamination of the sample.[163]

The digestions were performed using the Anton Paar Multiwave Microwave Sample Preparation System (1000 W) with 6 high-pressure tetrafluoromethaxil (TFM)-Ceramic Vessels (HF 50).[164] The maximum temperature was 260 °C and the maximum pressure was 75 bar. To ensure uniform sampling and improved precision, three sub-samples of each sample, both nuts and soil were digested. The defatted nut flour (0.5 g) was weighed into the TFM vessels. Five mL of 69% HNO_3 was added to each vessel and sealed. A leaf digestion programme (Section 3.3.5.1) was used for digestion of the nuts. For the digestion of soil samples, 0.3 g of soil finely crushed with pestle and mortar were weighed into the TFM vessels. Five mL of 69% HNO_3 was added to each vessel and sealed. A soil digestion programme (Section 3.3.5.2) was used for digestion of the soil samples.

3.3.5.1 Programme for the digestion of nuts: Leaf CRM TFM programme

The power was ramped to 500 W for the first 5 min., where it remained for the next 5 min. The power was then ramped to 650 W for 15 min. during which complete digestion of the samples occurred. The microwave power was then reduced and the bombs cooled by forced ventilation for 15 min. (Appendix 3). The digested samples were then removed from the TFM vessels and transferred to 25 mL volumetric flasks, diluted to the mark with double distilled water and stored in polyethylene bottles for analysis.[197]

3.3.5.2 Programme for the digestion of soil samples: Soil TFM programme

Soil samples were digested using a harsher digestion programme to digest most of the soil components. The power was ramped from 100 W to 600 W for 10 min. The power was then ramped from 600 W to 900 W over the next 12 min. during which digestion of the samples occurred. The microwave power was then reduced and the bombs cooled by forced ventilation

for 15 min. (Appendix 3). The digested samples were then removed from the TFM vessels and filtered through Mg free filter paper. This was done to remove the undigested silicates as HNO_3 will not digest silicate material. The samples were transferred to 25 mL volumetric flasks then diluted to the mark with double distilled water and stored in polyethylene bottles for analysis.

3.3.6 Extraction of bioavailable metals

A combination of chemical extractants was used to effectively release available metals from the soil fractions. Due to the distinctive extracting abilities of acidic ammonium acetate and EDTA the extractant solution chosen for the determination of potential nutrient availability from soil for uptake by Macadamia nut trees was an acidic ammonium acetate EDTA solution (Section 2.16.6). For good reproducibility, the soil / extractant ratio, temperature and duration of extraction were kept constant. The filtered sample solution was stored in polyethylene bottles for elemental analysis.

3.3.7 Analytical methods used for elemental analysis

All five purchased nuts, after acid digestion, were analysed for Ca, Cu, Cr, Fe, Mg, Mn, Se and Zn by Inductively Coupled Plasma-Optical Emission Spectrometry. All Macadamia nuts and soil samples obtained from the 8 different sites were analysed for Ca, Cu, Cr, Fe, Mg, Mn and Zn by ICP-OES. The detection of Se was omitted in this case since the Se levels in the purchased Macadamia nuts were found to be below the detection limit of the instrument. All measurements were performed using the Perkin Elmer Optima 5300 DV with axial plasma observation.

The analyte concentrations were obtained by reading off calibration curves. Calibration curves were obtained by introducing a blank and 4 standard solutions into the ICP and measuring the

intensity of the characteristic emission for each element. A plot of emission intensity versus concentration gave the calibration curve for an element. Working standard solutions for calibrations were prepared from spectroscopic grade stock standard solutions (1000 mg L^{-1}). All working standards were made up in double distilled water. Since samples were digested in HNO_3, a proportionate amount of HNO_3 was added to the standards to minimize the matrix effects. The analytical wavelengths were selected based on the minimum spectral interferences and maximum analytical performance. Table 10 shows the wavelengths chosen for each of the elements analysed by the ICP in this study. Initially the 3 most sensitive lines were chosen. From these lines, the lines with no interfering elements were chosen.

Table 10. Wavelengths selected for the chosen elements.

Elements	***Wavelengths (nm)***
Ca	317.94
Cr	267.71
Cu	324.76
Fe	238.21
Mg	279.08
Mn	259.37
Se	203.99
Zn	206.20

Total inorganic As was analysed using Hydride Generation-Atomic Absorption Spectrometry (HG-AAS). This was performed on a Perkin Elmer Analyst 100 with an MHS 15 Mercury Hydride System. Arsenic was detected by a hollow cathode lamp at the 193.7 nm line. The slit width was 0.7 nm and the QTA was heated in a lean, blue air-acetylene flame. Since the hydride is generated much more slowly from As(V) than from As(III), As(V) had to be pre-reduced to As(III) prior to determination to prevent interferences. The reliability of the above methods was verified by use of a certified reference material (CRM).

3.3.8 Certified reference materials

CRMs are sample materials obtained from an independent source that have been analysed by different laboratories to determine consensus levels of the analyte concentrations. CRMs are fundamental to laboratories monitoring the performance of their analytical work. These materials test the precision of the entire analytical method from sample preparation to analysis. They are a major tool for improving the confidence in, and the mutual recognition of test results and certificates in a global market.[198] Accuracy of the trace element measurements in this study, for the purpose of quality assurance, was tested by analysis of standard reference material, *lyophilized brown bread* (BCR 191), from the Community Bureau of Reference of the Commission of the European Communities. A certificate of analysis is provided in Appendix 4. This particular CRM was chosen due to matrix similarities. The CRM was provided as a fine dry powder which was similar to the defatted nut flour.

Analysis of the CRM was done to ensure that the digestion was complete, the instrument parameters were optimized, calibration errors were removed and any method imprecision's were eliminated. Working standards were prepared and introduced into the instrument to obtain calibration curves. The CRM sample was then analysed to give the concentrations of the analyte elements. The measured and certified values were compared and only when the measured values were consistent and in agreement with the certified values did analysis of the samples proceed.

3.3.9 Physicochemical properties of extracted nut oils

The saponification value was determined by the indicator method as specified by ISO 3657 (2002) (Section 2.16.1.2).[148] The iodine value was determined using Hanus reagent and was

according to the method specified by ISO 3961 (1996) (Section 2.16.1.3).[148] The acid value was as per method described by Akpan et al. (Section 2.16.1.1).[148]

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

The pH of the soil was determined by measuring a 1:1 soil / water suspension using a pH meter fitted with a glass electrode. The SOM was estimated using the wet chemistry extraction technique known as the Walkley-Black Method (Section 2.16.2).[150] The pH 7.0 ammonium acetate method was used for the determination of the CEC in the soil (Section 2.16.3).[151]

3.3.11 Statistical analysis of data

The significance of the plant-soil relationships were established by computing correlation coefficients (r) for the relationships between the concentrations of the elements in the Macadamia nuts and the total and bioavailable amounts in the soil. Correlation coefficients were evaluated by Spearman's test, using the Statistical Package for the Social Sciences (SPSS). The correlation coefficient (r) determines the extent to which values of two variables are linearly related to each other. The value of the correlation (r value) does not depend on the specific measurement units used. The correlation is high if it can be approximated by a straight line, called the regression line. The correlation coefficient can range from -1.0 to +1.0. The closer r is to +1 or -1, the more closely the two variables are related. If r is positive then as one variable gets larger the other gets larger. If r is negative then there is an inverse relationship between the variables which means as one variable gets larger, the other gets smaller. A test of significance, using the One-sample T-Test, was done on the proximate chemical composition of the Macadamia nuts from the different collection sites. A one sample t test compares the mean value with a hypothetical value. In most cases, the hypothetical value comes from theory.

CHAPTER 4

ELEMENTAL COMPOSITION AND CHEMICAL CHARACTERISTICS OF EDIBLE NUTS (ALMOND, BRAZIL, PECAN, MACADAMIA & WALNUT)

4.1 Introduction

The results and discussion in this chapter are done to achieve the first objective of this study which is to obtain a comparison on the chemical and physicochemical characteristics of various common edible tree nuts consumed in South African households. The comparison is only done for the 5 different tree nuts (almond, Brazil nuts, Macadamias, Pecans and walnuts) chosen in this study with no assumptions being made about the consumption of other tree nuts in South Africa. These nuts were chosen primarily because of their similarities in physical properties and because of their popularity amongst the consuming population. Initially, all nuts were analysed for the essential elements Ca, Cu, Cr, Fe, Mg, Mn, Se and Zn. The concentration of As was also determined since this element can be found in pesticides. The proximate chemical composition namely %oil, %ash, %protein and %carbohydrate in the nuts and the physicochemical properties namely acid value, iodine value and saponification value of the extracted nut oils were obtained for a holistic depiction of the composition of the five nuts studied. No further analysis was done on these nuts since the nuts were purchased and no other relevant information could be acquired. In this chapter, all tables contain the mean values with their standard deviations however, when reproducing these values in the discussion the standard deviations are omitted for fluency.

4.2 Quality Assurance

Table 12. Comparison of measured and certified values in the certified reference material.

Element	Wavelength (nm)	Concentration*	
		Certified**	Measured**
Cu	324.76	2.6 ± 0.1 μg g^{-1}	1.9 ± 0.8 μg g^{-1}
Fe	238.21	40.7 ± 2.3 μg g^{-1}	41.8 ± 1.3 μg g^{-1}
Mn	259.37	20.3 ± 0.7 μg g^{-1}	19.8 ± 1.5 μg g^{-1}
Zn	206.20	19.5 ± 0.5 μg g^{-1}	19.4 ± 0.7 μg g^{-1}
As	193.70	23.0 ng g^{-1}	23.6 ± 0.2 ng g^{-1}
Ca	317.94	0.41 mg g^{-1}	0.43 ± 0.02 mg g^{-1}
Mg	279.08	0.5 mg g^{-1}	0.51 ± 0.01 mg g^{-1}

***** Dry mass. ** Mean ± S.D, at 95% confidence interval, n = 6.

Results shown are the means of 6 separate sample preparations. Accuracy of the trace element measurements was corroborated by analysis of standard reference material, *lyophilized brown bread* (BCR 191) and the results are presented in Table 11. The values provided for Cu, Fe, Mn and Zn are certified. However, the values provided for As, Ca and Mg are indicative so no uncertainties were ascribed to them. These values, though insufficient for certification, are the arithmetic means of the accepted results submitted by some laboratories. The measured results are in good agreement with the certified values.

3.3 Comparison of Elemental Concentrations with Other Similar Studies

Table 12. Comparison of average concentrations of different essential elements found by various authors, in the defatted nut flour, in the five edible nuts chosen in this study.

Sample	References (Country)	Mass of Elements (μg g^{-1})							
		Cu	**Cr**	**Mg**	**Mn**	**Fe**	**Zn**	**Ca**	**Se**
WALNUT	**This Study, (RSA)**	**59**	**1.7**	**4833**	**128**	**72**	**54**	**2880**	
	Wuilloud et al[165], (USA)	222			68		189		
	Caĝlarirmak[162], (Turkey)	13		990	38.5	33	25	1055	
	Cabrera et al[166], (Spain)	2	0.4			23	29		
	Kannamkumarath et al[147], (USA)								0.4
	Holland et al[167], (UK)	15				29	27		
	Furr et al[168], (USA)	19	1.0	1794	30	73	46	668	
ALMOND	**This Study, (RSA)**	**24**	**0.9**	**5424**	**26**	**72**	**50**	**5392**	**0.004**
	Christian et al[169], (Nigeria)			400	9.5	49	0.5	320	ND
	Wuilloud et al, (USA)	200			4780		236		
	Cabrera et al, (Spain)	11	0.4			45	39		
	USDA[170], (USA)	11				43	34		
	Furr et al, (USA)	14	1.7	2297	14	54	32	2720	
BRAZIL	**This Study, (RSA)**	**59**	**1.3**	**9679**	**3.4**	**74**	**110**	**7433**	**36**
	Wuilloud et al, (USA)	186			12		547		
	Kannamkumarath et al, (USA)								35
	Furr et al, (USA)	18	0.6	3370	8	93	41	1592	11
PECAN	**This Study, (RSA)**	**36**	**2.0**	**4197**	**193**	**106**	**138**	**2088**	**ND**
	Kannamkumarath et al, (USA)								0.1
	Furr et al, (USA)	15	0.3	980	30	73	56	618	
MACADAMIA	**This Study, (RSA)**	**19**	**1.3**	**4887**	**88.6**	**68**	**38**	**3376**	

Table 12 serves to provide a comparison of the different concentrations found by various authors for the 8 essential elements (Ca, Cr, Cu, Fe, Mg, Mn, Se and Zn) chosen in this study. Whilst these results do bear some similarities to this study some differences amongst the concentrations obtained for a particular element in a particular nut are observed. Deriving diagnostic information on the chemical characteristics of a particular nut consumed in different countries, without looking at various contributing factors, is difficult. However, the values provided by other authors are indicative of the range for a similar type of nut and is useful as a guide.

From the table, it can be seen that there is no reported work on the elemental concentrations of edible tree nuts consumed in South Africa. A quick perusal of the table shows that all nuts in this study seem to have a higher level of Ca and Mg than the other studies. However, the concentrations of the other six elements are quite comparable to the values obtained by other authors for a specific nut. Unfortunately, no published information on the elemental concentrations in Macadamia nuts could be obtained for comparison.

4.4 Elemental Distribution in the Five Edible Nuts

The elemental concentrations and the dietary reference intakes (DRIs- expressed as μg/day) for the essential and toxic elements in the different nut samples analyzed are summarized in Table 13. The elemental distributions of the 8 detectable elements from the table are illustrated in Figures 9a-h. The distributions of the major elements are represented in Figure 10 and that of the minor elements are represented in Figure 11.

Table 13. Total elemental concentrations in the five edible nut samples expressed as μg g^{-1}.

	Almond	Brazil	Macadamia	Pecan	Walnut	DRI* (μg/day)	
						RDA**	UL***
As	0.013 ± 0.004	0.017 ± 0.002	0.019 ± 0.002	0.019 ± 0.001	0.024 ± 0.002	—	ND
Ca	5392.4 ± 57.2	7432.8 ± 10.2	3376.1 ± 15.0	2088.4 ± 32.7	2880.2 ± 35.9	10×10^6	2.5×10^6
Cr	0.94 ± 0.14	1.34 ± 0.19	1.26 ± 0.06	2.02 ± 0.07	1.74 ± 0.12	25-35	ND
Cu	23.74 ± 0.04	59.44 ± 0.51	18.96 ± 0.11	35.5 ± 0.05	59.14 ± 0.06	900	1.0×10^4
Fe	71.54 ± 0.36	74.26 ± 0.46	68.06 ± 0.27	105.86 ± 1.68	71.58 ± 1.13	8-18000	4.5×10^4
Mg	5424.1 ± 51.7	9678.5 ± 68.5	4886.5 ± 24.4	4197.0 ± 60.8	4833.0 ± 56.2	3.2×10^5	3.5×10^5
Mn	25.86 ± 0.48	3.40 ± 0.21	88.64 ± 0.51	192.60 ± 3.05	128.04 ± 1.56	18-2300	11000
Se	0.0039 ± 0.0007	36.1 ± 0.4	ND	ND	ND	55	400
Zn	49.72 ± 0.09	110.31 ± 1.25	38.46 ± 0.66	137.86 ± 0.39	54.26 ± 0.22	1.1×10^4	4.0×10^4

Mean ± S.D expressed as μg g^{-1} of sample, at 95% confidence interval, n = 3. ND- Not detected * DRI - Dietary Reference Intakes (expressed as μg/day). ** RDA - Recommended Dietary Allowance. *** UL - Tolerable Upper Intake level.

Very little or no Se was detected in most of the nut samples analyzed. However, high Se levels were detected in the Brazil nut sample with a concentration of 36.1 μg g^{-1}, which is comparable to the results obtained by Kannamkumarath et al.[147] These levels are below the tolerable limit for this element in most adults (Table 13). Two grams of Brazil nuts per day would be sufficient to meet the RDA for Se in most adults (Table 13). However, consuming more than 11 g of Brazil nuts per day will result in the Tolerable Upper Intake Level (ULs) being exceeded and this could likely lead to the risk of adverse effects.

With the almond and walnut samples setting the maximum and minimum limits, respectively the levels of As found in all the nut samples ranged from 0.013 to 0.024 μg g^{-1} (Table 13). These levels are considerably lower than the FDA limit for As in fruit and vegetable which is 1.4 μg g^{-1}.

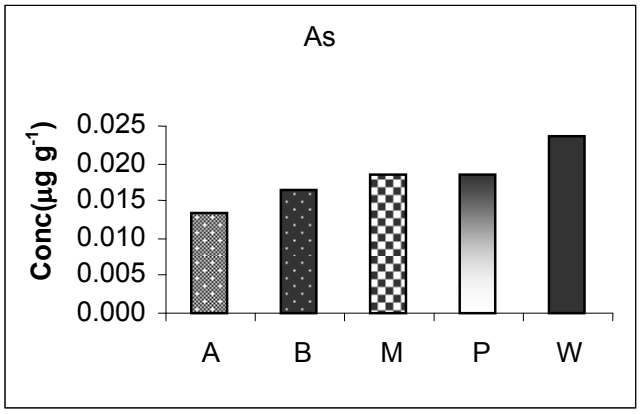


Fig.9a Distribution of As in the 5 different nuts

(A-almond, B- Brazil nut, M-Macadamia, P-pecan, W-Walnut)

It can be observed from Figure 9b that the Cr concentrations were around the same value for the different types of nuts. On average, the Cr concentration was found to be 1.46 μg g^{-1} with the highest Cr concentration emerging in the pecan nut sample (2.02 μg g^{-1}). It is also reported that As and Cr are usually not phytotoxic, even in cases of severe soil contamination in the field and these elements are also not taken up by plants in measurable quantities.[66]

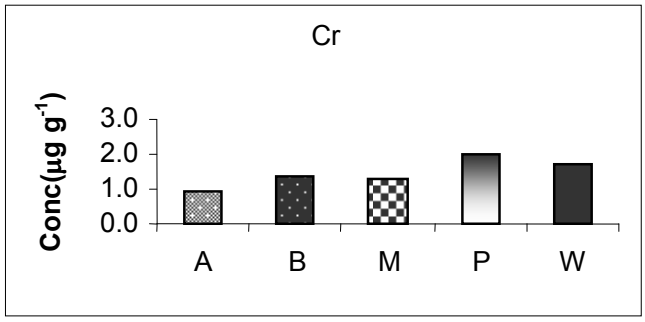


Fig.9b Distribution of Cr in the 5 different nuts

(A-almond, B- Brazil nut, M-Macadamia, P-pecan, W-Walnut)

The pecan nut sample had the highest Fe concentration of 105.86 μg g^{-1}. With the exception of the pecan nut, all the other nut samples had an Fe concentration close to 70 μg g^{-1} (Fig.9c).The Fe levels in the nuts investigated can contribute to the RDA for this element in most adults without posing the risk of toxicity.

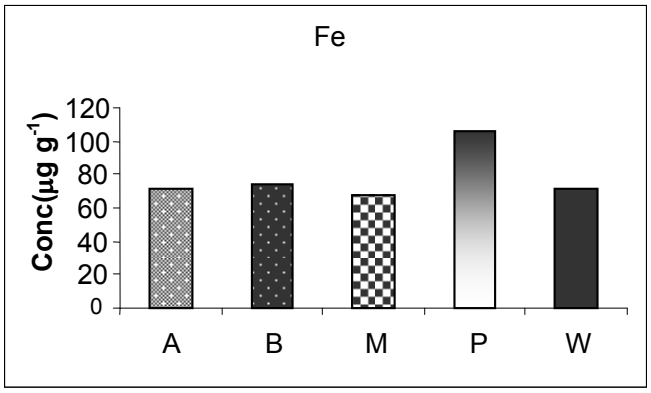


Fig.9c Distribution of Fe in the 5 different nuts

(A-almond, B- Brazil nut, M-Macadamia, P-pecan, W-Walnut)

The Cu concentrations in the different nut samples analyzed ranged between 18.96 μg g^{-1} and 59.44 μg g^{-1}. The most elevated concentrations were found in the walnut and Brazil nut samples and their concentrations were higher than those found in the almond and Macadamia nut samples by a factor of about 3 (Fig.9d). These results are in agreement with data obtained for similar types of nuts by Cabrera et al.[166]

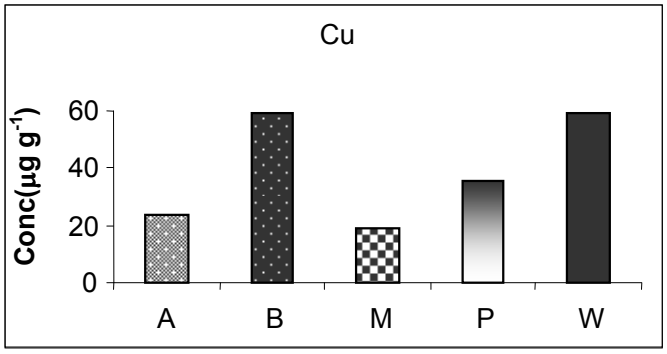


Fig.9d Distribution of Cu in the 5 different nuts

(A-almond, B- Brazil nut, M-Macadamia, P-pecan, W-Walnut)

Mg is an essential part of many enzyme systems and large amounts were found in the different nut samples studied, with the highest amount of 9678.5 μg g^{-1} being present in the Brazil nuts.

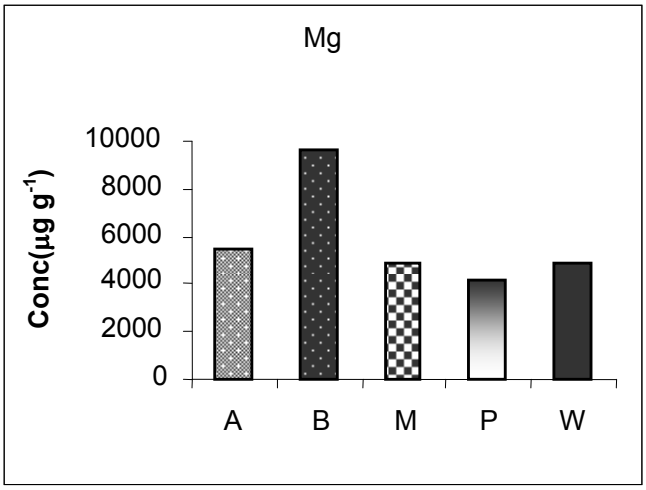


Fig.9e Distribution of Mg in the 5 different nuts

(A-almond, B- Brazil nut, M-Macadamia, P-pecan, W-Walnut)

Ca, an essential element required for bone formation and the development of strong teeth, was found to be present at significant levels in all the different nuts studied. The highest Ca level was found in the Brazil nut sample (7432.8 μg g^{-1}) and the lowest level was found in the pecan nut sample (2088.4 μg g^{-1}). From Fig.9f it is clear that the concentration in the Brazil nut sample is more than 2 times the concentrations in the Macadamia and walnut samples and more than 3 times the concentration in the pecan nut sample.

