THE ROLE OF UNDERUTILIZED CROPS IN ALLEVIATING *HIDDEN HUNGER*

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Abstract

The quantity, quality and variety of food ingested by humans largely determines the intake of the essential mineral micronutrients required for normal human physiological functioning, growth and development. Inadequate dietary intake, low bioavailability, and failure of the human body to utilize ingested essential minerals lead to mineral micronutrient deficiencies (MNDs), an invisible form of undernutrition also known as *hidden hunger*. With the aim of aiding future human nutrition policy planning, the extent of dietary MNDs of calcium (Ca), copper (Cu), iodine (I), iron (Fe), magnesium (Mg), selenium (Se) and zinc (Zn) were estimated by integrating food supply and composition, estimated average requirement and demographic data. National level dietary Ca, Mg and Zn deficiency risks between 1992 and 2011 were estimated for the populations of 145 countries. Globally, in 2011, 3.5 and 1.1 billion people were at risk of Ca and Zn deficiency, respectively, due to inadequate dietary supply; 14 million people were at risk of Mg deficiency during the same period. Ninety percent of those at risk of Ca and Zn deficiency in 2011 lived in Africa and Asia. Considering the limited policy-making relevance of the low resolution national estimates of mineral MNDs, sub-national level assessments of the prevalence of dietary mineral MNDs were made for Malawi in 2011 using a 7-day household dietary recall survey data (n = 12117). It was estimated that >50% of households in Malawi were at risk of energy, Ca, Se, or Zn deficiencies due to inadequate dietary supplies, but supplies of Fe, Cu and Mg were adequate for >80% of households. Interventions to address dietary mineral deficiencies, such as dietary diversification using underutilized multipurpose and hardy tree/shrub species (e.g. *Moringa* spp.), were considered. Greater than 78% of *Moringa* growing households in southern Ethiopia and Kenya use *M. oleifera* (MO) and *M. stenopetala* (MS) trees as a source of food. Increasing the dietary consumption of MO and MS leaves, as a fresh vegetable or in powdered form, can reduce the prevalence of mineral micronutrient deficiencies, most notably Se deficiency. Daily consumption of 100 g Kenyan MO or MS fresh leaves could provide 100% and 144%, respectively, of the Se recommended daily allowance for a healthy adult man. Research and development to promote the use of these species in the fight against *hidden hunger,* are necessary. Continuing to reduce mineral MND risks through dietary diversification, and food and agricultural interventions, including fortification, crop breeding and use of micronutrient fertilisers, will remain a significant challenge during the global Sustainable Development era.

List of published papers arising from the thesis research

Kumssa, D. B., Joy, E. J., Young, S. D., Odee, D. W., Ander, E. L. and Broadley, M. R. (2017) Variation in the mineral element concentration of *Moringa oleifera* Lam. and *M. stenopetala* (Bak. f.) Cuf.: Role in human nutrition. *PLOS ONE,* 12(4), pp. e0175503. doi: 10.1371/journal.pone.0175503.

Kumssa, D. B., Joy, E. J., Ander, E. L., Watts, M. J., Young, S. D., Walker, S. and Broadley, M. R. (2015) Dietary calcium and zinc deficiency risks are decreasing but remain prevalent. *Scientific Reports, 5*, pp. 10974. doi: 10.1038/srep10974.

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Joy, E. J. M., **Kumssa, D. B.**, Broadley, M. R., Watts, M. J., Young, S. D., Chilimba, A. D. C. and Ander, E. L. (2015) Dietary mineral supplies in Malawi: spatial and socioeconomic assessment. *BMC Nutrition, 1*(1), pp. 1-25. doi: 10.1186/s40795-015-0036-4.

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Abbreviations

ADCC	Allan D C Chilimba
AI	Adequate Intake
Al	Aluminium
AME	Adult Male Equivalent
ANOVA	Analysis of Variance
BO	*Brassica oleracea*
Ca	Calcium
CRM	Certified Reference Material
Cu	Copper
DALY	Disability-Adjusted Life-Year
DBK	Diriba Bekere Kumssa
DER	Dietary Energy Requirement
DRI	Dietary Reference Intake
DW	Dry-Weight
EA	Enumeration Area
EAR	Estimated Average Requirement
EAR-CP	Estimated Average Requirement Cut Point
EJMJ	Edward J M Joy
ELA	E Louise Ander
EP	Edible Portion
EPA	Extension Planning Area
ESRI	Environmental Systems Research Institute
FAO	Food and Agriculture Organization of The United Nations
FAOSTAT	Food and Agriculture Organization Statistics
FBS	Food Balance Sheet
Fe	Iron
FISP	Farm Input Subsidy Scheme
GADM	Global Administrative Areas
GNI-PPP	Gross National Income Based on Purchasing Power Parity
GPS	Geographical Position System
HF	Hydrofluoric Acid
I	Iodine
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IHME	Institute of Health Metrics and Evaluation
IHS3	Third Integrated Household Survey of Malawi
IOM	Institute of Medicine
KEN	Kenya
LL	Tolerable Lower Limit
LRNI	Lower Reference Nutrient Intake
MBO	Mbololo
Mg	Magnesium
MGH	*Moringa* Growing Households