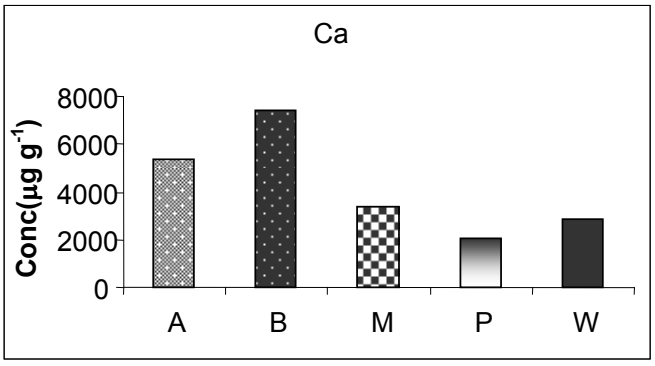


Fig.9f Distribution of Ca in the 5 different nuts

(A-almond, B- Brazil nut, M-Macadamia, P-pecan, W-Walnut)

The Zn levels in the different nut samples are within the range 38.46 μg g^{-1} and 137.86 μg g^{-1} (Fig.9g). Zn levels in Brazil and pecan nut samples appear to be comparable (110.31 μg g^{-1} and 137.86 μg g^{-1}) whilst those in walnut, almond and Macadamia nut samples show only marginal differences (38.46 to 54.26 μg g^{-1}). This observed variability of Zn in the nut samples could be a consequence of the variability of Zn in the soil or due to the differences in the nature of the nuts. Nuts and legumes are relatively good plant sources of Zn, but plant Zn concentrations may get enhanced, if grown in Zn-rich soils or treated with Zn-rich fertilizers.[171]

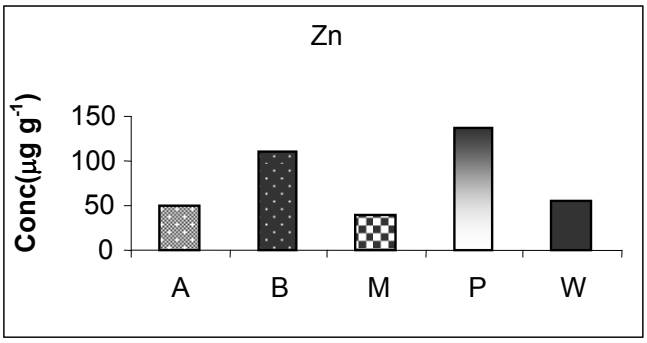


Fig.9g Distribution of Zn in the 5 different nuts

(A-almond, B- Brazil nut, M-Macadamia, P-pecan, W-Walnut)

Mn presented the greatest variability with regards to concentration amongst the different nut samples analyzed, with concentrations ranging from 3.40 to 192.60 μg g^{-1} in the order of Brazil, almond, Macadamia, walnut and pecan nut (Fig.9h). Wuilloud et al.[165] also found a large variation in the Mn concentration amongst different nut samples with concentrations ranging from 9 μg g^{-1} to 4780 μg g^{-1}. The difference in the Mn concentration could be due to the type of nut as well as factors such as environmental conditions under which the nuts grew or variations in the mineral concentration of soils.[165]

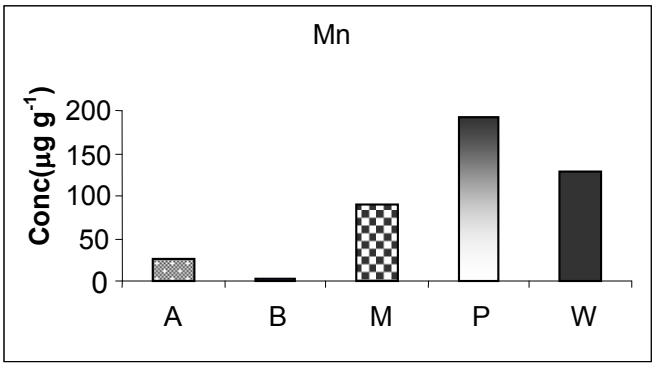


Fig.9h Distribution of Mn in the 5 different nuts

(A-almond, B- Brazil nut, M-Macadamia, P-pecan, W-Walnut)

Brazil nuts are known to have high levels of Ca and Mg which is confirmed by the results obtained in this study. With the exception of the almond sample, where the Ca and Mg levels appear to be almost the same (Fig.10), the levels of Mg are higher than Ca in the different nut samples. The data shows that Ca, Cu, Mg and Se levels are highest in the Brazil nuts analysed whilst Cr, Fe, Mn and Zn are highest in the pecan nuts analysed (Table 13). This suggests that amongst the 5 nuts studied, Brazil and pecan nuts are richer sources of these essential elements. However, an adequate serving of any one of these nuts would contribute to the RDA for most essential elements in most adults.

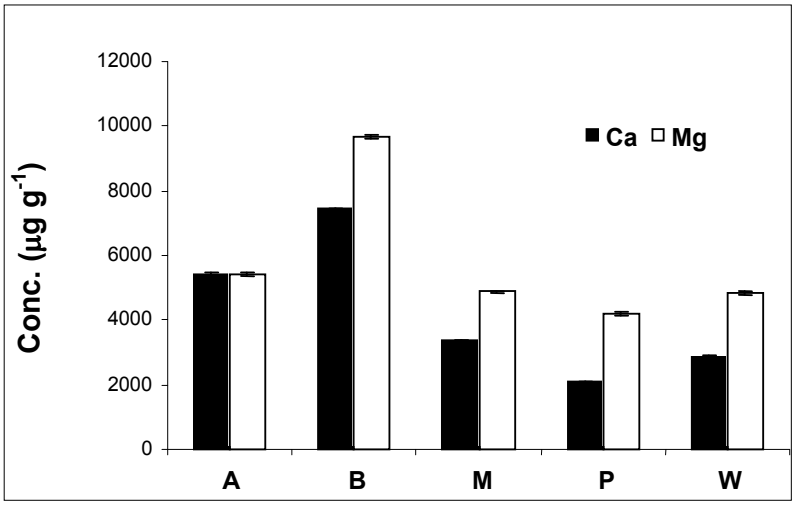


Fig.10 Distribution of major elements (Ca and Mg) in the different nut samples.

The concentrations of certain essential elements were found to be similar although obtained from different types of nuts. In particular, these elements are Cr, Cu, Fe and to a lesser extent, Zn. The similarity in the concentrations found for the different elements in the different types of nuts is an indication that the levels of essential elements in the plant are controlled. This natural phenomenon of the plant is to ensure that the typical concentrations sufficient for plant growth (Table 3) are not exceeded. These concentrations invariably are below the tolerable upper intake levels for most adults.

The elemental concentrations depicted in Figure 10 and Figure 11 clearly shows a trend that exists amongst the different types of nuts. The concentrations of the minor elements in the almond, Macadamia and walnut samples are in the decreasing order of Fe>Zn>Cu>Cr>As. In the Brazil and pecan nut samples the Zn concentration is higher than Fe and is in the decreasing order of Zn>Fe>Cu>Cr>As. In general, the order of the concentrations of the elements (both major and minor) in the nut samples is shown to be Mg>Ca>Fe>Cu>Cr>As>Se. Zn and Mn are omitted from the general order due to the variations in concentrations.

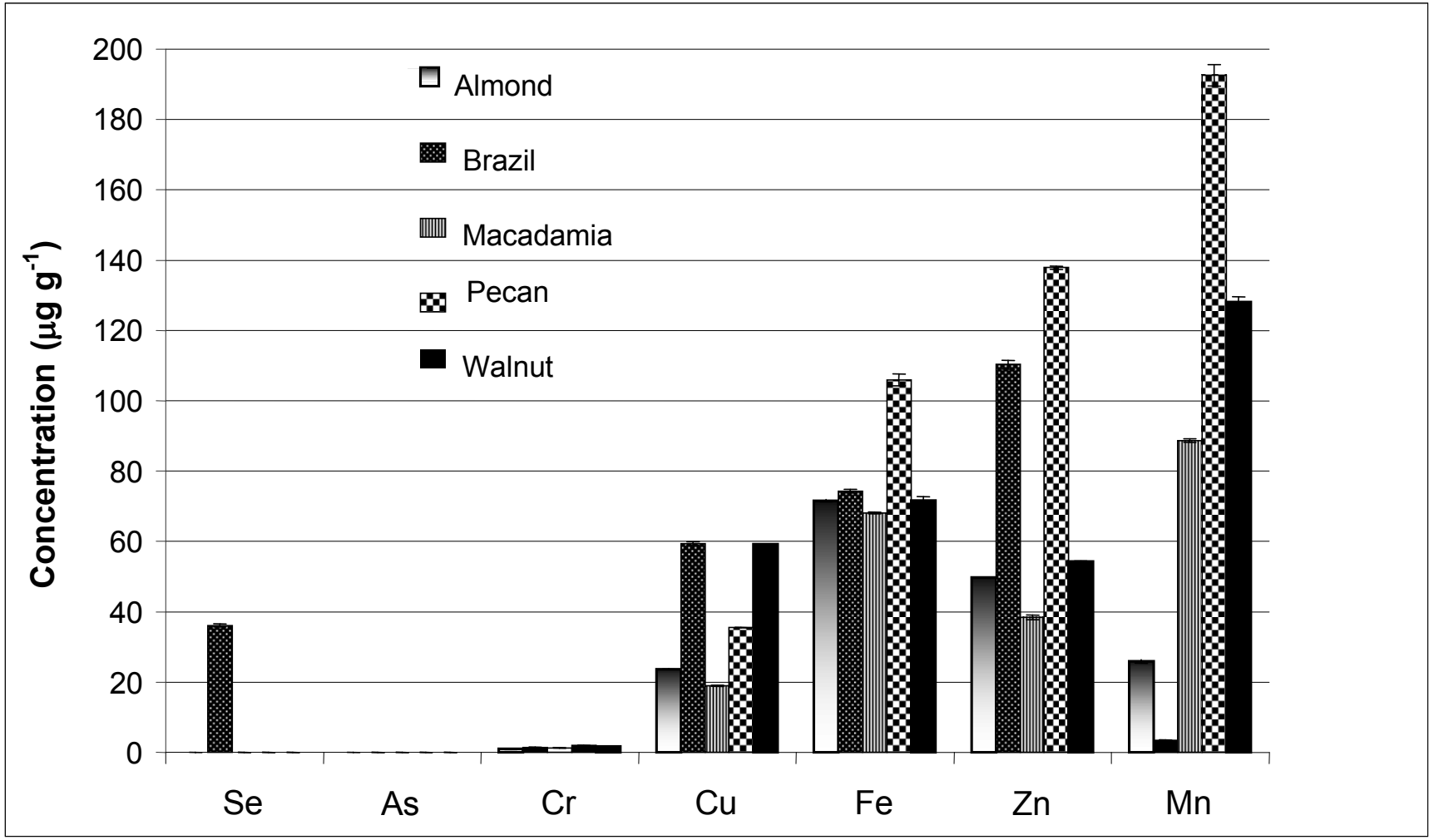


Fig.11 Distribution of minor elements (Se, As, Cr, Cu, Fe, Zn, Mn) in the different nut samples.

4.5 Proximate Chemical composition of the Five Edible Nuts

The proximate chemical composition of the different nut samples are presented in Table 14 and a graphical representation of this data is provided by Figure 12.

Table 14. Proximate chemical composition (g per100 g dry mass) of the nut samples analyzed.

Nut Sample	Oil	Ash	Protein	Carbohydrate*
Macadamia	76.0 ± 0.5	4.0 ± 0.1	13.0 ± 0.3	7
Pecan	65.0 ± 0.6	6.0 ± 0.1	8.0 ± 0.3	21
Brazil	65.0 ± 1.1	4.0 ± 0.2	22.0 ± 0.3	9
Walnut	57.0 ± 0.5	2.0 ± 0.1	14.0 ± 0.2	27
Almond	47.0 ± 0.4	5.0 ± 0.1	20.0 ± 0.2	28

* Carbohydrate obtained by subtracting the sum of oil, ash & protein from the total dry matter.

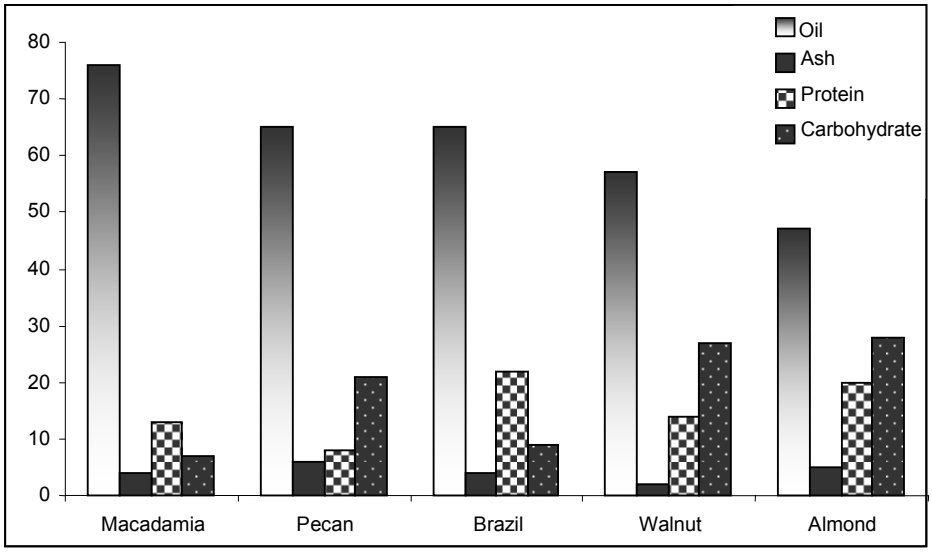


Fig.12 Percentage carbohydrate, protein, ash and oil in the 5 different nuts.

It can be seen that all the edible tree nuts are rich in oils (47.0 to 76.0 g/100g dry mass (DM)) with moderate amounts of protein (8.0 to 22.0 g/100g DM) and carbohydrate (7 - 28 g/100g DM). The highest content of oil was observed in the Macadamia nut sample (76.0 g/100g DM). This is in agreement with the results reported by Kaijser et al.[172] for four different cultivars of Macadamia nuts grown in New Zealand, which ranged from 69.1 to 78.4 g/100g DM. The proximate chemical composition of the walnut sample in this study is similar to the results obtained by Cağlarirmak[162] for the Güvenli variety grown in Turkey (oil - 57.32%, ash - 1.8%, protein - 13.16% and carbohydrate - 25.23%). The results of the proximate analysis of the Brazil nut sample per 100 g DM are as follows: 65.0 g oil, 4.0 g ash, 22.0 g protein and 9 g carbohydrate. Similar values are reported in earlier works by Ramos et al.[173] The pecan nut sample has the lowest protein content but relatively high amounts of oil and carbohydrate. The almond nut sample has the lowest oil content but the highest amount of carbohydrate with relatively high protein content.

4.6 Physicochemical Properties of the Five Edible Nuts

The characteristics of the extracted nut oils from the different nut samples are summarized in Table 15

Table 15**.** Physicochemical properties of the extracted nut oils.

Nut Sample	**Acid Value** mg KOH/g oil	**Iodine Value** g I_2 / 100 g oil	**Saponification Value** mg KOH/g oil
Macadamia	0.42 ± 0.01	78.3 ± 0.6	193.7 ±2.4
Pecan	1.07 ± 0.03	110.1 ± 1.1	185.1 ± 1.8
Brazil	1.45 ± 0.14	74.2 ± 0.3	192.4 ± 1.3
Walnut	1.35 ± 0.11	113.8 ± 2.8	190.4 ± 1.2
Almond	0.78 ± 0.03	93.6 ± 0.8	182.5 ± 2.6

Edible oils with high iodine values are usually less stable and more susceptible to oxidation. The iodine value provides a measure of the degree of oil unsaturation and is commonly used as a means of predicting shelf-life.[174] Storage capabilities are also dependent on the polyunsaturated fatty acid (PUFA) levels because PUFAs are more susceptible to oxidative degradation.[175] What this translates to is that although oils with PUFAs are good for health, they have a shorter shelf-life therefore they need to be consumed quickly. Lower iodine values were obtained for the Macadamia nut oil and Brazil nut oil. The values are 78.3 g I_2/100 g oil and 74.2 g I_2/100 g oil, respectively.

The iodine value for the Macadamia nut oil obtained in this work is similar to that obtained by Saleeb et al.[176] which was found to be 75.4 g I_2/100 g oil. The low iodine values indicate that these nuts will have a longer shelf-life than the other nuts studied in this work. This is especially true for Macadamia nut oils, which have very low levels of PUFAs, which means less oxidative degradation. Walnuts contain primarily PUFAs therefore the oil will be prone to oxidative degradation and this is confirmed by the highest iodine value obtained in this study (113.8 g I_2/100 g oil). The iodine values obtained in this study indicate that walnut and pecan nut oils would tend to become rancid much faster than Macadamia and Brazil nut oils. Since the quality of the oil affects the quality of the nuts, nuts with oils that have become rancid would reduce the quality of the nuts. These nuts would be unpalatable and no longer suitable for human consumption.

The acid value is an important indication of oil quality, with low acid values indicating good quality oil. The acceptable limit for acid value is reported to be 7 to 8 mg KOH/g oil.[192] This signifies that edible oils with acid values below this limit are suitable for human consumption. All the nut oils analysed in this study have acid values well below this limit suggesting that all 5 nuts are suitable for human consumption. The saponification value is highest for the Macadamia nut oil (193.7 mg KOH/g oil) and lowest for the almond oil (182.5 mg KOH/g oil). Macadamia nut oils possess high saponification values, a high oxidative stability plus a relatively high content of palmitoleic acid[10] which possesses physical properties similar to human sebum. This makes it a botanical alternative to mink oil (animal oil containing palmitoleic acid) and a desirable ingredient in medicinal products.

CHAPTER 5

CHEMICAL COMPOSITION OF EDIBLE MACADAMIA NUTS (MACADAMIA INTEGRIFOLIA) AND IMPACT OF SOIL QUALITY

5.1 Introduction

The results and discussion in this chapter is done to achieve the second objective of this study which is to determine the impact of soil quality parameters on the chemical characteristics of Macadamia nuts. In the previous chapter, a general comparison on the chemical characteristics of edible nuts purchased in South Africa was done. In this study Macadamia nuts were obtained from various locations in KwaZulu-Natal. The environmental conditions were recorded and soil samples were obtained from these locations. Analogous to the previous chapter, the concentrations of essential elements (excluding Se) and As were determined in the Macadamia nuts. The proximate chemical compositions of the nuts were also determined. Additionally, the total concentrations and bioavailability of the elements in conjunction with pH, SOM and CEC were determined in the soil. Statistical analysis was imperative to evaluate the impact of soil quality parameters on the chemical composition of Macadamia nuts. This analysis was undertaken to provide some diagnostic information on the chemical characteristics of nuts as a function of both soil quality and plant physiology. In this chapter, all tables contain the mean values with their standard deviations however, when reproducing these values in the discussion the standard deviations are omitted for fluency.

5.2 Elemental Distribution in Macadamia Nuts from the Different Sites

Table 16. Elemental concentrations (expressed as mean ± S.D, at 95% confidence interval, n = 3) for the chosen elements in the Macadamia nuts and corresponding soil samples.

Elements	Concentration (μg g^{-1})		
	Soil (Total)	**Soil (Bioavailable)**	**Macadamia**
UMHLANGA			
As	0.161 ± 0.004	0.005 ± 0.001	0.036 ± 0.001
Ca	3505.34 ± 22.42	2220.45 ± 2.15	3752.31 ± 74.44
Cr	49.91 ± 0.03	0.44 ± 0.003	1.59 ± 0.14
Cu	57.79 ± 0.22	14.97 ± 0.01	15.3 9 ± 0.09
Fe	25350.1 ± 96.8	184.29 ± 0.09	61.87 ± 1.26
Mg	1668.08 ± 7.10	161.54 ± 0.08	3175.48 ± 53.73
Mn	477.29 ± 3.34	304.99 ± 0.29	102.64 ± 2.41
Zn	45.94 ± 0.18	19.49 ± 0.01	30.51 ± 0.39
CHATSWORTH			
As	0.078 ± 0.002	0.003 ± 0.001	0.032 ± 0.002
Ca	8299.65 ± 87.35	4324.86 ± 4.15	1423.00 ± 27.82
Cr	60.38 ± 0.21	0.60 ± 0.001	4.40 ± 0.16
Cu	58.02 ± 0.25	5.53 ± 0.002	39.01 ± 1.08
Fe	20454.3± 105.3	233.20 ± 0.21	90.98 ± 2.35
Mg	3903.79 ± 25.01	47.53 ± 0.04	4956.14 ± 92.75
Mn	252.26 ± 1.44	166.95 ± 0.17	27.55 ± 0.83
Zn	35.62 ± 0.19	10.49 ± 0.004	70.52 ± 0.45

Elements	Concentration (μg g^{-1})		
	Soil (Total)	Soil (Bioavailable)	Macadamia
IFAFA			
As	0.104 ± 0.006	0.003 ± 0.001	0.024 ± 0.003
Ca	6173.04 ± 69.99	1378.81 ± 1.93	2168.80 ± 10.30
Cr	69.02 ± 0.8	0.87 ± 0.002	2.05 ± 0.06
Cu	49.039 ± 0.44	6.53 ± 0.003	20.27 ± 0.10
Fe	20198.7 ± 100.1	274.54 ± 0.37	91.67 ± 0.36
Mg	4655.73 ± 34.52	38.34 ± 0.04	4918.57 ± 19.87
Mn	386.917 ± 8.05	204.07 ± 0.29	54.63 ± 0.38
Zn	30.16 ± 0.74	8.87 ± 0.004	52.29 ± 0.44
HIBBERDENE			
As	0.105 ± 0.003	0.004 ± 0.001	0.032 ± 0.001
Ca	2529.46 ± 30.75	463.93 ± 0.39	1640.84 ± 22.32
Cr	6.30 ± 0.16	0.24 ± 0.001	3.07 ± 0.12
Cu	5.99 ± 0.32	2.20 ± 0.003	31.38 ± 0.25
Fe	3301.9 ± 20.5	51.25 ± 0.05	102.40 ± 1.43
Mg	817.34 ± 7.76	71.22 ± 0.09	4694.43 ± 53.63
Mn	15.31 ± 0.50	5.25 ± 0.01	169.92 ± 2.29
Zn	1.27 ± 0.29	0.75 ± 0.003	49.56 ± 0.22
PADDOCK			
As	0.192 ± 0.007	0.010 ± 0.003	0.040 ± 0.002
Ca	3342.46 ± 44.69	3318.80 ± 0.07	2672.50 ± 56.19
Cr	22.39 ± 0.33	0.57 ± 0.001	1.18 ± 0.15
Cu	61.82 ± 0.87	25.02 ± 0.01	8.33 ± 0.09
Fe	16804.2 ± 100.7	957.76 ± 0.08	85.26 ± 1.70
Mg	523.08 ± 0.89	153.38 ± 0.19	4220.92 ± 80.89
Mn	102.85 ± 0.45	67.34 ± 0.09	16.69 ± 0.76
Zn	242.66 ± 1.65	198.61 ± 0.26	46.05 ± 0.51

Elements	Concentration (μg g^{-1})		
	Soil (Total)	Soil (Bioavailable)	Macadamia
UVONGO			
As	0.266 ± 0.005	0.016 ± 0.001	0.058 ± 0.003
Ca	1842.34 ± 20.20	1443.51 ± 1.28	2648.95 ± 18.73
Cr	19.29 ± 0.09	0.61 ± 0.002	1.75 ± 0.24
Cu	22.06 ± 0.28	11.76 ± 0.002	22.02 ± 0.06
Fe	4497.5 ± 21.7	422.98 ± 0.32	62.19 ± 0.25
Mg	452.38 ± 1.73	189.65 ± 0.13	3618.82 ± 30.29
Mn	81.60 ± 0.52	80.57 ± 0.06	32.69 ± 0.58
Zn	16.80 ± 0.39	9.43 ± 0.001	33.41 ± 0.20
SOUTHBROOM			
As	0.158 ± 0.003	0.008 ± 0.001	0.025 ± 0.002
Ca	4108.07 ± 10.14	695.69 ± 0.64	2220.31 ± 37.55
Cr	37.74 ± 0.13	2.06 ± 0.0003	1.68 ± 0.07
Cu	22.07 ± 0.03	6.24 ± 0.002	16.39 ± 0.10
Fe	8870.6 ± 24.1	290.52 ± 0.29	78.01 ± 1.58
Mg	1372.93 ± 5.68	157.17 ± 0.15	4014.66 ± 57.21
Mn	47.48 ± 0.10	17.62 ± 0.02	10.21 ± 0.47
Zn	3.86 ± 0.56	3.83 ± 0.005	40.44 ± 0.39
PORT EDWARD			
As	0.156 ± 0.006	0.007 ± 0.001	0.048 ± 0.002
Ca	1655.53 ± 8.05	1428.71 ± 1.37	3081.35 ± 10.27
Cr	21.91 ± 0.45	0.99 ± 0.002	1.67 ± 0.10
Cu	24.25 ± 0.61	8.59 ± 0.004	10.35 ± 0.05
Fe	9034.1 ± 45.5	717.20 ± 0.68	81.57 ± 0.47
Mg	695.06 ± 8.33	271.28 ± 0.21	3648.60 ± 1.07
Mn	103.82 ± 1.25	55.15 ± 0.06	216.40 ± 0.39
Zn	307.34 ± 1.51	295.95 ± 0.39	54.15 ± 0.25

The elemental concentrations for the essential and toxic elements in the Macadamia nuts and corresponding soil samples are summarized in Table 16. A quick perusal of Table 16 shows that the total and especially the bioavailable soil concentrations of As at all sites are extremely low. Soil-metal interactions significantly reduce the leachability and bioaccessibility of As from soils.[144] This could explain, in part, why on average about 4.5% of the total metal content was bioavailable.

Alkaline materials like $CaCO_3$ that increase soil pH favor the oxidation of Cr(III)to Cr(VI).[177] This can cause a higher Cr mobility and uptake by vegetation.[179] This is observed at Chatsworth where the level of Ca in the soil is highest resulting in the highest concentration of Cr in the Macadamia nuts. Cr is a metal not generally considered of high environmental risk as most Cr in soils occur as Cr(III) which is generally strongly sorbed by soils and regarded as relatively inert.[145] This appeared to be the case in this study where on average about 2.2% of the total metal content was bioavailable.

The total soil concentration of Fe at all sites is extremely high. At Umhlanga the total soil concentration of Fe is found to be as high as 25350.1 $\mu g\ g^{-1}$. However, these elevated concentrations are not bioavailable since the amount of Fe found to be available to plants for uptake does not exceed 1000 $\mu g\ g^{-1}$ at any site.

Generally, the plant roots are reported to absorb Cu and Zn by a similar mechanism.[61] This causes antagonistic interferences to one, when the other is in excess in the root zone. On inspection of the concentrations of Cu and Zn in Macadamia nuts, it is apparent that Macadamia nuts have a proclivity for higher Zn uptake (Table 16). When comparing the soil concentrations of Cu and Zn to determine if any site exhibited an excess of either element in the root zone, 2 sites were identified (Table 16). These sites are Paddock and Port Edward and show high total and bioavailable soil concentrations of Zn compared to the other sites. Consequently, the Cu

concentrations are found to be lowest in the nuts at these sites, although relatively high in the soil. Specifically, at Paddock, the soil concentrations of Cu (both bioavailable and total) are highest but the concentrations of Cu in the nuts are lowest. This site clearly demonstrates the antagonistic effect of Zn on Cu. Although Macadamia nuts have a proclivity for higher Zn uptake, an interrelationship with Cu nonetheless exists. This antagonistic effect of Zn on Cu was also observed by Chaudhry et al.[180] , in rice plants where increased soil Zn concentrations markedly reduced Cu contents in rice.

The availability and uptake of Fe is a complex subject as many factors of soil and plant can influence the Fe level in the plant. The level of Fe in the soil is lowest at Hibberdene. However, the concentration of Fe in the nuts, interestingly, is highest. Fe is known to compete with Zn and Cu in their ionic forms.[76] The extent of this competition is dependent on the bioavailability of Zn and Cu, which is found to be lowest for these two elements at this particular site. The lack of interference from Zn and Cu with Fe in the soil could possibly have resulted in an increased uptake of Fe at this site.

A prominent feature on inspection of Table 16 is the lack of correlation between the concentration of Mn in the soil and Mn uptake. It is reported that the Mn content of plants is frequently more closely related to soil pH than to the concentration of Mn in the soil.[74] This study confirms the incongruity between the concentration of Mn in the soil and plant.

On closer inspection of Table 16, it is observed that Chatsworth and Hibberdene have the higher nut concentrations of most of the elements. A contributing factor to the higher uptake of elements could be the level of Ca in the soil. At Chatsworth there is an extremely high level of Ca in the soil although lowest in the nuts. In the presence of high concentrations of Ca it is likely that the plant excludes Ca and preferentially takes up more of the other available elements. The soil concentrations of most of the elements studied are lowest at Hibberdene.

When the soil concentration of an element essential for plant growth is low the plant tends to accumulate the element. This could be another reason why the concentrations of the elements in the nuts at Hibberdene are high.

At all sites, the total concentration of Ca in the soil (1655.53 μg g^{-1} to 8299.65 μg g^{-1}) is much greater than that of Mg (425.38 μg g^{-1} to 4655.73 μg g^{-1}) (Table 16). This also holds true for the bioavailable concentrations. Despite the higher Ca levels in the soil there is a lower concentration of Ca in the nuts (1423.00 μg g^{-1} to 3752.31 μg g^{-1}) showing evidence of an exclusion of this element. Despite the lower levels of Mg in the soil there appears to be relatively higher concentration of Mg in the nuts (3175.48 μg g^{-1} to 4956.14 μg g^{-1}), resulting in a higher accumulation of this element. This characteristic is evident at all the chosen sites. Macadamia nuts appear to have an affinity for higher Mg uptake.

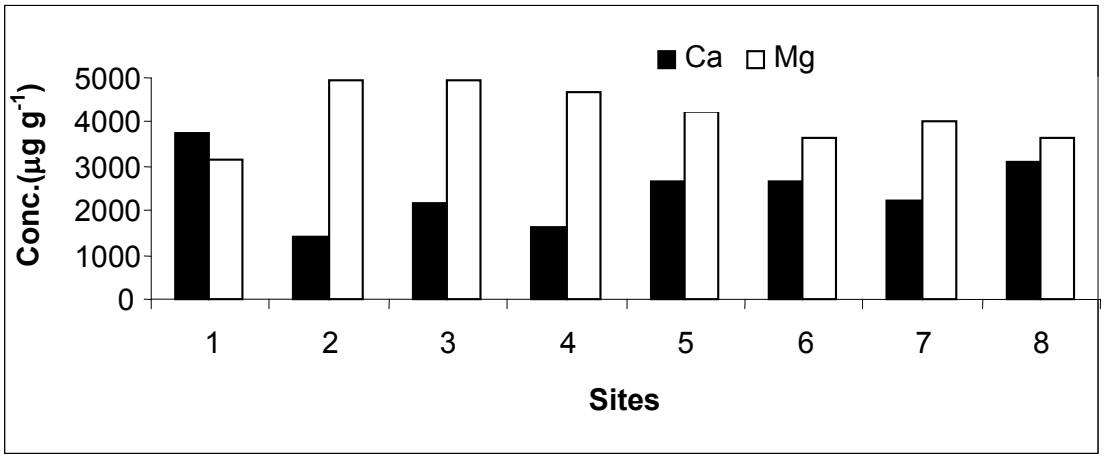


Fig. 13 Distribution of major elements in the Macadamia nut samples at the 8 different sites*.

* Sites: 1 - Umhlanga, 2 - Chatsworth, 3 - Ifafa, 4 - Hibberdene, 5 - Paddock, 6 - Uvongo, 7 - Southbroom and 8 - Port Edward.

The elemental distributions of the two major elements are illustrated in Figure 13. As the level of Mg in the nuts decreases, the level of Ca increases. This verifies that a relationship between Ca and Mg does exist in the Macadamia nuts and confirms this relationship to be antagonistic. A strong Ca and Mg antagonism was also observed by Jarvan et al. in their work on the mineral composition of vegetable leaves including leaves of lettuce, cucumber, tomatoes and paprika.[137]

The elemental distributions for the minor elements are illustrated in Figure 14. From Figure 14, it can be observed that, irrespective of the varying concentrations in the soils, the As and Cr concentrations varied in narrow ranges for the different nut samples analyzed. The As concentration is below 0.058 μg g^{-1}, whilst that of Cr is within the range 1.18 μg g^{-1} to 4.40 μg g^{-1}. Cr is reported to be one of the few elements for which no accumulation against the concentration gradient has been evident at any point in the biological cycle from soil to plant to animal.[178]

The highest concentrations for Cu and Zn are 39.01 μg g^{-1} and 70.52 μg g^{-1}, respectively and these concentrations were found in the samples obtained from the same site which is Chatsworth. The minimum and maximum limits of Fe found in the nuts are 61.87 μg g^{-1} and 102.40 μg g^{-1}, respectively.

Mn presented the greatest variability with regards to concentration in the Macadamia nuts with concentrations ranging from 10.21 to 216.40 μg g^{-1}, which were obtained at Southbroom and Port Edward, respectively. Consistent with the results found in this study, the Mn concentration in pecan nuts obtained from different sites, in another study, were also found to be immensely different from one another.[78] The reason for this is not well understood but the consensus is that plants seem to have less control on the concentrations of Mn taken up.[78]

The uptake trends in the Macadamia nuts are visibly evident from Figure 14. This trend shows the concentration of minor elements in the Macadamia nuts to be in the decreasing order of Fe > Zn > Cu > Cr > As. The exclusion of Mn from this order is due to the large array of variability of Mn in the nuts.

By simultaneously inspecting Figure 13 and 14 we observe a trend pertaining to the elemental concentrations in the Macadamia nuts. The trend shows the concentrations of the elements in the Macadamia nuts to be in the decreasing order of Mg > Ca > Fe > Zn > Cu > Cr > As.

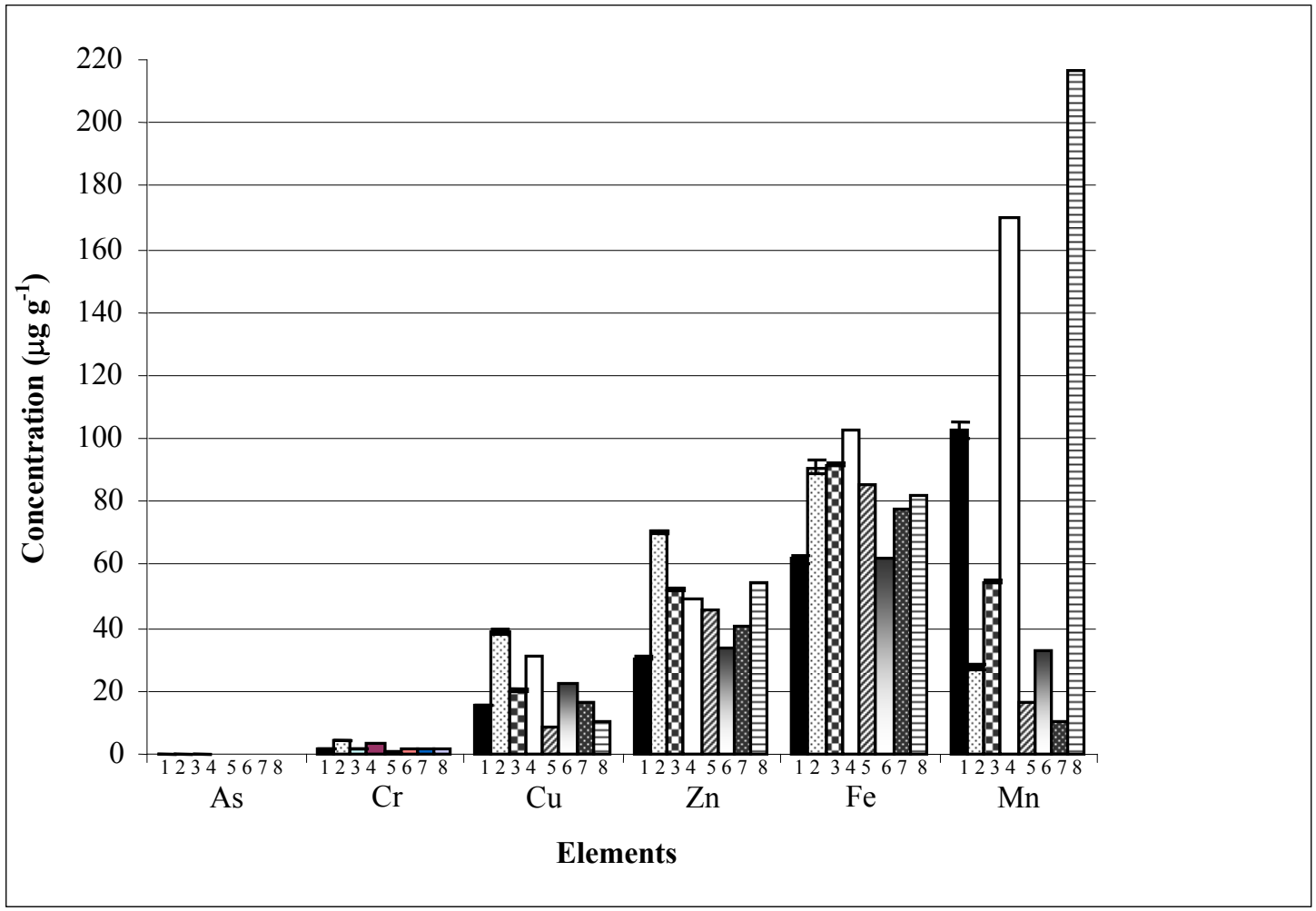


Fig.14 Distribution of minor elements in the Macadamia nut samples at the 8 different sites*.

*Sites: 1 - Umhlanga, 2 - Chatsworth, 3 - Ifafa, 4 - Hibberdene, 5 - Paddock, 6 - Uvongo, 7 - Southbroom and 8 - Port Edward.

5.3. Bioaccumulation Factors (BAFs) obtained at the Different Sites

The BAFs obtained for each element studied at the 8 different sites are plotted in Figures 15a-15h. The BAFs suggest that when the soil concentrations (total and bioavailable) of an element essential for plant growth is below the physiological requirement level, indicated by the dotted line running vertically down each figure, the plant tends to accumulate the element until the required level is reached. Conversely, at soil concentrations (total and bioavailable) that exceed the physiological requirement levels of the plant, uptake of the associated element is inhibited thereby partially excluding the element. This trend was also observed by Timperley et al.[185] who suggested that a plot of relative accumulation as a function of total soil content indicated essentiality of the element if a rectangular hyperbola was produced whereas it indicated non-essentiality if a linear plot parallel to the x-axis was obtained. The physiological requirement levels of plants, in general, are presented in Table 3 (Section 2.10). In this section a comparison of the BAFs obtained by using the total concentrations of the elements in the soil is done to determine accumulation or exclusion of the elements by the plant.

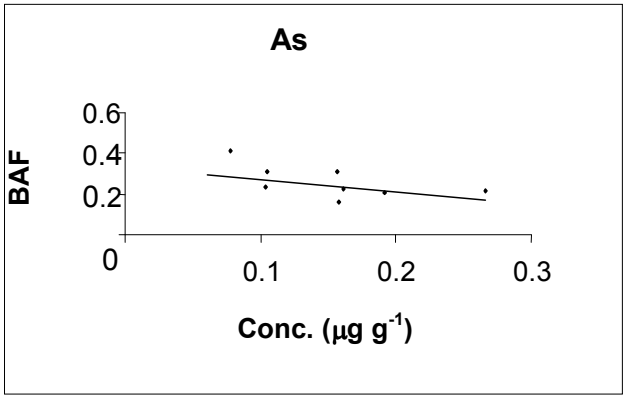


Fig.15a Bioaccumulation Factor vs. Total Concentration of As in soil.

A negative linear relationship is obtained for the plot of BAF vs. Total concentration of As in soil (Fig.15a). This shows that the uptake of As is reduced as the total concentration of As in the soil is increased. The linear relationship is vastly different from the relationships observed by the essential elements in this study thereby affirming the non-essentiality of As for plant growth. There is no accumulation of As since there is no physiological requirement level for the plant to maintain. However, the levels of As are kept at a minimum to prevent associated phytotoxities. In this study none of the sites had extremely high levels of soil As or even levels that would be considered toxic.

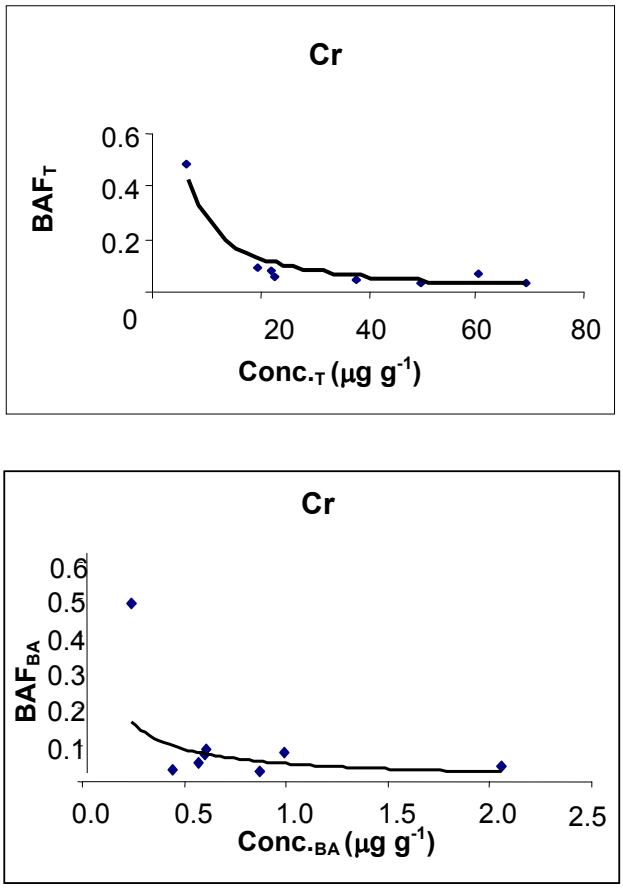


Fig.15b Bioaccumulation Factors (BAF_{BA}, BAF_T) vs. Total ($Conc_T$) and Bioavailable ($Conc_{BA}$) Concentrations of Cr in soil.

The accumulation and exclusion of essential elements, characteristic of most plants, to meet the physiological requirement levels are also observed for Cr (Fig.15b). This behavior illustrates the likelihood of this element being important to the growth of Macadamia nuts, although essentiality of Cr for plant growth, in general, is not yet established. Another observation is that the BAFs for Cr, in all cases, are low even though the trends for Cr are similar to those of the essential elements studied in this work.

The BAFs displayed by Zn and Mg are similar. Zn and Mg showed high BAFs at most sites therefore suggesting an accumulation of these elements (Table 17a and 17b).

Table 17a. Zn concentrations in nuts and soil with bioaccumulation factors (BAFs).

Sites*	Zn Concentration (μg g $^{-1}$)			BAF	
	Soil (T)	Soil (BA)	Macadamia	[plant]/[soil]$_{BA}$	[plant]/[soil]$_{T}$
1	**45.94 ± 0.18**	19.49 ± 0.01	30.51 ± 0.39	1.565	**0.664**
2	**35.62 ± 0.19**	10.49 ± 0.004	70.52 ± 0.45	6.721	**1.979**
3	**30.16 ± 0.74**	8.87 ± 0.004	52.29 ± 0.44	5.891	**1.734**
4	**1.27 ± 0.29**	0.75 ± 0.003	49.56 ± 0.22	65.515	**38.774**
5	**242.66 ± 1.65**	198.61 ± 0.26	46.05 ± 0.51	0.232	**0.190**
6	**16.80 ± 0.39**	9.43 ± 0.001	33.41 ± 0.20	3.543	**1.988**
7	**3.86 ± 0.56**	3.83 ± 0.005	40.44 ± 0.39	10.546	**10.462**
8	**307.34 ± 1.51**	295.95 ± 0.39	54.15 ± 0.25	0.183	**0.176**

* Sites: 1 - Umhlanga, 2 - Chatsworth, 3 - Ifafa, 4 - Hibberdene, 5 - Paddock, 6 - Uvongo, 7 - Southbroom and 8 - Port Edward. BA- Bioavailable. T- Total.

The typical concentration of Zn required for plant growth, in general, is 20-50 μg g $^{-1}$.[59] Sites 2, 3, 4, 6 and 7 had total soil Zn concentrations ranging between 1.27 μg g $^{-1}$ and 35.62 μg g $^{-1}$. These values are below the physiological requirement level of the plant thereby explaining the high BAFs. Sites 5 and 8 are the only 2 sites with high total soil concentrations of Zn. Although the amounts of Zn that are bioavailable at these sites are also high, it is evident from the concentrations of Zn in the nuts obtained from these sites, that the plant partially excluded Zn. This further corroborates the assertion that the plant either adopts a mechanism of exclusion or accumulation to meet its physiological requirement levels.

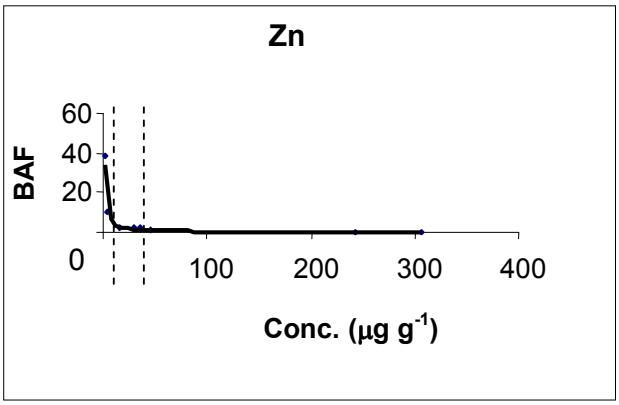


Fig.15c Bioaccumulation Factor vs. Total Concentration of Zn in soil.

Table 17b. Mg concentrations in nuts and soil with bioaccumulation factors (BAFs).

Sites*	Mg Concentration (μg g $^{-1}$)			BAF	
	Soil (T)	Soil (BA)	Macadamia	[plant]/[soil]$_{BA}$	[plant]/[soil]$_{T}$
1	**1668.08 ± 7.10**	161.54 ± 0.08	3175.48 ± 53.73	19.66	**1.90**
2	**3903.79 ± 25.01**	47.53 ± 0.04	4956.14 ± 92.75	104.270	**1.270**
3	**4655.73 ± 34.52**	38.34 ± 0.04	4918.57 ± 19.87	128.259	**1.056**
4	**817.34 ± 7.76**	71.22 ± 0.09	4694.43 ± 53.63	65.914	**5.744**
5	**523.08 ± 0.89**	153.38 ± 0.19	4220.92 ± 80.89	27.519	**8.069**
6	**452.38 ± 1.73**	189.65 ± 0.13	3618.82 ± 30.29	19.081	**7.999**
7	**1372.93 ± 5.68**	157.17 ± 0.15	4014.66 ± 57.21	25.542	**2.924**
8	**695.06 ± 8.33**	271.28 ± 0.21	3648.60 ± 1.07	13.449	**5.249**

* Sites: 1 - Umhlanga, 2 - Chatsworth, 3 - Ifafa, 4 - Hibberdene, 5 - Paddock, 6 - Uvongo, 7 - Southbroom and 8 - Port Edward. BA- Bioavailable. T- Total.

The typical concentration of Mg required for plant growth, in general, is 2000 μg g $^{-1}$.[59] Six of the 8 sites had total soil Mg concentrations less than 2000 μg g $^{-1}$ (Fig.15d and Table 17b).

These six sites had BAFs ranging from 1.90 to 8.069. A surprising observation was that even when soil concentrations were as high as 4655 μg g $^{-1}$, the plant tended to accumulated Mg. Two possible explanations for this tendency are that the physiological requirement level for Mg in Macadamia nuts is not the same as the general level but perhaps higher (somewhere between 4000 to 5000 μg g $^{-1}$) or that the plant has an overall proclivity for Mg accumulation hence making it a good dietary source of Mg.

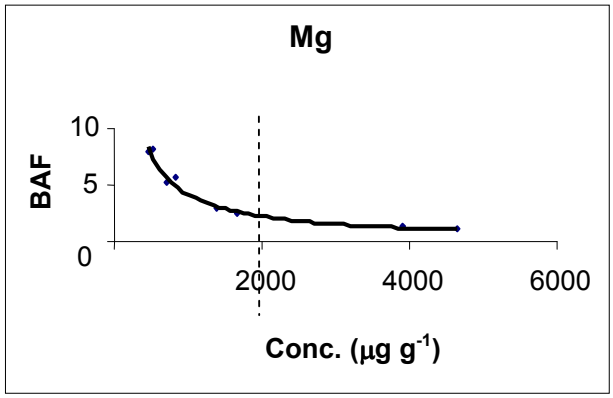


Fig.15d Bioaccumulation Factor vs. Total Concentration of Mg in soil.

The BAFs displayed by Zn and Mg are somewhat different from those of Ca, Cu, Fe and Mn. Unlike Zn and Mg that were accumulated at most sites, Ca, Cu, Fe and Mn were excluded at most sites.

Table 17c. Ca concentrations in nuts and soil with bioaccumulation factors (BAFs).

Sites*	Ca Concentration (μg g $^{-1}$)			BAF	
	Soil (T)	Soil (BA)	Macadamia	$[plant]/[soil]_{BA}$	$[plant]/[soil]_{T}$
1	**3505.34 ± 22.42**	2220.45 ± 2.15	3752.31 ± 74.44	1.690	**1.070**
2	**8299.65 ± 87.35**	4324.86 ± 4.15	1423.00 ± 27.82	0.329	**0.171**
3	**6173.04 ± 69.99**	1378.81 ± 1.93	2168.80 ± 10.30	1.573	**0.351**
4	**2529.46 ± 30.75**	463.93 ± 0.39	1640.84 ± 22.32	3.537	**0.649**
5	**3342.46 ± 44.69**	3318.80 ± 0.07	2672.50 ± 56.19	0.805	**0.800**
6	**1842.34 ± 20.20**	1443.51 ± 1.28	2648.95 ± 18.73	1.835	**1.438**
7	**4108.07 ± 10.14**	695.69 ± 0.64	2220.31 ± 37.55	3.192	**0.540**
8	**1655.53 ± 8.05**	1428.71 ± 1.37	3081.35 ± 10.27	2.157	**1.861**

* Sites: 1 - Umhlanga, 2 - Chatsworth, 3 - Ifafa, 4 - Hibberdene, 5 - Paddock, 6 - Uvongo, 7 - Southbroom and 8 - Port Edward. BA- Bioavailable. T- Total.

In the instance of Ca, the typical concentration required for plant growth, in general, is 5000 μg g $^{-1}$.[59] It would appear, from Fig.15e, that the physiological requirement level in Macadamia nuts is around 3000 μg g $^{-1}$. When the total soil concentration of Ca falls below this level, as demonstrated by sites 6 and 8 in this study, the plant tends to accumulate Ca and when the total soil concentration of Ca exceeds this value, as demonstrated by most of the other sites in this study, the plant tends to exclude it. The total soil concentration of Ca at site 4 is lower than that at site 1 but there is a higher accumulation of Ca at site 1 with a BAF of 1.070. At site 4 it is also noticed that most of the soil concentrations of the other elements are low compared to the other sites. Since there is a lack of competition in the soil it would appear that the bioavailability and uptake of Ca was reduced. At site1 most of the soil concentrations of the

other elements are relatively high compared to the other sites. At this site the competition in the soil was greater and it appears as though Ca has out competed the other elements for uptake resulting in a higher concentration of Ca and lower concentrations of the other elements in the Macadamia nuts.

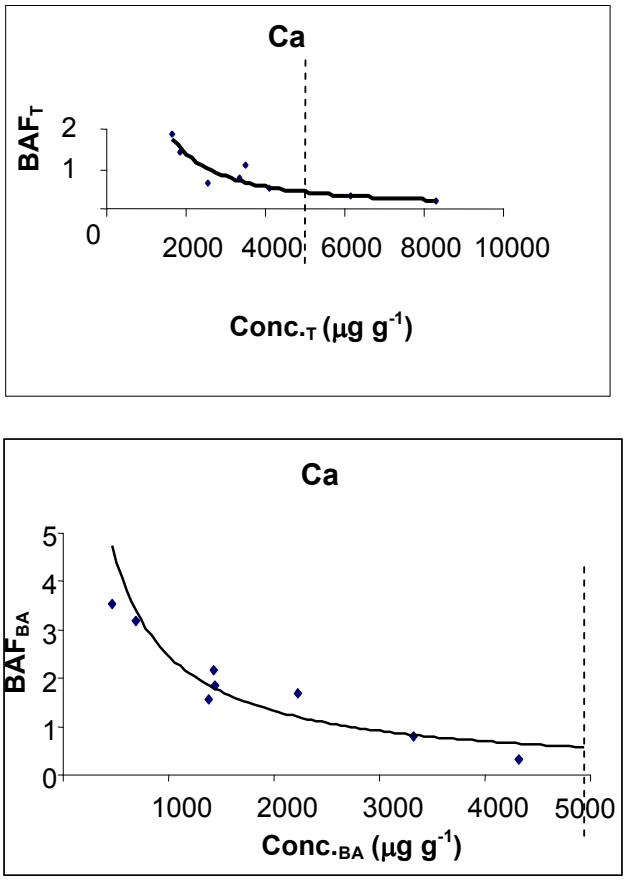


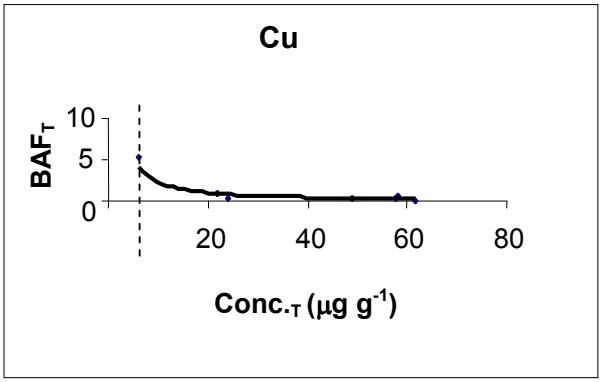
Fig.15e Bioaccumulation Factors (BAF_{BA}, BAF_T) vs. Total ($Conc_T$) and Bioavailable ($Conc_{BA}$) Concentrations of Ca in soil.

Table 17d. Cu concentrations in nuts and soil with bioaccumulation factors (BAFs).

Sites*	Cu Concentration (μg g $^{-1}$)			BAF	
	Soil (T)	Soil (BA)	Macadamia	[plant]/[soil]$_{BA}$	[plant]/[soil]$_{T}$
1	**57.79 ± 0.22**	14.97 ± 0.01	15.3 9 ± 0.09	1.027	**0.266**
2	**58.02 ± 0.25**	5.53 ± 0.002	39.01 ± 1.08	7.050	**0.670**
3	**49.039 ± 0.44**	6.53 ± 0.003	20.27 ± 0.10	3.101	**0.413**
4	**5.99 ± 0.32**	2.20 ± 0.003	31.38 ± 0.25	14.201	**5.238**
5	**61.82 ± 0.87**	25.02 ± 0.01	8.33 ± 0.09	0.333	**0.135**
6	**22.06 ± 0.28**	11.76 ± 0.002	22.02 ± 0.06	1.872	**0.998**
7	**22.07 ± 0.03**	6.24 ± 0.002	16.39 ± 0.10	2.623	**0.743**
8	**24.25 ± 0.61**	8.59 ± 0.004	10.35 ± 0.05	1.204	**0.427**

* Sites: 1 - Umhlanga, 2 - Chatsworth, 3 - Ifafa, 4 - Hibberdene, 5 - Paddock, 6 - Uvongo, 7 - Southbroom and 8 - Port Edward. BA- Bioavailable. T- Total.

The typical concentration of Cu required for plant growth, in general, is 6 μg g $^{-1}$.[59] On inspection of Fig.15f and Table 17d it is observed that site 4 has a high BAF (5.238). Further investigation into this deviation confirms that the total soil concentration of this element at site 4 is slightly below the physiological requirement level of the plant and much lower than the total soil concentrations at the other sites. The BAF of Cu at Site 6 is almost equal to 1. This could mean that the physiological requirement level of the plant could possibly be closer to 20 than 6 μg g $^{-1}$. Figure 15f further illustrates the likelihood of the physiological requirement level of the plant being closer to 20 μg g $^{-1}$.



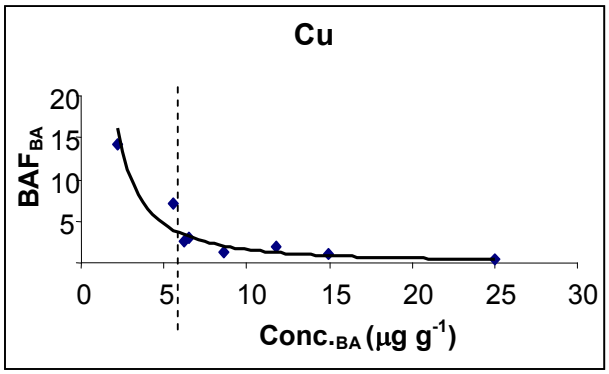


Fig.15f Bioaccumulation Factors (BAF_{BA}, BAF_T) vs. Total ($Conc_T$) and Bioavailable ($Conc_{BA}$) Concentrations of Cu in soil.

Table 17e. Fe concentrations in nuts and soil with bioaccumulation factors (BAFs).

Sites*	Fe Concentration (μg g $^{-1}$)			BAF	
	Soil (T)	Soil (BA)	Macadamia	$[plant]/[soil]_{BA}$	$[plant]/[soil]_T$
1	25350.1 ± 96.8	184.29 ± 0.09	61.87 ± 1.26	0.336	0.002
2	20454.3± 105.3	233.20 ± 0.21	90.98 ± 2.35	0.390	0.004
3	20198.7 ± 100.1	274.54 ± 0.37	91.67 ± 0.36	0.334	0.005
4	3301.9 ± 20.5	51.25 ± 0.05	102.40 ± 1.43	1.998	0.031
5	16804.2 ± 100.7	957.76 ± 0.08	85.26 ± 1.70	0.089	0.005
6	4497.5 ± 21.7	422.98 ± 0.32	62.19 ± 0.25	0.147	0.014
7	8870.6 ± 24.1	290.52 ± 0.29	78.01 ± 1.58	0.268	0.009
8	9034.1 ± 45.5	717.20 ± 0.68	81.57 ± 0.47	0.114	0.009

The typical concentration of Fe required for plant growth, in general, is 100 μg g^{-1}.[59] All of the chosen sites had extremely high total soil Fe concentrations (Table 17e, Fig.15g). Since the plants physiological requirement levels were well exceeded, the plant adopted the mechanism for exclusion thereby exhibiting low BAFs. Site 1 had the highest total soil concentration of Fe therefore the lowest bioaccumulation factor was obtained at this site (0.002). Site 4 had the lowest total soil concentration of Fe therefore the highest BAF was obtained at this site (0.031).

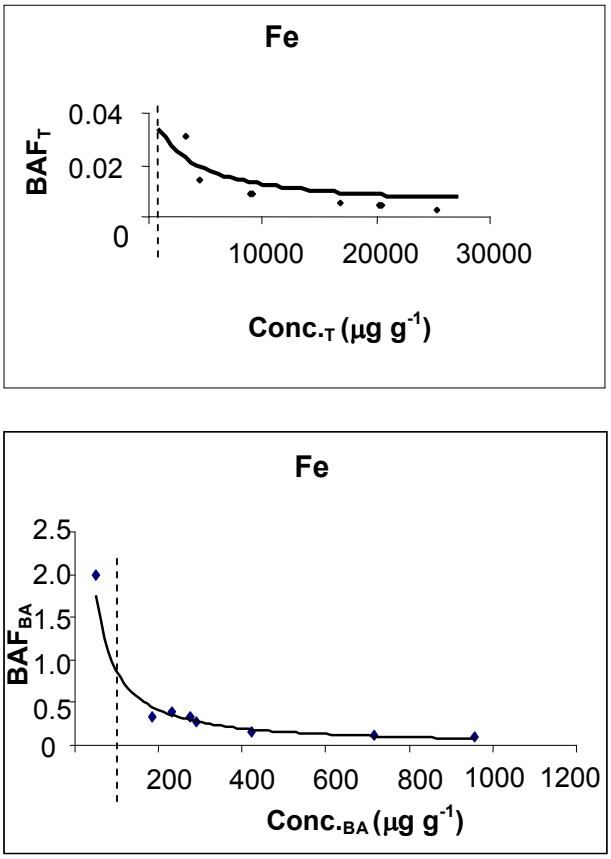


Fig.15g Bioaccumulation Factors (BAF_{BA}, BAF_T) vs. Total ($Conc_T$) and Bioavailable ($Conc_{BA}$) Concentrations of Fe in soil.

Table 17f. Mn concentrations in nuts and soil with bioaccumulation factors (BAFs).

Sites*	Mn Concentration (μg g $^{-1}$)			BAF	
	Soil (T)	Soil (BA)	Macadamia	[plant]/[soil]$_{BA}$	[plant]/[soil]$_{T}$
1	477.29 ± 3.34	304.99 ± 0.29	102.64 ± 2.41	7.200	0.215
2	252.26 ± 1.44	166.95 ± 0.17	27.55 ± 0.83	0.165	0.109
3	386.917 ± 8.05	204.07 ± 0.29	54.63 ± 0.38	0.268	0.141
4	15.31 ± 0.50	5.25 ± 0.01	169.92 ± 2.29	32.312	11.091
5	102.85 ± 0.45	67.34 ± 0.09	16.69 ± 0.76	0.248	0.162
6	81.60 ± 0.52	80.57 ± 0.06	32.69 ± 0.58	0.406	0.401
7	47.48 ± 0.10	17.62 ± 0.02	10.21 ± 0.47	0.579	0.215
8	103.82 ± 1.25	55.15 ± 0.06	216.40 ± 0.39	3.923	2.084

*Sites: 1 - Umhlanga, 2 - Chatsworth, 3 - Ifafa, 4 - Hibberdene, 5 - Paddock, 6 - Uvongo, 7 - Southbroom and 8 - Port Edward. BA- Bioavailable. T- Total.

The typical concentration of Mn required for plant growth, in general, is 50 μg g $^{-1}$.[59] Site 4 and site 8 are the only sites that display evidence of Mn accumulation (Table 17f, Fig 15h). Not surprisingly, the total soil Mn concentration at site 4 is less than the physiological requirement level of the plant. However, this does not hold true for site 8. The total soil concentrations of Mn at site 5 and site 8 are similar. The soil concentrations at both these sites are above the physiological requirement level of the plant. However, the plant tended to accumulate Mn at site 8 and not at site 5. The total soil concentrations of the other elements at both these sites are also similar, except for Fe (Table 16). It is reported that Fe interferes with Mn uptake.[76] At site 5 the concentration of Fe is found to be 16804.2 μg g $^{-1}$ and at site 8 it is found to be 9034.1 μg g $^{-1}$. The reduced interference of Fe on the mobility and uptake of Mn at site 8 as opposed to site 5 could possibly have led to an elevated concentration of Mn in the nuts at this

site. Another contributing factor to the elevated uptake of Mn at site 8, could be the lack of interference from Ca in the soil since this site has the lowest concentration of Ca in the soil. At site 7, the concentration of Mn in the soil is slightly below the physiological requirement level of the plant. However, the BAF at this site is low and the concentrations in the nuts are the lowest. This site undoubtedly displays an anomaly that could be a consequence of anything from the intrinsic properties of the plant to the extrinsic factors contributing to its growth. It is also important to consider the fact that the accumulation and exclusion theory does not apply to all cases (metals, soils and plants) and site 7, for Mn levels, may be one of these exceptions.

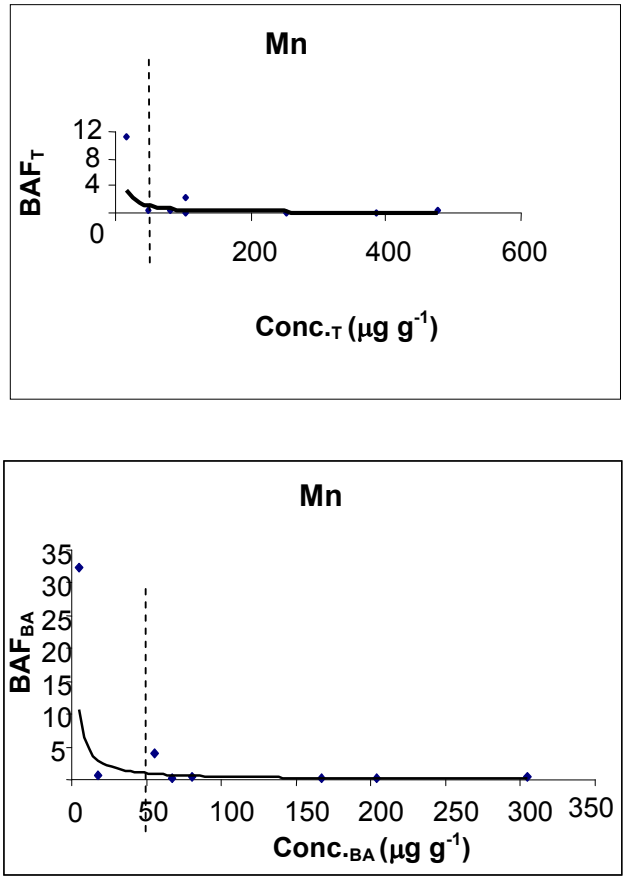


Fig.15h Bioaccumulation Factors (BAF_{BA}, BAF_T) vs. Total ($Conc_T$) and Bioavailable ($Conc_{BA}$) Concentrations of Mn in soil.

5.4. Proximate Chemical Composition of Macadamia Nuts

The proximate chemical composition of the Macadamia nuts analysed from the 8 different sites are presented in Table 18 and a graphical representation of this data is provided by Figure 16.

Table 18. Proximate chemical composition (g per100 g dry mass) of the nut samples analyzed.

Sites*	**Oil**	**Ash**	**Protein**	**Carbohydrate****
1	78.1 ± 1.3	3.0 ± 0.1	12.0 ± 0.1	6.9
2	75.5 ± 1.8	3.8 ± 0.1	6.2 ±0.3	14.5
3	76.1 ± 1.1	4.0 ± 0.1	7.8 ± 0.2	12.1
4	73.6 ± 0.4	4.2 ±0.1	8.3 ± 0.2	13.9
5	75.2 ± 2.2	3.9 ± 0.1	8.6 ± 0.2	12.3
6	78.4 ± 1.9	2.8 ± 0.1	12.5 ± 0.1	6.3
7	74.3 ± 2.6	4.0 ± 0.1	6.6 ± 0.3	15.1
8	76.5 ± 2.4	3.6 ± 0.1	4.9 ± 0.3	15.0
Mean ± SD	76.0 ± 1.7	3.7 ± 0.1	8.4 ± 0.2	12.0
Sigma (2-tailed)	NS***	NS	NS	NS

* Sites: 1 - Umhlanga, 2 - Chatsworth, 3 - Ifafa, 4 - Hibberdene, 5 - Paddock, 6 - Uvongo, 7 - Southbroom and 8 - Port Edward**.**

**Carbohydrate obtained by subtracting the sum of oil, ash & protein from the total dry mass.

*** NS - No statistically significant differences in samples (One-sample T-Test, P≤ 0.05)

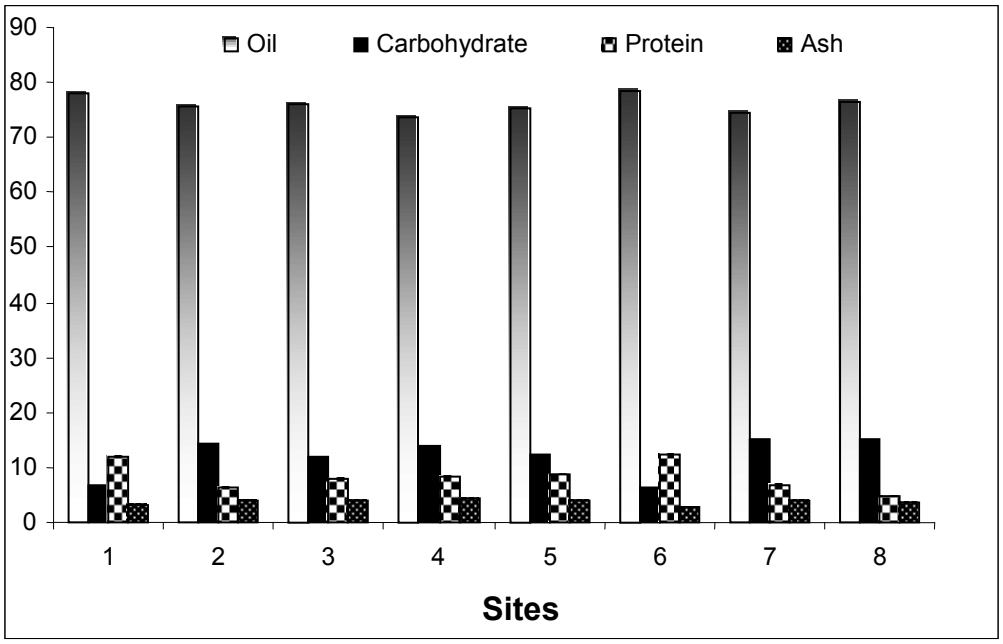


Fig.16 Percentage ash, protein, carbohydrate and oil in the Macadamia nuts.

* Sites: 1 - Umhlanga, 2 - Chatsworth, 3 - Ifafa, 4 - Hibberdene, 5 - Paddock, 6 - Uvongo, 7 - Southbroom and 8 - Port Edward.

It can be seen that the Macadamia nuts are rich in oils with the oil content of the Macadamia nuts ranging from 73.6 to 78.4 g/100g DM. In the previous chapter, a high content of oil was also observed in the Macadamia nuts and the value obtained previously (76.0 g/100g DM) is similar to the average value obtained here (76.0 g/100g DM). The average values for the content of ash, protein and carbohydrate were found to be 3.7 g/100g DM, 8.4 g/100g DM and 12.0 g/100g DM, respectively. According to the One-sample T-Test there were no statistically significant differences in the percentage oil, ash, protein and carbohydrate of Macadamia nuts from the different sites chosen.

5.5 CEC, SOM and pH of Soil Samples from the Different Sites

The soil properties CEC, SOM and pH of the soil samples are presented in Table 19.

Table 19. Cation exchange capacity (CEC), soil organic matter (SOM) and pH of soil samples obtained from the 8 different sites.

Sites*	**CEC** meq./100g	**SOM** %	**pH**
1	10.36 ± 0.04	5.95 ± 0.05	6.76 ± 0.03
2	8.20 ± 0.03	4.73 ± 0.10	6.25 ± 0.03
3	8.99 ± 0.03	4.49 ± 0.04	4.95 ± 0.02
4	12.99 ± 0.05	6.37 ± 0.04	6.81 ± 0.04
5	3.70 ± 0.01	1.25 ± 0.10	5.30 ± 0.03
6	6.79 ± 0.03	3.75 ± 0.05	6.16 ± 0.02
7	7.17 ± 0.02	3.62 ± 0.05	5.10 ± 0.02
8	6.52 ± 0.04	3.45 ± 0.10	5.46 ± 0.03

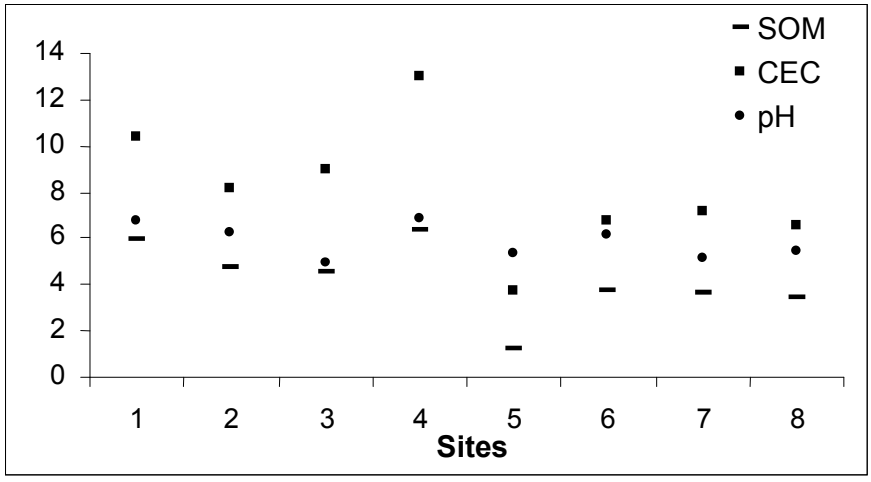


Fig.17 Comparison of SOM (%), CEC (meq/100g) and pH in soil.

*Sites: 1 - Umhlanga, 2 - Chatsworth, 3 - Ifafa, 4 - Hibberdene, 5 - Paddock, 6 - Uvongo, 7 - Southbroom and 8 - Port Edward.

The pH of the soils ranged between 4.95 and 6.81. From the soil pH it is quite evident that Macadamia nuts grow in slightly acidic soils with the ideal level being 5.5.[183] The CEC of the soil ranged between 3.70 and 12.99 meq/100g and the SOM ranged between 1.25 and 6.37% with site 5 having the lowest CEC and SOM values and site 4 having the highest values. According to the norms for soil analysis, the ideal level of organic carbon for optimal growth of Macadamia trees is 4.0%.[183] 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.[32] From Table 19 we see that sites 1 and 4 have pH values greater than 6.5 and not surprisingly these 2 sites have the highest CECs. A perusal of Figure 17 confirms a positive relationship between the CEC of the soil and the SOM. In most cases, as the CEC value increased so too did the SOM, although not to the extent of the CEC value. An increase in SOM would increase soils CEC since mineral cations adsorb to the negative surface charges of organic soil particles. The results obtained for the SOM and CEC at sites 4 and 5 further accentuates the positive relationship between these 2 soil parameters. Although not clearly distinguishable by Figure 17, there also appears to be a relationship between the pH and CEC as well as pH and SOM. Correlation analysis will be done on this data to obtain more clarity on this relationship.

5.6 Statistical Analysis of Data

Table 20. Inter-item correlation matrix for the elemental concentrations in the Macadamia nuts and soil (Total and Bioavailable). (P≤ 0.05)

	AsN	AsB	AsT	CaN	CaB	CaT	CrN	CrB	CrT	CuN	CuB	CuT	FeN	FeB	FeT	MgN	MgB	MgT	MnN	MnB	MnT	ZnN	ZnB	ZnT	SOM	CEC	pH
AsB	0.52	1.00																									
AsT	0.68	0.96	1.00																								
CaN	0.75	0.81	0.91	1.00																							
CaB	0.10	0.06	-0.01	-0.08	1.00																						
CaT	-0.58	-0.34	-0.49	-0.54	0.58	1.00																					
CrN	-0.37	-0.58	-0.72	**-0.76**	0.37	0.65	1.00																				
CrB	-0.41	0.02	-0.02	-0.03	-0.33	0.06	-0.28	1.00																			
CrT	-0.32	-0.05	-0.13	-0.07	0.40	0.83	0.26	0.17	1.00																		
CuN	-0.32	-0.60	-0.69	**-0.77**	0.20	0.57	**0.94**	-0.31	0.20	1.00																	
CuB	0.46	0.60	0.68	0.58	0.44	-0.25	-0.61	-0.19	-0.13	-0.65	1.00																
CuT	0.06	0.33	0.27	0.22	0.82	0.58	0.00	-0.21	0.66	-0.12	0.58	1.00															
FeN	-0.77	-0.61	-0.75	**-0.76**	0.00	0.36	**0.54**	-0.13	-0.03	**0.42**	-0.42	-0.14	1.00														
FeB	0.24	0.17	0.27	0.35	0.31	-0.32	-0.54	0.07	-0.29	-0.70	0.74	0.28	-0.13	1.00													
FeT	0.00	0.39	0.29	0.27	0.64	0.62	0.06	-0.16	0.80	-0.06	0.34	0.92	-0.13	-0.02	1.00												
MgN	-0.82	-0.75	-0.87	**-0.89**	0.20	0.67	**0.66**	-0.10	0.29	**0.62**	-0.42	0.07	**0.87**	-0.24	0.04	1.00											
MgB	0.65	0.45	0.58	0.71	-0.21	-0.75	-0.64	0.24	-0.50	-0.70	0.36	-0.24	-0.60	0.57	-0.31	-0.83	1.00										
MgT	-0.54	-0.40	-0.49	-0.41	0.32	0.89	0.51	0.02	0.91	0.46	-0.38	0.48	0.33	-0.41	0.61	0.63	-0.73	1.00									
MnN	0.24	0.02	0.02	0.24	-0.40	-0.50	0.01	-0.29	-0.41	-0.09	-0.32	-0.46	0.21	-0.06	-0.28	-0.21	0.35	-0.29	1.00								
MnB	0.28	0.38	0.36	0.41	0.41	0.44	0.04	-0.31	0.75	0.04	0.18	0.71	-0.37	-0.27	0.87	-0.16	-0.25	0.56	-0.13	1.00							
MnT	0.15	0.33	0.30	0.37	0.34	0.47	0.03	-0.24	0.80	0.01	0.12	0.70	-0.26	-0.28	0.89	-0.08	-0.30	0.64	-0.09	0.98	1.00						
ZnN	-0.55	-0.66	-0.78	**-0.68**	0.46	0.64	**0.75**	-0.08	0.29	**0.53**	-0.39	0.17	**0.74**	0.01	0.13	**0.76**	-0.44	0.54	0.09	-0.11	-0.05	1.00					
ZnB	0.26	0.16	0.21	0.40	0.15	-0.42	-0.40	0.01	-0.35	-0.64	0.44	0.08	0.02	0.84	-0.07	-0.28	0.67	-0.41	0.45	-0.26	-0.24	0.17	1.00				
ZnT	0.26	0.20	0.24	0.42	0.24	-0.36	-0.40	-0.03	-0.29	-0.64	0.52	0.19	0.02	0.87	0.03	-0.26	0.63	-0.36	0.39	-0.19	-0.17	0.18	0.99	1.00			
SOM	0.00	-0.04	-0.13	-0.14	-0.29	0.14	0.49	-0.32	0.16	0.60	**-0.66**	-0.27	0.12	**-0.91**	0.05	0.08	-0.40	0.28	0.40	0.35	0.35	-0.01	**-0.61**	-0.63	1.00		
CEC	-0.13	-0.10	-0.20	-0.23	-0.40	0.08	0.45	-0.34	0.05	0.57	**-0.66**	-0.35	0.29	**-0.89**	-0.05	0.20	-0.47	0.23	0.43	0.21	0.23	0.00	**-0.59**	-0.62	**0.97**	1.00	
pH	0.47	0.19	0.15	0.03	0.08	-0.13	0.42	-0.67	-0.26	0.51	-0.16	-0.14	-0.12	-0.53	-0.04	-0.19	-0.12	-0.21	0.32	0.22	0.11	-0.14	-0.36	-0.36	**0.70**	**0.65**	1.00

AsN - $[As]_{Nut}$
AsB - $[Soil\ As]_{Bioavailable}$
AsT - $[Soil\ As]_{Total}$
SOM - Soil Organic Matter
CEC - Cation Exchange Capacity

A correlation matrix for the concentrations of elements in the nuts and soil (total and bioavailable) are presented in Table 20. Statistically, no significant positive correlation could be established between the bioavailable concentrations of elements in soil and their associated nut concentrations. For the total soil concentrations of elements and their related nut concentrations, a moderate correlation was found for Mg only ($r = 0.63$).

Antagonism occurs when the plant takes up two different elements by the same mechanism. An increase in the total soil concentration of one of the elements would reduce the uptake of the other element. The negative correlations obtained for Mn in the nut with the total soil concentrations of Ca ($r = -0.5$), Cr ($r = -0.4$), Cu ($r = -0.46$) and, to a lesser extent, Fe ($r = -0.28$) is evidence of an antagonistic relationship between Mn and these 4 elements. This reveals that as total soil Ca, Cr, Cu and Fe increases, the uptake of Mn is reduced. A stronger negative correlation is obtained between Cu in the nut and total soil Zn ($r = -0.64$). An assertion was made earlier (after close scrutiny of the concentration data) on the presence of antagonism between Zn and Cu and this negative correlation reinforces this assertion.

On examination of the BAFs in conjunction with the correlation coefficients (r) for the total soil and nut concentrations of essential elements, it was observed that a negative correlation indicated exclusion of the element by the plant. On inspection of the soil concentrations of the excluded essential elements, it was noticed that most of the soil concentrations exceeded the physiological requirement levels of the plant. The essential elements that fall into this category are Ca, Fe, Cu and Mn, as observed by Figures 15e-h. Conversely, a positive correlation between total soil and nut concentrations of essential elements indicated an accumulation of these elements by the plant as most of the soil samples were deficient in these elements and did not meet the plant's physiological requirement levels. The elements exhibiting this behaviour are Zn and Mg as observed by Figures 15c and 15d.

Table 21. Intercorrelation between the total (T) soil concentrations of the elements Ca, Cr, Cu, Fe, Mg and Mn extracted from Table 20.

	CaT	**CrT**	**CuT**	**FeT**	**MgT**
CrT	0.83				
CuT	0.58	0.66			
FeT	0.62	0.80	0.92		
MgT	0.89	0.91	0.48	0.61	
MnT	0.47	0.80	0.70	0.89	0.64

There is a statistically significant positive intercorrelation between the total soil concentrations of the elements Cr, Cu, Fe, Mg and Mn (Fig. 18). This means that the total soil concentrations of each element are positively related to the total soil concentrations of the other 4 elements eg. Mn in the soil is positively correlated to Cr, Cu, Fe and Mg in the soil. At the same time, there is a significant positive correlation between the total soil concentrations of Ca with the total soil concentrations of these 5 elements (Table 21). Since the total soil concentrations of these 5 elements are closely related, although from different sites, according to Lombnaes et al., this indicates that these elements are linked to a related geological parent material.[181] It is also known that soils obtain their structure and minerals from their parent material and since these soils were obtained from the same region , it is likely that the parent material is the same.

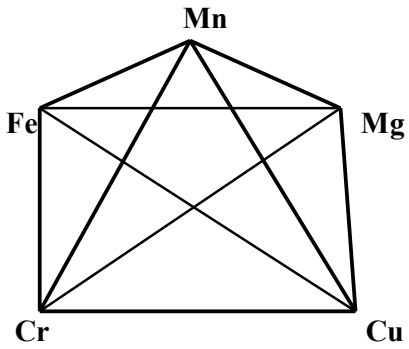


Fig. 18 Diagram showing the intercorrelations between Cr, Cu, Fe, Mg and Mn in the soil.

In an earlier work by Lombnaes et al.,[181] an intercorrelation between Cu, Mn and Zn was found in the soil. However, no corresponding relationship for these nutrients was found in the cereal crops studied, thus illustrating the limited value of total soil micronutrients in determining phytoavailability in cereals. In this study a positive intercorrelation between Cr, Cu, Fe, Mg and Mn was found in the soil and a corresponding positive intercorrelation for these nutrients was found in the nuts, except that Mn in the soil was replaced by Zn in the nuts (Fig.19). This means that nut concentrations of each element are positively related to the nut concentrations of the other 4 elements eg. Zn in the nuts is positively correlated to Cr, Cu, Fe and Mg in the nuts. Unlike the work done by Lombnaes et al. on cereal crops, this study illustrates the usefulness of total soil concentrations of micronutrients in determining phytoavailability in Macadamia nuts.

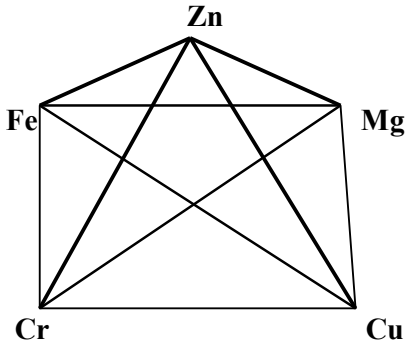


Fig. 19 Diagram showing the intercorrelations between Cr, Cu, Fe, Mg and Zn in the nuts.

Table 22. Intercorrelation between the total nut (N) concentrations of the elements Ca, Cr, Cu, Fe, Mg and Zn extracted from Table 20.

	CaN	**CrN**	**CuN**	**FeN**	**MgN**
CrN	-0.76				
CuN	-0.77	0.94			
FeN	-0.76	0.54	0.42		
MgN	-0.89	0.66	0.62	0.87	
ZnN	-0.68	0.75	0.53	0.74	0.76

Interestingly, there appears to be a statistically significant negative correlation between the concentration of Ca in the nuts with the concentration of Cr, Cu, Fe, Mg and Zn in the nuts. The negative relationship between Ca and Mg in Macadamia nuts was already observed in Fig.13 which is reaffirmed by the negative correlation obtained in Table 20 (r = -0.89). Since Mg correlates positively with Cr, Cu, Fe and Zn, it stands to reason that these elements would correlate negatively to Ca. The fact that Mn was replaced by Zn indicates that Zn needs to correlate more than Mn with the essential elements in the nuts, for nutrient balance. This could be so, since Zn is essential for the kernel formation of the nuts. The Mn levels in the nuts, as observed by Fig. 14, are also not controlled in the nuts. The synergy between Cr, Cu, Fe, Mg and Zn might be vital to optimize the formation of the nuts hence the concentrations of these elements are controlled and their proportions are maintained in the nuts.

Table 23. Significant intercorrelations between the total (T) and bioavailable (B) soil concentrations extracted from Table 20.

	MnB	FeB	ZnB	CaB	MgB	CuB	SOM	pH
MnT	**0.98**							
CrT	**0.75**							
FeT	**0.87**			**0.64**				
CuT	**0.71**			**0.82**				
MgT	**0.56**							
ZnT		**0.87**	**0.99**		**0.63**	**0.52**		
SOM		**-0.91**	**-0.61**			**-0.66**		**0.70**
CEC		**-0.89**	**-0.59**			**-0.66**	**0.97**	**0.65**

McBride et al.[85] showed that soil pH and total soil Zn were highly significant contributors to Zn availability while organic matter was not. Zhao et al.[86] found that organic matter in addition to soil pH and total soil Zn were significant predictors of Zn solubility. In this study, we see that total soil Zn is more important than any other single property in predicting Zn availability ($r = 0.99$). It is reported that Mn availability is more closely related to soil pH than to the concentration of Mn in the soil.[74] In this study, we find Mn availability to be more closely related to Mn in the soil ($r = 0.98$) than to pH ($r = 0.22$). The uptake of Mn is also more affected by CEC ($r = 0.43$) and SOM ($r = 0.40$) than pH (Table 20).

Total soil Ca and Cu are positively correlated to Ca and Cu availability, respectively, where $r = 0.58$ in both cases. This indicates that total soil concentrations of these elements would be able to predict their availability, to some extent. McBride et al. found total soil Cu to be a

significant predictor of soluble Cu.[85] Total soil Cr would not be able to predict available Cr (r = 0.17) and total soil Fe would not be able to predict available Fe (r = -0.02) since there is no correlation between the total and bioavailable soil concentrations of these 2 elements. There is a significant negative correlation between total soil Mg and available Mg (r = -0.73). This significant inverse relationship demonstrates that as the total soil concentration of Mg increases, the availability of Mg is reduced.

Synergism usually occurs when 2 elements compete for the same adsorption site in the soil. When the total soil concentration of one of the elements increases, the bioavailability of the other element also increases. A synergistic relationship is observed between Mn and Cr, Fe, Cu plus Mg since an increase in the total soil concentrations of these 4 elements increases the availability of Mn. As total soil Fe and Cu increases, the availability of Ca also increases. A synergistic relationship is also observed between Zn and Fe, Cu as well as Mg since an increase in total soil Zn increases the availability of Fe, Cu and Mg.

A positive intercorrelation between the three soil parameters pH, SOM and CEC is obtained. A negative correlation between the availability of Cu (r = -0.66), Fe (r = -0.91) and Zn (r = -0.61) with SOM and CEC is observed (Table 23). It is likely that organic matter, which contains strong ligands, would chelate metals thus hindering the availability of metals in soil. It is also reported that as the organic matter content of soil increases, the availability of Cu, Fe and Zn decreases.[65] Fe deficiency has also been seen in soils high in organic matter.[74] This could be explained by the fact that the strongest negative correlation between availability and SOM is obtained for Fe. A high CEC means that the quantity of negatively charged sites on soil surfaces that can retain positively charged ions by electrostatic forces are high.[35] If the CEC is high then more of the metal cations would adsorb to the negative surface charges of soil particles thus hindering the metals availability. This could explain the negative correlation between CEC and the availability of Cu, Fe and Zn.

The pH is known to impact on plant growth through its influence on the availability of essential plant nutrients and on the concentration of elements toxic to plants.[182] No significant positive correlation between pH and availability or uptake of the elements was found in this study. However, in the case of Cr availability, a negative correlation ($r = -0.67$) was observed with soil pH. Negative correlations were also observed between pH and the availability of Cu, Fe and Zn. Metals like Cu, Fe and Zn are tightly bound to the soil at high pH and are therefore more available at low pH levels.[32] This means that as the pH increases, the availability of Cu, Fe and Zn decreases which would explain the negative correlation.

CHAPTER 6

CONCLUSIONS

Firstly, the elemental composition and chemical characteristics of five edible tree nuts (almond, Brazil, pecan, Macadamia and walnut) were determined. This was achieved by obtaining the total elemental concentrations of the essential elements Ca, Cr, Cu, Fe, Mg, Mn, Se and Zn in the nuts. In agreement with literature data, a high level of Se was found in Brazil nuts making these nuts a good dietary source of this element. The Cr, Cu and Fe concentrations showed little difference amongst the different nuts studied, illustrating the likelihood of these concentrations being controlled in the nuts. The Zn concentrations in the almond, Macadamia and walnut samples were comparable whilst those in the Brazil and pecan nut samples were higher and more closely related. There was an apparent difference in the Mn concentrations amongst all nuts studied with no observable trend with the concentrations of the other elements.

Secondly, an investigation into the impact of soil quality on the chemical characteristics of edible Macadamia nuts grown in South African soils was undertaken. The study showed that the elemental concentrations of the essential trace elements namely Cu, Cr, Fe and Zn as well as arsenic were controlled in the Macadamia nuts as their concentrations were restricted to a small range of variation. Ca uptake trends in the Macadamia nuts were similar, whilst an affinity to high Mg uptake was observed. The Mn levels, although not phytotoxic, varied considerably in Macadamia nuts from different sites. The data showed total soil Mn to be a significant predictor of available Mn. However, there was no relationship between total soil Mn or available Mn with Mn in the nuts. Statistical analysis of data also showed that the availability and uptake of Mn was not significantly correlated to the pH of the soil. The uptake of Mn is possibly more affected by

the CEC, SOM and total soil concentrations of the other elements present, especially Ca, Cr, Cu and Fe, since an antagonistic relationship was observed between Mn and these 4 elements. The antagonistic effect of Zn on Cu was also observed. Additionally, there was evidence of synergistic relationships in soil since total soil concentrations of some elements increased the availability of others.

The accumulation or exclusion of the essential elements to meet the probable physiological requirement levels of the plant was quite evident. This is characteristic of most plants given that these mechanisms help prevent phytotoxicities and deficiencies of nutrients that limit plant growth. Statistical analyses of the data revealed a positive intercorrelation between Cu, Cr, Fe, Mg and Zn in the nuts indicating that the concentrations of these 5 elements are controlled in nuts and their proportions need to be maintained.

The proximate chemical composition of the five different edible nuts purchased in South Africa suggests that the oil content is high and could satisfy the calorie needs of the consuming population, whilst the protein and carbohydrate content are adequate to supplement other dietary sources. This study confirms that almonds, Brazil nuts, pecans, Macadamia nuts and walnuts consumed in South African households are good for health and should readily be incorporated into the diet.

The data in this work demonstrates that the competition effects (both antagonistic and synergistic) amongst the elements in the soil influences the availability and uptake of these elements by the plant. This consequently affects the elemental composition of the Macadamia nuts produced. However, uptake and distribution of elements in Macadamia nuts primarily dependent on the plants inherent controls which ensure that the physiological requirement levels of the plant are acquired and that a nutrient balance is maintained in the nuts. Macadamia nuts prove to have good control on elemental uptake and are low in the toxic metals investigated.

FURTHER WORK

1. Selenium distribution and speciation in Brazil nuts.

   Further work can be carried out by means of ion-pairing HPLC–ICP–MS for selenium speciation in Brazil nuts. This additional work can reaffirm that the primary species found in nuts is Se-methionine.
2. Elemental distribution in other types of tree nuts.
3. A detailed study on compositional and quality analysis of oils from tree nuts.
4. Investigations of elemental distribution in various food stuff.
5. Speciation analysis of the elements studied in this dissertation.
6. Comparative study of tree nut oils from South Africa with those from other countries.

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APPENDIX 1

Journal of Environmental Science and Health Part B (2007) 42, 585–591
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ISSN: 0360-1234 (Print); 1532-4109 (Online)
DOI: 10.1080/03601230701391591

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Elemental composition and chemical characteristics of five edible nuts (almond, Brazil, pecan, macadamia and walnut) consumed in Southern Africa

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The total elemental concentrations and proximate chemical composition of five different tree nuts, almond (*Prunus dulcus*), Brazil (*Bertholletia excelsa*), pecan (*Carya pecan*), macadamia (*Macadamia integrifolia*) and walnut (*Juglans nigra*) that are consumed in South African households were investigated. In addition, six physicochemical properties of the extracted nut oils, namely acid value, iodine value, saponification value, refractive index, density and specific gravity were evaluated. A high concentration of Se (36.1 ± 0.4 $\mu g\ g^{-1}$) was found in the Brazil nuts only. With maximum and minimum limits being set by the almond and pecan nut samples, Cr ranging from 0.94 ± 0.14–2.02 ± 0.07 $\mu g\ g^{-1}$ was detected in the nut samples. Generally, the order of the concentrations of the elements in all the nut samples is found to be Mg>Ca>Fe>Cu>Cr>As>Se. The concentrations of Mn and Zn showed greater variation amongst the different types of nuts. The extracted oils showed low acid values and high saponification values with the macadamia nut sample having the highest oil content (76.0 ± 0.5 g per 100 g of sample), the lowest acid value (0.42 ± 0.01 mg KOH per g of oil) and highest saponification value (193.7 ± 2.4 mg KOH per g of oil). The present findings are useful in calculating the Dietary Reference Intakes of these nutrients.

Keywords: Elemental composition; chemical characteristics; edible nuts, almond (*Prunus dulcus*); Brazil (*Bertholletia excelsa*); pecan (*Carya pecan*); macadamia (*Macadamia integrifolia*); walnut (*Juglans nigra*)

Introduction

The world consumption of low-fat and healthy foods is increasing as more people are recognizing the need for a healthy lifestyle.[1] The search for foods that contribute to a healthy diet and that aid in disease prevention has led to the identification of plant foods that have important phytonutrients, micronutrients, proteins, fiber and sterols. Edible tree nuts are such foods, since they contain nutrients that work independently or synergistically with one another or in conjunction with other foods. There is also increasing evidence that the consumption of whole foods due to the effects of their phytonutrients is better than the consumption of nutrients taken as dietary supplements.[2] People are therefore looking to plants instead of dietary supplements to obtain their dietary requirements for a healthy existence.

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Received January 23, 2007.

Scientific evidence has revealed significant and consistent results which indicate that nuts exert protective effects on human health. Most clinical studies on nuts have been devoted to the relationship between nuts and coronary heart disease, while epidemiological investigations have reported on correlations between nuts and cancer.[3] Direct experimental evidence also shows that nuts help reduce low density lipoprotein cholesterol without changing the high density lipoprotein cholesterol.[4] Further studies also report that nuts in the diet help reduce the risk of Type 2 diabetes by almost 30%.[5] Due to the protective role of nuts in the diet, nuts have been included as part of the dietary plan clinically proven to significantly reduce blood pressure which is supported by the National Heart, Lung and Blood Institute.[6]

Essential elements are vital for various metabolic processes and toxic element if present in relatively high quantities, adversely affect these processes.[7] Although essential elements are only required in trace amounts, their deficiencies in the human population are widespread, affecting up to two billion people.[8] The determination of the elemental distribution in food is therefore necessary to evaluate the total dietary intake of the essential elements which is

required for any deficiencies to be detected. This information is also useful in detecting heavy metal contamination in food so that the health risks caused by exposure to toxic elements may be averted.

Some of the essential trace elements that serve as cofactors for many physiological and metabolic functions are Cr, Cu, Fe, Mn, Se and Zn. Cr deficiency disturbs the metabolism, whilst an excess of Cr may be toxic.[9] Cu deficiency leads to high blood pressure and infertility. Cr is obtained mostly from meat, vegetable and unrefined sugar. Cu is obtained mostly from shellfish, organ meats and nuts.[10] Fe is obtained mostly from cereals, bread and beef. A deficiency of Fe causes anemia whilst an excess of iron may increase your risk of developing cancer or heart attacks. Tea, nuts and dried fruit contain the largest concentrations of Mn. The major source of dietary Se is food. Se may help resist arthritis, prevent heart disease and inhibit cancer but an excess can produce toxicity with symptoms of depression, gastrointestinal disturbances and excessive tooth decay.[11] Meat, fish and poultry are the major food sources of Zn. Zn deficiency is a common problem with some of its symptoms being hair loss, white streaks on the nails and poor wound healing. The U.S. Food and Drug Administration (FDA) limit for the toxic element As in fruit and vegetable is set at 1.4 $\mu g\ g^{-1}$. Most foods contain less than 1.0 $\mu g\ g^{-1}$. However, As poisoning from the ingestion of contaminated foods have been reported. Symptoms include pigmented skin lesions, gangrene of the feet, paralysis and skin cancer.[12]

Given the importance of a healthy lifestyle and the contribution of tree nuts to assist in maintaining this lifestyle, the aim of this research is to expand the knowledge on the chemical characteristics of various edible tree nuts consumed in South African households. The concentrations of the essential elements viz. Ca, Cu, Cr, Fe, Mg, Mn, Se, Zn and the toxic element As is determined. Furthermore, the proximate chemical composition of the nuts as well as the physicochemical properties of the extracted nut oils is evaluated. This is done to provide a comparative study on various aspects of the different edible tree nuts.

Materials and methods

Reagents and standards

All chemicals used were supplied by Merck and Sigma Chemical Companies and were of analytical-reagent grade. Double distilled water was used throughout the experiments. Working standard solutions for calibrations were prepared from spectroscopic grade stock standard solutions (1000 mg L^{-1}). All glassware and other equipment were cleaned with 6.0 M HNO_3 and rinsed off with double distilled water to prevent contamination.

Collection and preparation of nut samples

Nut samples that are commercially available in South Africa were purchased from the local supermarket in KwaZulu-Natal, Durban. Due to the large variation of nuts available as a result of flavoring and processing, only raw, unflavored nuts were used to minimize the sample variability. The selected nuts were almond (*Prunus dulcus*), Brazil (*Bertholletia excelsa*), pecan (*Carya pecan*), macadamia (*Macadamia integrifolia*) and walnut (*Juglans nigra*) nuts. The nut samples were washed with double distilled water, dried in an oven at 40°C overnight and stored in polyethylene bags.

Proximate chemical composition

The lipid content was determined by grinding a known mass of the nut samples in a commercial food processor and by extracting with 90 mL of a 2:1 chloroform-methanol mixture, after mixing together for 15 min.[13] The mixture was then filtered and the dry residue was reground to repeat the extraction. This was done three times. Thereafter, the chloroform-methanol extracts were combined, the solvents were evaporated and the oils were quantified gravimetrically and reported as mass of oil in sample. The defatted nut flour obtained by this process was sieved through a 500 μm mesh, packed in polyethylene bags and stored in a cool dry place for analysis.[14]

The ash content was determined by incineration of known masses of the defatted nut samples in a muffle furnace at 600°C for six hours. Nitrogen in the defatted nut samples was determined by the Kjeldahl Method. The nitrogen value obtained was multiplied by a conversion factor and reported as mass of protein in sample. The conversion factor for walnuts and pecan nuts is 6.25, almonds 5.18, Brazil nuts 5.46 and for macadamia nuts it is 5.3. The available carbohydrate was obtained by difference. This was done by subtracting the amount of oil, ash and protein from the total dry matter.[15]

Analysis of essential and toxic elements

The defatted nut flour samples were digested prior to analysis using the microwave-assisted closed vessel digestion technique. This method facilitates rapid dissolution of the sample matrix, requires low oxidizing reagent use and causes minimal contamination of the sample.[16] The digestions were performed using the Anton Paar Multiwave Microwave Sample Preparation System (1000 W) with 6 high-pressure tetra fluoro methaxil (TFM)-Ceramic Vessels (HF 50).[17] Three sub-samples of the defatted nut flour (0.5 g) were weighed into the TFM vessels. Five mL of 69% HNO_3 was added to each vessel and sealed. The leaf certified reference material (CRM) TFM programme was used for digestion of the nut samples. The power was ramped to

500 W for the first 5 min., where it remained for the next 5 min. The power was then ramped to 650 W for 15 min during which complete digestion of the samples occurred. The digested samples were transferred to 25 mL volumetric flasks, diluted to the mark with double distilled water and stored in polyethylene bottles for analysis.

All samples obtained from the acid digestion were analyzed for Ca, Cu, Cr, Fe, Mg, Mn, Se and Zn by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). This technique was the preferred method of analysis because of its multi-element determination capability, its high dynamic linear range and its sensitivity. All measurements were performed using the Perkin Elmer ICP-OES with radial plasma observation. The analytical wavelengths were selected based on the minimum spectral interferences and maximum analytical performance.

Total inorganic As was analyzed using Hydride Generation Atomic Absorption Spectrometry (HG-AAS). This was performed on a Perkin Elmer Analyst 100 with an MHS 15 Mercury Hydride System. Arsenic was detected by an electrodeless discharge lamp at the 193.7 nm line. The reliability of the above methods was corroborated by using the certified reference material, *lyophilized brown bread* (BCR 191), which was prepared under the sponsorship of the Commission of the European Communities.

Physicochemical properties of extracted nut oils

The saponification value was determined by the indicator method as specified by ISO 3657 (1988).[18] The iodine value was determined using Hanus reagent and was according to the method specified by ISO 3961. [18] The acid value was determined as per procedure described by Akpan et al.[18] A refractometer was used to obtain the refractive index of the extracted oils and the refractive index was reported after three determinations. Density bottles were used to determine the densities of the oils. The specific gravity was calculated as the ratio of the density of the oil to the density of water at the same temperature.[18]

Table 1. Comparison of measured and certified values in the certified reference material

	*Concentration**	
Element	*Certified***	*Measured***
Cu	$2.6 \pm 0.1\ \mu g\ g^{-1}$	$2.6 \pm 0.1\ \mu g\ g^{-1}$
Fe	$40.7 \pm 2.3\ \mu g\ g^{-1}$	$40.6 \pm 2.1\ \mu g\ g^{-1}$
Mn	$20.3 \pm 0.7\ \mu g\ g^{-1}$	$20.3 \pm 0.8\ \mu g\ g^{-1}$
Zn	$19.5 \pm 0.5\ \mu g\ g^{-1}$	$19.5 \pm 0.4\ \mu g\ g^{-1}$
As	$23.0\ ng\ g^{-1}$	$23.6 \pm 0.2\ ng\ g^{-1}$
Ca	$0.41\ mg\ g^{-1}$	$0.42 \pm 0.01\ mg\ g^{-1}$
Mg	$0.5\ mg\ g^{-1}$	$0.51 \pm 0.01\ mg\ g^{-1}$

*Dry mass. **Mean ± std. dev., at 95% confidence interval, n = 6.

Results and discussion

The reliability of the method was corroborated by using the certified reference material, *lyophilized brown bread* (BCR 191) and the results are presented in Table 1. The values provided for As, Ca and Mg are indicative, since insufficient data was available for certification. These values are the arithmetic means of the accepted results submitted by the laboratories and no uncertainties were ascribed to them. The measured results are in good agreement with the certified values.

The elemental concentrations and the Dietary Reference Intakes (expressed as μg/day) (DRIs) for the essential and toxic elements in the different nut samples analyzed are

Table 2. Total elemental concentrations in five edible nut samples expressed as $\mu g\ g^{-1}$ of sample

						DRI (μg/day)	
	Almond	*Brazil*	*Macadamia*	*Pecan*	*Walnut*	*RDA**	*UL***
As	0.013 ± 0.004	0.017 ± 0.002	0.019 ± 0.002	0.019 ± 0.001	0.024 ± 0.002	—	ND
Ca	5392.4 ± 57.2	7432.8 ± 10.2	3376.1 ± 15.0	2088.4 ± 32.7	2880.2 ± 35.9	10×10^6	2.5×10^6
Cr	0.94 ± 0.14	1.34 ± 0.19	1.26 ± 0.06	2.02 ± 0.07	1.74 ± 0.12	25–35	ND
Cu	23.74 ± 0.04	59.44 ± 0.51	18.96 ± 0.11	35.5 ± 0.05	59.14 ± 0.06	900	1.0×10^4
Fe	71.54 ± 0.36	74.26 ± 0.46	68.06 ± 0.27	105.86 ± 1.68	71.58 ± 1.13	8–18000	4.5×10^4
Mg	5424.1 ± 51.7	9678.5 ± 68.5	4886.5 ± 24.4	4197.0 ± 60.8	4833.0 ± 56.2	3.2×10^5	3.5×10^5
Mn	25.86 ± 0.48	3.40 ± 0.21	88.64 ± 0.51	192.60 ± 3.05	128.04 ± 1.56	18–2300	11000
Se	0.0039 ± 0.0007	36.1 ± 0.4	ND	ND	ND	55	400
Zn	49.72 ± 0.09	110.31 ± 1.25	38.46 ± 0.66	137.86 ± 0.39	54.26 ± 0.22	1.1×10^4	4.0×10^4

Mean ± std. dev. expressed as $\mu g\ g^{-1}$ of sample, at 95% confidence interval, n = 6.
ND: Not detected.
*RDA: Recommended Dietary Allowance. **UL: Tolerable Upper Intake Level.
DRI: Dietary Reference Intakes (expressed as μg/day) are the recommended intakes for adults according to the Food and Nutrition Board, Institute of Medicine, National Academies.[28]

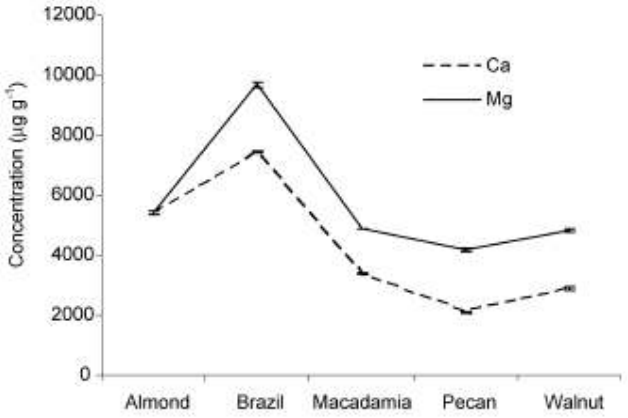

Fig. 1. Elemental distribution in the different nut samples.

summarized in Table 2.[19] The elemental distributions of the minor elements from the table are illustrated in Figure 1 and those of the major elements are represented in Figure 2. In general, the order of the concentrations of the elements in the nut samples from Figure 1 and Figure 2 is shown to be Mg>Ca>Fe>Cu>Cr>As>Se. Very little or no Se was detected in most of the nut samples analyzed. However, high Se levels were detected in the Brazil nut sample with a concentration of $36.1 \pm 0.4\ \mu g\ g^{-1}$, which is comparable to the results obtained by Kannamkumarath et al.[20] These levels are below the tolerable limit for this element in most adults. The tolerable levels are set at a limit below which toxic effects should not occur. Two g of Brazil nuts per day would be sufficient to meet the recommended dietory allowance (RDA) for Se in most adults. However, consuming more than 11 g of Brazil nuts per day will result in the Tolerable Upper Intake Level (ULs) being exceeded and this could likely lead to the risk of adverse effects. With the walnut and almond samples setting the minimum and maximum limits, the levels of As found in the nut samples ranged from 0.013 ± 0.004–$0.024 \pm 0.002\ \mu g\ g^{-1}$. These levels are considerably lower than the FDA limit for As in fruit and vegetable. It can be observed from Figure 1 that the Cr concentrations were around the same value for the different types of nuts. With the exception of the pecan nut sample that had a high Fe concentration of $105.86 \pm 1.68\ \mu g\ g^{-1}$, all the other nut samples had iron concentration close to $70\ \mu g\ g^{-1}$. The Cr and Fe levels in the nuts investigated can contribute to the RDA for these elements in most adults without posing the risk of toxicity. The Cu

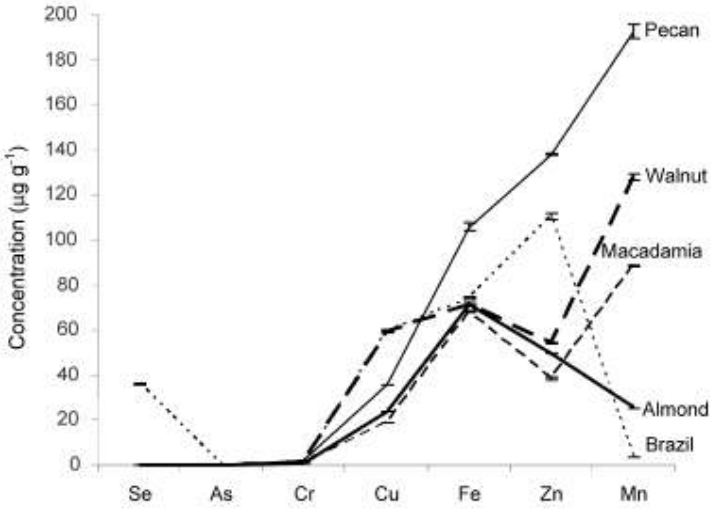

Fig. 2. Distribution of Ca and Mg in the different nut samples.

concentrations also showed little difference amongst the different nut samples analyzed which ranged between 18.96 ± 0.11–59.44 ± 0.51 $\mu g\ g^{-1}$. The most elevated concentrations were found in the walnut and Brazil nut samples, while lower levels of Cu was noticed in the macadamia nut sample. These results are in agreement with data obtained for similar types of nuts by Cabrera et al.[21]

Zn levels in Brazil and pecan nut samples appear to be comparable (110.31 ± 1.25 $\mu g\ g^{-1}$ and 137.86 ± 0.39 $\mu g\ g^{-1}$) whilst those in walnut, almond and macadamia nut samples show only slight variations (38.46 ± 0.66–54.26 ± 0.22 $\mu g\ g^{-1}$). The variability of Zn in the nut samples could be a consequence of the variability of Zn in the soil or due to the different nature of the nuts. Nuts and legumes are relatively good plant sources of Zn, but plant Zn concentrations may be enhanced, if grown in Zn-rich soils or treated with Zn-rich fertilizers.[22]

Mn presented a wide concentration variation amongst the different nut samples analyzed, with concentrations ranging from 3.40 ± 0.21–192.60 ± 3.05 $\mu g\ g^{-1}$ in the order of Brazil, almond, macadamia, walnut and pecan nuts. Wuilloud et al. also found a large variation in the Mn concentration amongst different nut samples with concentrations ranging from 9 $\mu g\ g^{-1}$– 4780 $\mu g\ g^{-1}$.[13] The difference in the Mn concentration could be due to the type of nut as well as factors such as environmental conditions under which the nuts grew or variations in the mineral concentration of soils.[13]

Ca, a macro element required for bone formation and the development of strong teeth, was found to be present at significant levels in all the different nuts studied. The highest Ca level was found in the Brazil nut sample (7432.8 ± 10.2 $\mu g\ g^{-1}$) and the lowest level was found in the pecan nut sample (2088.4 ± 32.7 $\mu g\ g^{-1}$). Mg is an essential part of many enzyme systems and large amounts were found in the different nut samples studied, with the highest amount of 9678.5 ± 68.50 $\mu g\ g^{-1}$ being present in the Brazil nut sample. Brazil nuts are known to have high levels of Ca and Mg which is confirmed by the results obtained in this study. With the exception of the almond nut sample, where the Ca and Mg levels appear to be almost the same (Fig. 2), there is a proportionate amount of Ca to Mg in the different nut samples with the Mg levels being higher than the Ca levels. An adequate serving of any one of these nuts would satisfy the RDA for these two elements in most adults.

Table 3. Proximate chemical composition (g per 100 g dry mass) of the nut samples analyzed.

Sample	*Oils*	*Ash*	*Protein*	*Carbohydrate**
Macadamia	76.0 ± 0.5	4.0 ± 0.1	13.0 ± 0.3	7.0 ± 0.2
Pecan	65.0 ± 0.6	6.0 ± 0.1	8.0 ± 0.3	21.0 ± 0.8
Brazil	65.0 ± 1.1	4.0 ± 0.2	22.0 ± 0.3	9.0 ± 0.4
Walnut	57.0 ± 0.5	2.0 ± 0.1	14.0 ± 0.2	27.0 ± 1.4
Almond	47.0 ± 0.4	5.0 ± 0.1	20.0 ± 0.2	28.0 ± 0.6

*Carbohydrate obtained by subtracting the amount of oil, ash and protein from the total dry matter (100 g).

The proximate chemical composition of the different nut samples are presented in Table 3. It can be seen that the edible tree nuts are rich in oils [47.0 ± 0.4–76.0 ± 0.5 g/100g dry mass (DM)], proteins (8.0 ± 0.3–22.0 ± 0.3 g/100g DM) and carbohydrates (7–28 g/100g DM).

The highest content of oil was observed in the macadamia nut sample (76.0 ± 0.5 g/100g DM). This is within the results reported by Kaijser et al. for four different cultivars of macadamia nuts grown in New Zealand, which ranged from 69.1–78.4 g/100g DM.[23] The proximate chemical composition of the walnut sample in this study is similar to the results obtained by Calarirmak[15] for the Güvenli variety grown in Turkey (oil-57.32%, ash-1.8%, protein-13.16% and carbohydrate-25.23%). The results of the proximate analysis of the Brazil nut sample per 100 g DM are as follows: 65.0 ± 1.1 g oil, 4.0 ± 0.2 g ash, 22.0 ± 0.3 g protein and 9 g carbohydrate. Similar values are reported in earlier works by Ramos et al.[24] The pecan nut sample has the lowest protein content but relatively high amounts of oil and carbohydrate. The almond nut sample has the lowest oil content but the highest amount of carbohydrate with relatively high protein content.

The characteristics of the extracted nut oils are summarized in Table 4. Edible oils with high iodine values are usually less stable and more susceptible to oxidation. The iodine value provides a measure of the degree of oil unsaturation and is commonly used as a means of predicting shelf-life.[25] Storage capabilities are also dependent on the polyunsaturated fatty acid (PUFA) levels because PUFAs are more susceptible to oxidative degradation.[26] Lower iodine values were obtained for the macadamia nut oil and Brazil nut oil. The values are 78.3 ± 0.6 g I_2/100 g oil and

Table 4. Physicochemical properties of the extracted nut oils

Nut sample	*Acid value mg KOH/g oil*	*Iodine value g I_2/100 g oil*	*Saponification value mg KOH/g oil*	*Refractive index*	*Density g/ml (25°C)*	*Specific gravity (25°C)*
Macadamia	0.42 ± 0.01	78.3 ± 0.6	193.7 ± 2.4	1.4690	0.9125 ± 0.0002	0.9152 ± 0.0002
Pecan	1.07 ± 0.03	110.1 ± 1.1	185.1 ± 1.8	1.4719	0.9210 ± 0.0001	0.9238 ± 0.0001
Brazil	1.45 ± 0.14	74.2 ± 0.3	192.4 ± 1.3	1.4725	0.9110 ± 0.0002	0.9137 ± 0.0002
Walnut	1.35 ± 0.11	113.8 ± 2.8	190.4 ± 1.2	1.4765	0.9140 ± 0.0002	0.9168 ± 0.0002
Almond	0.78 ± 0.03	93.6 ± 0.8	182.5 ± 2.6	1.4716	0.9100 ± 0.0001	0.9127 ± 0.0001

74.2 ± 0.3 g I_2/100 g oil respectively. The iodine value for the macadamia nut oil obtained in this work is similar to that obtained by Saleeb et al. which was found to be 75.4 g I_2/100 g oil.[27] The low iodine values indicate that these nuts will have a longer shelf-life than the other nuts studied in this work. This is especially true for macadamia nut oils, which have very low levels of PUFAs, which means less oxidative degradation. Walnuts contain primarily PUFAs therefore the oil will be prone to oxidative degradation and this is confirmed by the higher iodine value obtained in this study (113.8 ± 2.8 g I_2/100 g oil).

The acid value, which is an important indication of oil quality, suggests that the levels of acidity in the nuts are low, making the nuts suitable for human consumption. The saponification value is highest for the macadamia nut oil (193.7 ± 2.4 mg KOH/g oil) and lowest for the almond oil (182.5 ± 2.6 mg KOH/g oil).The high saponification values obtained for the different nut oils is a possible reason why these oils can be incorporated into many skincare products. The refractive index, density and specific gravity of the different oil samples showed little variation amongst the different types of nuts.

Conclusions

In this work, the total elemental concentrations of the essential elements in different types of nuts were determined. Because the bioavailability, retention and fate of Se in the human body is selenium-species-dependent, natural products rich in Se are recommended as supplements.[28] In agreement with literature data, a high level of Se was found in the Brazil nut sample making it a good dietary source of this element. The Cr, Cu and Fe concentrations showed little difference amongst the different nut samples and the values obtained indicate that these nuts can contribute to the RDA for these elements in most adults and do not represent any toxicological risk. The Zn concentrations in the different nut samples are comparable. However, those of Mn are significantly different. The difference in the Mn concentration could be due to the type of nut as well as factors such as the availability of the element in the soil as determined by soil pH, cation exchange capacity and organic matter and the total elemental content of the soil as determined geochemically or by environmental contamination. To this end, further research on the impact of soil quality on the chemical characteristics of macadamia nuts grown in South African soils will be undertaken in our laboratory.

The proximate chemical composition of the nut samples studied suggests a high oil content that can satisfy the calorie needs of the consuming population whilst the protein and carbohydrate content is adequate to supplement other dietary sources. The data reported in this work confirms that nuts consumed in South African households are good for health and should readily be incorporated into the diet.

Acknowledgments

Authors thank the University of KwaZulu-Natal for various forms of support extending in performing this work.

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APPENDIX 2

Journal of Environmental Science and Health Part A (2007) 42, 2097–2104
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ISSN: 1093-4529 (Print); 1532-4117 (Online)
DOI: 10.1080/10934520701627074

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Chemical composition of edible Macadamia nuts (*Macadamia integrifolia*) and impact of soil quality

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The distribution of eight selected elements (As, Ca, Cr, Cu, Fe, Mg, Mn and Zn) in edible Macadamia nuts (*Macadamia integrifolia*) from eight sampling sites in the south east coast region of South Africa is investigated. The levels of the elements in all the Macadamia nuts are found to be in the decreasing order of Mg > Ca > Fe > Zn > Cu > Cr > As. The exception is Mn, which exhibited large variability with concentrations in nuts ranging from $10.21 \pm 0.47\ \mu g\ g^{-1}$ to $216.4 \pm 0.4\ \mu g\ g^{-1}$. The impact of soil quality parameters: soil pH, cation exchange capacity, soil organic matter and elemental concentrations, as well as interactions in the soil on the elemental composition in the nuts are also studied. At the Ifafa site, south of Durban, typical elemental concentrations (in $\mu g\ g^{-1}$ dry weight) in the (nuts and soil) are Mg (4920 and 4656), Ca (2169 and 6173), Fe (92 and 20200), Zn (52 and 30), Cu (20 and 49), Cr (2.0 and 69.0) and As (0.024 and 0.104). The maximum concentration of Mg in the nuts is observed to be $4956.1 \pm 92.8\ \mu g\ g^{-1}$, while that of As is below $0.058 \pm 0.003\ \mu g\ g^{-1}$. Ca and Mg levels in nuts are antagonistic. Further, when bioavailable levels of Fe, Cu and Zn in soil are low, the bioaccumulation factor for Fe in nuts is high. Although, the soil quality parameters have an influence on the elemental uptake by the Macadamia nut, the results show that uptake and distribution of metals in the nuts are primarily dependent on the plants inherent controls that ensure the physiological well-being of the plant. Macadamia nuts prove to have good control on elemental uptake and are low in the toxic metals investigated.

Keywords: Elemental composition; chemical characteristics; edible nuts; Macadamia nuts; nutrition; South Africa.

Introduction

Plant nutrition depends on many factors that include the ability of the soil to supply these nutrients, the rate of absorption of nutrients by the plant, the distribution of nutrients to functional sites and the nutrient mobility within the plant.[1] The uptake of elements by plants depends on the interactions amongst the different elements in the soil which are bound to soil particles that in turn varies according to organic matter content, pH, cation exchange capacity and clay content of the soil matrix.[2] Since the availability of micronutrients in the soil changes with soil conditions, making generalizations on uptake of elements by plants is particularly difficult.

There is sufficient scientific evidence that shows consistency across different studies to support the health benefit claims of the use of edible nuts from trees. In the search for bioactive components in foods that favorably affect cardiovascular disease risk, nuts have begun to attract much attention.[3] There is substantial evidence that nuts have favorable effects on coronary heart disease. The possible mechanisms whereby nuts may improve lipid profiles do not rely exclusively on the beneficial action of unsaturated fatty acids, but may include the effects of fiber, essential elements like Cu, Mg and Se, plant proteins, plant sterols and phenolic compounds.[4] Garg et al. have demonstrated that Macadamia nut consumption as part of a healthy diet favorably modifies the plasma lipid profile in hypercholesterolemic men despite the diet being high in fat. These results in association with other published reports on the beneficial effects of tree nuts on biomarkers of coronary artery disease, allow a prudent recommendation for the inclusion of Macadamia nuts as part of a heart healthy diet.[5]

The Macadamia nut industry in the Southern African region is rapidly expanding, especially in the KwaZulu-Natal province of South Africa, with numerous plantations stretching across the South Coast and further inland. Growing Macadamia nut trees is also becoming ever more popular among home growers in South Africa. South Africa's 2006 Macadamia nut production was 19,500 MT wet-in-shell (WIS) and is projected to reach about 44,000

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Received April 23, 2007.

MT (WIS) in 2010. The production is expected to further double by 2016, when all new plantations would reach full production.[6] The need for assessing the quality of the nuts produced in South Africa especially by the plantations is crucial in the face of the escalating export market. In particular, the elemental accumulation in the nuts as a function of their location and soil quality parameters needs to be evaluated. Such information would make valuable contributions to food science and agriculture yet there is a general lack of this information. To overcome this paucity in information this study was undertaken to determine the chemical characteristics of the Macadamia nuts from different locations in the KwaZulu-Natal coast region and the impact of soil quality parameters on these characteristics. Previously, we have reported on the elemental composition and chemical characteristics of five edible nuts, namely Almond, Brazil, Pecan, Macadamia and Walnut nuts that are commonly consumed by the population in the Southern African region.[7]

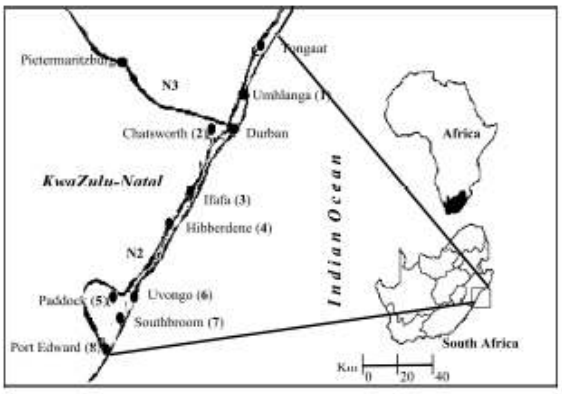

Fig. 1. Map of the selected sampling sites in KwaZulu-Natal, South Africa. Sites: 1 – Umhlanga, 2 – Chatsworth, 3 – Ifafa, 4 – Hibberdene, 5 – Paddock, 6 – Uvongo, 7 – Southbroom and 8 – Port Edward.

Materials and methods

Reagents and standards

All chemicals used were supplied by Merck and Sigma and were of analytical-reagent grade. Double distilled water was used throughout the experiments. Working standard solutions for calibrations were prepared from spectroscopic grade stock standard solutions (1000 mg L^{-1}). All glassware and other equipment were cleaned with 6.0 M HNO_3 and rinsed off with double distilled water to prevent contamination.

Collection and preparation of Macadamia nut and soil samples

The Macadamia nut and soil samples were collected from 8 different sites in KwaZulu-Natal, represented in Figure 1. The chosen sites were: Site 1–Umhlanga, Site 2–Chatsworth, Site 3–Ifafa, Site 4–Hibberdene, Site 5–Paddock, Site 6–Uvongo, Site 7–Southbroom and Site 8–Port Edward. Soils are inherently variable therefore random sampling needs to be done for correct representation. This was achieved by taking samples 7″–18″ deep, from 6 points within the drip-line of the tree canopy with the use of a hand auger.

Extraction of oils

The oil content was determined by grinding a known mass of the Macadamia nut samples in a commercial food processor and by extracting with 90 mL of a 2:1 chloroform-methanol mixture, after mixing together for 15 min.[8] The mixture was then filtered and the residue was dried, reground and extracted again. This was done 3 times thereafter the chloroform-methanol extracts were combined and the solvents were evaporated using a rotary evaporator. The collected oils were quantified gravimetrically and reported as percentage oil in sample. The defatted nut flour obtained by this process was sieved through a 500 μm mesh, packed in polyethylene bags and stored in a cool dry place for analysis.[9]

Analysis of essential and toxic elements

The sample analysis requires a preliminary step in which an organic matrix is broken down and the analyte is extracted into solution. The microwave-assisted closed vessel digestion technique was used for digestion of the Macadamia nut and soil samples. This method facilitates rapid dissolution of the sample matrix, requires low oxidizing reagent use and causes minimal contamination of the sample.[10] The digestions were performed using the Anton Paar Multiwave Microwave Sample Preparation System (1000 W) with 6 high-pressure tetrafluoromethaxil (TFM)-Ceramic Vessels (HF 50).[11] Three sub-samples of the defatted nut flour (0.5 g) were weighed into the TFM vessels. Five mL of 69% HNO_3 was added to each vessel and sealed. The same procedure applied to the soil samples (0.3 g). The nut and soil digests were transferred to 25 mL volumetric flasks, diluted to the mark with double distilled water and stored in polyethylene bottles for analysis.

All samples obtained from the acid digestions were analysed for Ca, Cr, Cu, Fe, Mg, Mn and Zn by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). This technique was the preferred method of analysis because of its multi-element determination capability, its high dynamic linear range and its sensitivity. All measurements were performed using the Perkin-Elmer ICP-OES with radial plasma observation. The analytical wavelengths were selected based on the minimum spectral

interferences and maximum analytical performance. The instrument of choice for the determination of total inorganic As was the Perkin Elmer Analyst 100 Atomic Absorption Spectrometer with an MHS 15 Mercury Hydride System. Arsenic was detected by an electrodeless discharge lamp at the 193.7 nm line.

Physicochemical properties of extracted nut oils

A refractometer was used to obtain the refractive index of the extracted oils and the refractive index was reported after three determinations. Density bottles were used to determine the densities of the oils. The specific gravity was calculated as the ratio of the density of the oil to the density of water at the same temperature.[12]

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

The pH of the soil was determined by measuring a 1:1 soil/water suspension using a pH meter fitted with a glass electrode. The SOM was estimated using the wet chemistry extraction technique known as the Walkley-Black Method.[13] The pH 7.0 ammonium acetate method was used for the determination of the CEC in the soil.[14]

Statistical analysis of data

The significance of the plant-soil relationships was established by computing correlation coefficients (r) for the relationships between the concentrations of the elements in the Macadamia nuts and the total and bioavailable amounts in the soil. Correlation coefficients were evaluated by Spearman's test, using the SPSS statistical package.

Results and discussion

The analyses were carried out in triplicate. Accuracy of the trace element measurements was corroborated by concurrent analysis of standard reference material, *lyophilized brown bread* (BCR 191), from the Community Bureau of Reference of the Commission of the European Communities. The values provided for As, Ca and Mg are indicative, so no uncertainties were ascribed to them. The measured results are in good agreement with the certified values (Table 1).

The elemental concentrations and bioaccumulation factors for the essential and toxic elements in the Macadamia nuts and corresponding soil samples are summarized in Table 2. The elemental distributions for the minor elements are illustrated in Figure 2, whilset those of the two major elements are illustrated in Figure 3. A perusal of Figures 2 and 3 shows that the concentrations of the elements in the nut samples are in the decreasing order as follows: Mg > Ca > Fe > Zn > Cu > Cr > As .The exclusion of Mn from this order is due to the large array of variability of Mn in the nut samples, with concentrations ranging from 10.21 ± 0.47 $\mu g\ g^{-1}$ to 216.4 ± 0.4 $\mu g\ g^{-1}$. It is reported that the Mn content of plants is frequently more closely related to soil pH than to the concentration of Mn in the soil,[15] but this association between soil pH and Mn uptake is not evident in this study. However, a strong dissociation between soil Mn and Mn uptake is apparent.

On inspection of Figure 2, it can be observed that irrespective of the varying concentrations in the soils, the As and Cr concentrations varied in narrow ranges for the different nut samples analyzed. The As concentration was below 0.058 ± 0.003 $\mu g\ g^{-1}$, while that of Cr was within

Table 1. Comparison of measured and certified values in the certified reference material (*lyophilized brown bread* — (BCR 191)) and adequate concentrations of essential nutrients sufficient for plant growth[23]

	*Concentration**		
Element	*Certified*[@]	*Measured*[@]	*Essential nutrients plants*[#] ($\mu g\ g^{-1}$)
Cu	2.6 ± 0.1 $\mu g\ g^{-1}$	2.6 ± 0.1 $\mu g\ g^{-1}$	6
Fe	40.7 ± 2.3 $\mu g\ g^{-1}$	40.6 ± 2.1 $\mu g\ g^{-1}$	100
Mn	20.3 ± 0.7 $\mu g\ g^{-1}$	20.3 ± 0.8 $\mu g\ g^{-1}$	50
Zn	19.5 ± 0.5 $\mu g\ g^{-1}$	19.5 ± 0.4 $\mu g\ g^{-1}$	20
As	23.0 ng g^{-1}	23.6 ± 0.2 ng g^{-1}	ND
Ca	0.41 mg g^{-1}	0.42 ± 0.01 mg g^{-1}	5000
Mg	0.5 mg g^{-1}	0.51 ± 0.01 mg g^{-1}	2000

* per dry mass. [@] Mean ± standard deviation, at 95% confidence interval, n = 6.
[#] Adequate concentrations of essential nutrients that may be required by plants.
ND—Not determined.

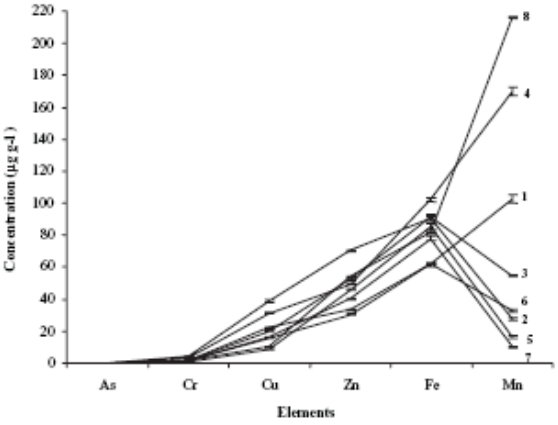

Fig. 2. Concentration of minor elements in the Macadamia nuts. Sites: 1 – Umhlanga, 2 – Chatsworth, 3 – Ifafa, 4 – Hibberdene, 5 – Paddock, 6 – Uvongo, 7 – Southbroom and 8 – Port Edward.

Table 2. Elemental concentrations and bioaccumulation factors for the essential and toxic elements in the Macadamia nut samples and corresponding soil samples

		Concentration ($\mu g\ g^{-1}$)			*Bioaccumulation factors*	
Sites[•]	*Elements*	*Soil (Total)*	*Soil (bioavailable)*	*Macadamia nuts*	$[plant]/[soil]_{BA}$	$[plant]/[soil]_{T}$
1	As	0.161 ± 0.004	0.005 ± 0.001	0.036 ± 0.001	7.2	0.22
	Ca	3505.3 ± 22.4	2220.5 ± 2.2	3752.3 ± 74.4	1.7	1.1
	Cr	49.91 ± 0.14	0.44 ± 0.01	1.59 ± 0.04	3.57	0.03
	Cu	57.79 ± 0.22	14.97 ± 0.01	15.3 9 ± 0.09	1.03	0.27
	Fe	25350 ± 97	184.3 ± 0.1	61.9 ± 1.3	0.3	0.002
	Mg	1668.1 ± 7.1	161.5 ± 0.1	3175.5 ± 53.7	19.7	1.90
	Mn	477.3 ± 3.3	305.0 ± 0.3	102.6 ± 2.4	0.3	0.22
	Zn	45.9 ± 0.2	19.5 ± 0.1	30.5 ± 0.4	1.6	0.7
2	As	0.078 ± 0.002	0.003 ± 0.001	0.032 ± 0.002	10.67	0.41
	Ca	8300 ± 87	4324.9 ± 4.2	1423.0 ± 27.8	0.3	0.2
	Cr	60.38 ± 0.21	0.60 ± 0.01	4.40 ± 0.16	7.33	0.07
	Cu	58.02 ± 0.25	5.53 ± 0.01	39.01 ± 1.08	7.05	0.67
	Fe	20454 ± 105	233.2 ± 0.2	91.0 ± 2.4	0.4	0.004
	Mg	3903.8 ± 25.0	47.5 ± 0.1	4956.1 ± 92.8	104.3	1.27
	Mn	252.3 ± 1.4	167.0 ± 0.2	27.55 ± 0.83	0.2	0.1
	Zn	35.62 ± 0.19	10.49 ± 0.004	70.52 ± 0.45	6.7	2.0
3	As	0.104 ± 0.006	0.003 ± 0.001	0.024 ± 0.003	8.0	0.23
	Ca	6173.0 ± 70.0	1378.8 ± 1.9	2168.8 ± 10.3	1.6	0.4
	Cr	69.02 ± 0.8	0.87 ± 0.01	2.05 ± 0.06	2.35	0.03
	Cu	49.04 ± 0.44	6.53 ± 0.01	20.27 ± 0.10	3.10	0.41
	Fe	20199 ± 100	274.5 ± 0.4	91.7 ± 0.4	0.3	0.005
	Mg	4655.7 ± 34.5	38.34 ± 0.04	4918.6 ± 19.9	128.3	1.1
	Mn	386.9 ± 8.05	204.1 ± 0.29	54.63 ± 0.38	0.27	0.14
	Zn	30.16 ± 0.74	8.87 ± 0.004	52.29 ± 0.44	5.89	1.73
4	As	0.105 ± 0.003	0.004 ± 0.001	0.032 ± 0.001	8.0	0.31
	Ca	2529.5 ± 30.8	463.9 ± 0.4	1640.8 ± 22.3	3.6	0.7
	Cr	6.30 ± 0.16	0.24 ± 0.001	3.07 ± 0.12	12.36	0.49
	Cu	5.99 ± 0.32	2.20 ± 0.01	31.38 ± 0.25	14.20	5.24
	Fe	3301.9 ± 20.5	51.33 ± 0.05	102.4 ± 1.4	2.0	0.03
	Mg	817.3 ± 7.8	71.2 ± 0.1	4694.4 ± 53.6	65.9	5.7
	Mn	15.31 ± 0.50	5.25 ± 0.01	169.9 ± 2.3	32.3	11.1
	Zn	1.27 ± 0.29	0.75 ± 0.003	49.56 ± 0.22	65.5	38.8
5	As	0.192 ± 0.007	0.010 ± 0.003	0.040 ± 0.002	4.0	0.208
	Ca	3342.5 ± 44.7	3318.8 ± 0.1	2672.5 ± 56.2	0.8	0.8
	Cr	22.39 ± 0.33	0.57 ± 0.001	1.18 ± 0.15	2.05	0.05
	Cu	61.82 ± 0.87	25.02 ± 0.01	8.33 ± 0.09	0.33	0.14
	Fe	16804 ± 101	957.8 ± 0.1	85.3 ± 1.7	0.09	0.005
	Mg	523.1 ± 0.9	153.4 ± 0.2	4220.9 ± 80. 9	27.5	8.0
	Mn	102.9 ± 0.45	67.3 ± 0.1	16.69 ± 0.76	0.25	0.16
	Zn	242.7 ± 1.7	198.6 ± 0.3	46.1 ± 0.5	0.23	0.19
6	As	0.266 ± 0.005	0.016 ± 0.001	0.058 ± 0.003	3.63	0.22
	Ca	1842.3 ± 20.2	1443.5 ± 1.3	2649.0 ± 18.7	1.8	1.4
	Cr	19.29 ± 0.09	0.61 ± 0.002	1.75 ± 0.24	2.86	0.09
	Cu	22.06 ± 0.28	11.76 ± 0.002	22.02 ± 0.06	1.87	1.0
	Fe	4497.5 ± 21.7	423.0 ± 0.3	62.2 ± 0.3	0.15	0.01
	Mg	452.4 ± 1.7	189.7 ± 0.1	3618.8 ± 30.3	19.1	8.0
	Mn	81.6 ± 0.5	80.6 ± 0.1	32.7 ± 0.6	0.41	0.40
	Zn	16.80 ± 0.39	9.43 ± 0.001	33.41 ± 0.20	3.54	1.99

(Continued on next page)

Table 2. *(Continued)*

Sites*	Elements	Concentration ($\mu g\ g^{-1}$)			Bioaccumulation factors	
		Soil (Total)	*Soil (bioavailable)*	*Macadamia nuts*	$[plant]/[soil]_{BA}$	$[plant]/[soil]_{T}$
7	As	0.158 ± 0.003	0.008 ± 0.001	0.025 ± 0.002	3.13	0.16
	Ca	4108.1 ± 10.1	695.7 ± 0.6	2220.3 ± 37.6	3.19	0.54
	Cr	37.74 ± 0.13	2.06 ± 0.0003	1.68 ± 0.07	0.81	0.05
	Cu	22.07 ± 0.03	6.24 ± 0.002	16.39 ± 0.10	2.62	0.74
	Fe	8870.7 ± 24.1	290.5 ± 0.3	78.0 ± 1.6	0.27	0.01
	Mg	1372.9 ± 5.7	157.2 ± 0.2	4014.7 ± 57.2	25.5	2.9
	Mn	47.48 ± 0.10	17.62 ± 0.02	10.21 ± 0.47	0.58	0.22
	Zn	3.86 ± 0.56	3.83 ± 0.005	40.44 ± 0.39	10.55	10.46
8	As	0.156 ± 0.006	0.007 ± 0.001	0.048 ± 0.002	6.86	0.31
	Ca	1655.5 ± 8.1	1428.7 ± 1.4	3081.4 ± 10.3	2.16	1.86
	Cr	21.91 ± 0.45	0.99 ± 0.002	1.67 ± 0.10	1.71	0.08
	Cu	24.25 ± 0.61	8.59 ± 0.004	10.35 ± 0.05	1.20	0.43
	Fe	9034.1 ± 45.5	717.2 ± 0.7	81.6 ± 0.5	0.11	0.01
	Mg	695.1 ± 8.3	271.3 ± 0.2	3648.6 ± 1.1	13.5	5.3
	Mn	103.8 ± 1.3	55.15 ± 0.06	216.4 ± 0.4	3.923	2.084
	Zn	307.3 ± 1.5	296.0 ± 0.4	54.2 ± 0.3a	0.18	0.18

*Sites: 1 – Umhlanga, 2 – Chatsworth, 3 – Ifafa, 4 – Hibberdene, 5 – Paddock, 6 – Uvongo, 7 – Southbroom and 8 – Port Edward.

the range 1.18 ± 0.15 $\mu g\ g^{-1}$ to 4.40 ± 0.16 $\mu g\ g^{-1}$. Cr is reported to be one of the few elements for which no accumulation against the concentration gradient has been evident at any point in the biological cycle from soil to plant to animal.[16]

Generally, the plant roots are reported to absorb Cu and Zn by a similar mechanism.[17] This causes antagonistic interferences to one, when the other is in excess in the root zone. Although the Macadamia nut has a proclivity for higher Zn uptake, an interrelationship between these elements nonetheless subsists and is evident at 2 of the sites chosen in this study. The soil Zn concentrations at Site 5 and Site 8 are found to be higher than those at the other sites. Consequently, the Cu concentrations are found to be lowest in the nuts, although relatively high in the soil. This antagonistic effect of Zn on Cu was also observed by Chaudhry et al.[18] in rice plants, where increased soil Zn concentrations markedly reduced Cu contents in rice.

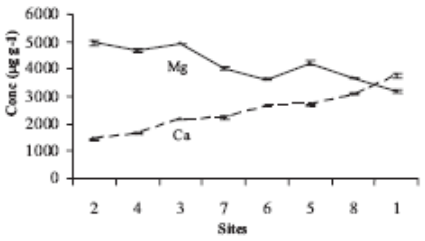

Fig. 3. Concentration of major elements in the Macadamia nuts.

The iron availability and uptake is a complex subject as many factors of soil and plant can influence the Fe level in the plant. The maximum and minimum limits of Fe found in the nuts are 102.40 ± 1.43 $\mu g\ g^{-1}$ and 61.87 ± 1.26 $\mu g\ g^{-1}$, respectively. The level of Fe in the soil is lowest at Hibberdene (Site 4), however the concentration of Fe in the nuts, interestingly, is highest. Fe is known to compete with Zn and Cu in their ionic forms.[19] The extent of this competition is dependent on the bioavailability of Zn and Cu, which is found to be lowest for these two elements at this particular site. The lack of interference from Zn and Cu in the soil, results in an increased uptake of Fe at this site.

An antagonism between Ca and Mg is detected in the Macadamia nuts (Fig. 3). Despite the higher Ca levels in the soil (1655.53 ± 8.05 $\mu g\ g^{-1}$ to 8299.65 ± 87.35 $\mu g\ g^{-1}$) than that of Mg (425.38 ± 1.73 $\mu g\ g^{-1}$ to 4655.73 ± 34.52 $\mu g\ g^{-1}$), there appears to be relatively higher accumulation of Mg in the nut, as evidenced by the elevated bioaccumulation factors. The bioaccumulation factors suggest that when the soil concentration of an element essential for plant growth is below the physiological requirement level, the plant tends to accumulate the element until the required level is reached (Figure 4a and 4b). The physiological requirements of plants, in general, are presented in Table 1. At soil concentrations that exceed the required levels of the plant, uptake of the associated element is inhibited thereby partially excluding the element. This behavior is also observed for Cr thus illustrating the likelihood of this element being essential to the growth of the Macadamia nut, although essentiality of Cr for plant growth, in general, is not yet established. The above relationship is not observed

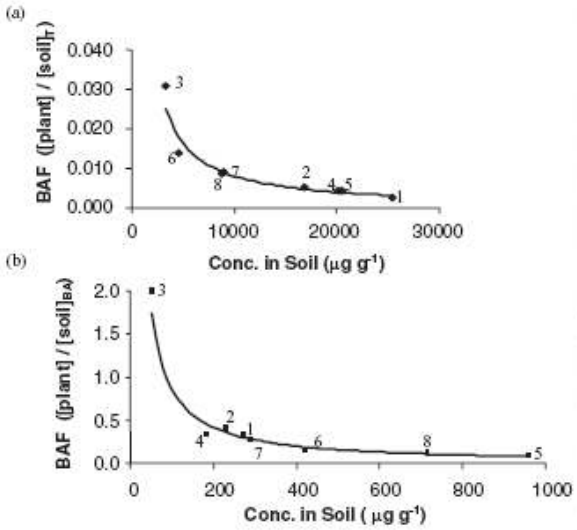

Fig. 4. (a) Total concentration of Fe in the soil versus bioaccumulation factor (BAF), (b) Bioavailable concentration of Fe in the soil versus bioaccumulation factor (BAF). Sites: 1 – Umhlanga, 2 – Chatsworth, 3 – Ifafa, 4 – Hibberdene, 5 – Paddock, 6 – Uvongo, 7 – Southbroom and 8 – Port Edward.

for As. Arsenic is usually not phytotoxic, even in cases of severe soil contamination and this element is also not taken up by plants in measurable amounts.[20] This is shown to be the case in the Macadamia nut since the uptake of As by the Macadamia nut is reduced as the total concentration of As in the soil is increased.

A correlation matrix for the total concentrations of elements in the nut and soil samples and the bioavailable concentrations are presented in Table 3. Statistically, no significant positive relationship could be established between the total soil concentrations of elements and their respective concentrations in the nuts. Surprisingly, that was also true for the bioavailable concentrations in the soil and the associated nut concentrations. However, on examination of the bioaccumulation factors in conjunction with the correlation coefficients for the total soil and nut concentrations of essential elements, it was observed that a negative correlation exists implying the exclusion of an element by the plant. On inspection of the soil concentrations of the excluded essential elements, it was noticed that most of the soil concentrations exceeded the physiological requirement levels of the plant (Table 1). The essential elements that fall into this category are Ca, Mn, Cu and Fe. Conversely, a positive correlation between total soil and nut concentrations of essential elements indicated an accumulation of these elements by the plant, although most of the soil samples were deficient in these elements and did not meet the plant's physiological requirements. The elements exhibiting this behavior were Zn and Mg.

There is a statistically significant positive intercorrelation between the total soil concentrations of the elements Cr, Cu, Fe, Mg and Mn. At the same time, there is a noteworthy positive correlation between the total concentrations of Ca in the soil with these 5 elements. This indicates that these elements, although from different sites, are linked to a related geological parent material.[21] In an earlier work by Lombnaes et al.,[21] an intercorrelation between Cu, Mn and Zn was found in the soil. However, no corresponding relationship for these nutrients was found in the cereal crops studied, thus illustrating the limited value of total soil micronutrients in determining phytoavailability in cereals. In this study a corresponding positive intercorrelation was found for the total nut concentrations of the same elements, except that Mn was replaced by Zn. Interestingly, there appears to be a statistically significant negative correlation between Ca in the nuts and these five elements. These results indicate that Zn needs to correlate more with the other elements in the nut, than with Mn for the nutrient balance. This could be so, since Zn is essential for the kernel formation of the nut. The synergy and correlation between Cr, Cu, Fe, Mg and Zn might be vital to optimize the formation of the nut.

The characteristics of the extracted Macadamia nut oils and the soil properties of the soil samples analyzed from the 8 different sites are presented in Table 4. The oil content in the Macadamia nut samples studied were on average around 75.9 ± 1.7%. The refractive index, specific gravity and density of the oils were not significantly different amongst the various Macadamia nut samples studied. The pH of the soils ranged between 4.95 and 6.81. The pH value is known to impact on the plant growth through its influence on the availability of essential plant nutrients and toxic elements to plants.[22] No direct relationship between pH and availability or uptake of the elements was found in this study. However, a positive intercorrelation between the three soil parameters pH, SOM and CEC was obtained. A negative correlation between the availability of Cu, Fe and Zn with the organic matter and CEC of the soil was also observed.

Conclusions

The study showed that the elemental concentrations of the essential trace elements namely Cu, Cr, Fe and Zn as well as the toxic element arsenic were well controlled in the Macadamia nuts as their concentrations were restricted to a small range of variation. Ca uptake trends in the Macadamia nut samples were similar, whilst an affinity to high Mg uptake was observed. The Mn levels, although not phytotoxic, varied considerably in the different Macadamia nut samples. The accumulation or exclusion of the essential elements to meet the probable physiological requirement

Table 3. Inter-item correlation matrix for the elemental concentrations in the Macadamia nuts as well as total and bioavailable concentrations in the soil

	AsN	*AsB*	*AsT*	*CaN*	*CaB*	*CaT*	*CrN*	*CrB*	*CrT*	*CuN*	*CuB*	*CuT*	*FeN*	*FeB*	*FeT*	*MgN*	*MgB*	*MgT*	*MnN*	*MnB*	*MnT*	*ZnN*	*ZnB*	*ZnT*	*SOM*	*CEC*	*pH*
AsN	1.00																										
AsB	0.52	1.00																									
AsT	0.68	0.96	1.00																								
CaN	0.75	0.81	0.91	1.00																							
CaB	0.10	0.06	−0.01	−0.08	1.00																						
CaT	−0.58	−0.34	−0.49	−0.54	0.58	1.00																					
CrN	−0.37	−0.58	−0.72	−0.76	0.37	0.65	1.00																				
CrB	−0.41	0.02	−0.02	−0.03	−0.33	0.06	−0.28	1.00																			
CrT	−0.32	−0.05	−0.13	−0.07	0.40	0.83	0.26	0.17	1.00																		
CuN	−0.32	−0.60	−0.69	−0.77	0.20	0.57	0.94	−0.31	0.20	1.00																	
CuB	0.46	0.60	0.68	0.58	0.44	−0.25	−0.61	−0.19	−0.13	−0.65	1.00																
CuT	0.06	0.33	0.27	0.22	0.82	0.58	0.00	−0.21	0.66	−0.12	0.58	1.00															
FeN	−0.77	−0.61	−0.75	−0.76	0.00	0.36	0.54	−0.13	−0.03	0.42	−0.42	−0.14	1.00														
FeB	0.24	0.17	0.27	0.35	0.31	−0.32	−0.54	0.07	−0.29	−0.70	0.74	0.28	−0.13	1.00													
FeT	0.00	0.39	0.29	0.27	0.64	0.62	0.06	−0.16	0.80	−0.06	0.34	0.92	−0.13	−0.02	1.00												
MgN	−0.82	−0.75	−0.87	−0.89	0.20	0.67	0.66	−0.10	0.29	0.62	−0.42	0.07	0.87	−0.24	0.04	1.00											
MgB	0.65	0.45	0.58	0.71	−0.21	−0.75	−0.64	0.24	−0.50	−0.70	0.36	−0.24	−0.60	0.57	−0.31	−0.83	1.00										
MgT	−0.54	−0.40	−0.49	−0.41	0.32	0.89	0.51	0.02	0.91	0.46	−0.38	0.48	0.33	−0.41	0.61	0.63	−0.73	1.00									
MnN	0.24	0.02	0.02	0.24	−0.40	−0.50	0.01	−0.29	−0.41	−0.09	−0.32	−0.46	0.21	−0.06	−0.28	−0.21	0.35	−0.29	1.00								
MnB	0.28	0.38	0.36	0.41	0.41	0.44	0.04	−0.31	0.75	0.04	0.18	0.71	−0.37	−0.27	0.87	−0.16	−0.25	0.56	−0.13	1.00							
MnT	0.15	0.33	0.30	0.37	0.34	0.47	0.03	−0.24	0.80	0.01	0.12	0.70	−0.26	−0.28	0.89	−0.08	−0.30	0.64	−0.09	0.98	1.00						
ZnN	−0.55	−0.66	−0.78	−0.68	0.46	0.64	0.75	−0.08	0.29	0.53	−0.39	0.17	0.74	0.01	0.13	0.76	−0.44	0.54	0.09	−0.11	−0.05	1.00					
ZnB	0.26	0.16	0.21	0.40	0.15	−0.42	−0.40	0.01	−0.35	−0.64	0.44	0.08	0.02	0.84	−0.07	−0.28	0.67	−0.41	0.45	−0.26	−0.24	0.17	1.00				
ZnT	0.26	0.20	0.24	0.42	0.24	−0.36	−0.40	−0.03	−0.29	−0.64	0.52	0.19	0.02	0.87	0.03	−0.26	0.63	−0.36	0.39	−0.19	−0.17	0.18	0.99	1.00			
SOM	0.00	−0.04	−0.13	−0.14	−0.29	0.14	0.49	−0.32	0.16	0.60	−0.66	−0.27	0.12	−0.91	0.05	0.08	−0.40	0.28	0.40	0.35	0.35	−0.01	−0.61	−0.63	1.00		
CEC	−0.13	−0.10	−0.20	−0.23	−0.40	0.08	0.45	−0.34	0.05	0.57	−0.66	−0.35	0.29	−0.89	−0.05	0.20	−0.47	0.23	0.43	0.21	0.23	0.00	−0.59	−0.62	*0.97*	1.00	
pH	0.47	0.19	0.15	0.03	0.08	−0.13	0.42	−0.67	−0.26	0.51	−0.16	−0.14	−0.12	−0.53	−0.04	−0.19	−0.12	−0.21	0.32	0.22	0.11	−0.14	−0.36	−0.36	*0.70*	*0.65*	1.00

AsN – $[As]_{Nut}$.
AsB – $[Soil\ As]_{Bioavailable}$.
AsT – $[Soil\ As]_{Total}$.
SOM – Soil Organic Matter.
CEC – Cation Exchange Capacity.

of the plant is quite evident. This is characteristic of most plants given that these mechanisms help prevent phytotoxicities and deficiencies of nutrients that limit plant growth. Some antagonism between Cu and Zn levels in the soils was observed. It was also established that the bioaccumulation factor of Fe was high, when the bioavailability of Cu, Fe and Zn in the soil was low. Statistical analyses of the data revealed a correlation between Cu, Cr, Fe, Mg and Zn in the nut. These results indicate that Macadamia nuts grown in South African soils can contribute to the Recommended Dietary Allowance for these essential elements in most adults without posing the risk of toxicity. The data in this work show that the intercorrelations as well as the antagonistic and synergistic relationships between the elements in the soil influence the availability and uptake by the plant, which subsequently affects the chemical characteristics of the Macadamia nuts produced. However, the uptake of elements by the Macadamia nut tree is primarily dependent on its inherent controls which ensure that the physiological requirements of the plant are attained and that a nutrient balance in the nut is maintained. Macadamia nuts prove to have good control on elemental uptake and are low on the toxic metals investigated.

Acknowledgments

The authors appreciate the assistance of the technical staff in the School of Chemistry at UKZN (Durban) and in particular those who work in the instrument laboratory. The authors acknowledge Kishore Singh, Julian Subramoney and Indrani Moonsamy for providing the homegrown Macadamia nut samples as well as Nisha Singh and Shakira Shaik for sharing their research laboratory. The authors especially acknowledge Mr. Barry Moses from Ocean Agriculture, Port Shepstone, for obtaining the Macadamia nut and soil samples from the plantations in KwaZulu-Natal.

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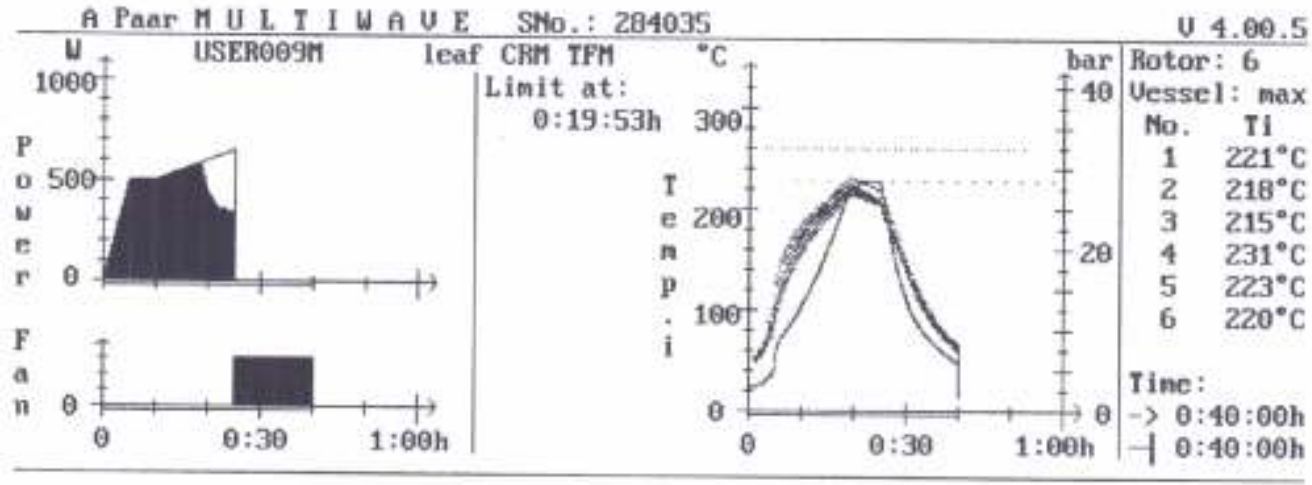
APPENDIX 3



Method: USER009M
Note:

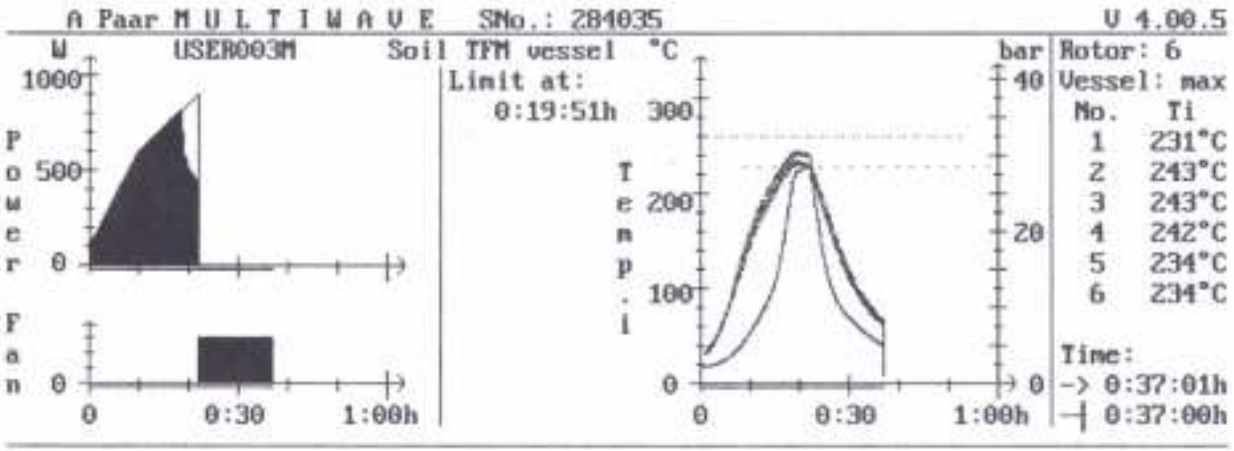
Sample (USER): leaf CRM TFM
Note: leaf TFM digestion

Ph	Power W	Time mm:ss	Power W	Fan
1	0	5:00	500	0
2	500	5:00	500	0
3	500	15:00	650	0
4	0	15:00	0	3
5	0	0:00	0	0
6	0	0:00	0	0
7	0	0:00	0	0
8	0	0:00	0	0

Ves	Weight max	Reag 1 HNO3	Reag 2 ———	Reag 3 ———	Remark
	0.500g	5.00 ml	0.00 ml	0.00 ml	
1					
2					
3					
4					
5					
6					

Run started on 06-07-08 at 14:15

a



Method: USER003M
Note:
soil samples

Sample (USER): Soil TFM vessel
Note: Soil digest for TFM

Ph	Power W	Time mm:ss	Power W	Fan
1	100	10:00	600	0
2	600	12:00	900	0
3	0	15:00	0	3
4	0	0:00	0	0
5	0	0:00	0	0
6	0	0:00	0	0
7	0	0:00	0	0
8	0	0:00	0	0

Ves	Weight max	Reag 1 HNO3	Reag 2 ------	Reag 3 ------	Remark
	0.300g	5.00 ml	0.00 ml	0.00 ml	
1					
2					
3					
4					
5					
6					

Run started on 06-07-13 at 17:20

APPENDIX 4

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

APPENDIX 5

List of Chemicals

Saarchem Analytical Reagent, As_2O_3, Merck Chemicals (Pty) Ltd.

Riedel-de Haën, HNO_3 (69%), Merck Chemicals (Pty) Ltd.

BDH, HCl (58%), Merck Chemicals (Pty) Ltd.

BDH, H_2SO_4 (39%), Merck Chemicals (Pty) Ltd.

Saarchem Analytical Reagent, Se powder, Merck Chemicals (Pty) Ltd.

1000 ppm stock solutions:

Fluka, 1.000g/L Ca Atomic Spectroscopy Standard Solution, Capital Lab Supplies.

Fluka, 1.000g/L Cu Atomic Spectroscopy Standard Solution, Capital Lab Supplies.

Fluka, 1.000g/L Cr Atomic Spectroscopy Standard Solution, Capital Lab Supplies.

Fluka, 1.000g/L Fe Atomic Spectroscopy Standard Solution, Capital Lab Supplies.

Fluka, 1.000g/L Mg Atomic Spectroscopy Standard Solution, Capital Lab Supplies.

Fluka, 1.000 g/L Mn Atomic Spectroscopy Standard Solution, Capital Lab Supplies.

Fluka, 1.000g/L Zn Atomic Spectroscopy Standard Solution, Capital Lab Supplies.