MJW	Michael J Watts
MND	Micronutrient Deficiency
MO	*Moringa oleifera*
MQW	MilliQ Water
MRB	Martin R Broadley
MS	*Moringa stenopetala*
N	Nitrogen
NDNS	National Diet and Nutrition Survey
NHANES	National Health and Nutrition Examination Survey
NSO	National Statistical Office
PA	Phytic Acid (Phytate)
PAL	Physical Activity Level
RDA	Recommended Daily Allowance
RNI	Recommended/Reference Nutrient Intake
S. ETH	Southern Ethiopia
S.D.	Standard Deviation
SDY	Scott D Young
Se	Selenium
SF	Syringe Filter
SPSS	Statistical Package for Social Sciences
SR	Standard Reference
SSA	Sub-Saharan Africa
SUCT	Single Use Centrifuge Tube
SW	Sue Walker
TAG	Trace Analysis Grade
TFCT	Tanzania Food Composition Table
Ti	Titanium
TMAH	Tetramethylammonium Hydroxide
UIC	Urinary Iodine Concentration
UK	United Kingdom
UL	Tolerable Upper Limit
UN	United Nations
URNI	Upper Reference Nutrient Intake
US	United States
USA	United States of America
USDA	United States Department of Agriculture
V	Vanadium
WHO	World Health Organization
WtdEAR	Weighted Estimated Average Requirement
YLD	Years of Life Lost Due to Disability
Zn	Zinc

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CHAPTER 1. INTRODUCTION

1.1 Human malnutrition

The quantity, quality and variety of food ingested by humans largely determines the intake of the various essential nutrients. A balanced diet is required to provide all essential nutrients for normal growth and development and to maintain the physiological reactions taking place in the body at an optimal level. Neither undernutrition nor overconsumption of the various essential nutrients, also known as malnutrition, is desirable. Human malnutrition is an abnormal physiological condition caused by inadequate, unbalanced or excessive consumption of dietary macronutrients (carbohydrates, fats and proteins) and/or micronutrients (vitamins and minerals) (FAO 2015). Malnutrition impacts on the health of individuals and can constrain economic development (Stein 2010).

Cognizant of the widespread prevalence of malnutrition, in November 1996, Global Leaders gathered at the World Food Summit in Rome and pledged to eradicate hunger[1] in all countries no later than 2015 (FAO 1996b). Similarly, in September 2000, at the Millennium Summit, a target was set to halve the proportion of hungry people between 1990 and 2015 (FAO 2012). However, notwithstanding these global initiatives to combat malnutrition, 0.8 billion people were undernourished in the period 2013-15 (FAO *et al.* 2015). There have been improvements in the proportion of undernourished people but with variation across regions. Between 1990-92 and 2011-13, the proportion of undernourished population decreased from

[1] In this thesis, hunger or undernourishment will be used interchangeably to refer to inadequate intake of dietary energy.

32 to 27 % in sub-Saharan Africa (SSA), from 27 to 18 % in southern Asia (SA), from 21 to 12 % in eastern Asia (EA), from 30 to 11 % in south-eastern Asia (SEA) and from 4 to 3 % in north Africa (NA) (UN 2013a). Similarly, the proportion of children under the age of five who were underweight had fallen from 50 to 31 % in SA, 29 to 21 % in SSA, 31 to 17 % in SEA, and 10 to 5 % in NA. The EA, SA, SEA and SSA regions accounted for 750 of the 842 million people with undernourishment in the 2011-13 which was 89 % of the global undernourishment proportion (FAO *et al.* 2013). The proportion of hungry people in all developing countries declined from 23.3 % in 1990-92 to 12.9 % in 2013-15 while improvements remaining uneven across various regions (FAO *et al.* 2015). At the end of the Millennium Development Goals, on 25 September 2015, as part of the new Sustainable Development Goals, the United Nations adopted 17 goals with specific targets to be reached until 2030 (UN 2015). At the top of these was a goal to end hunger, achieve food security and improve nutrition. Recently, on 1 April 2016, the United Nations (UN) General Assembly proclaimed UN *Decade of Action on Nutrition 2016 - 2025* calling on various governmental, non-governmental, and international organizations to collaborate in order to eradicate hunger and malnutrition (FAO and WHO 2016).

Undernourishment has typically been assessed based solely on dietary energy supply, which although the most important requirement, does not fully represent the multidimensional characteristics of food security. The Food and Agriculture Organization of the United Nations (FAO) defines food security as "a situation when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food which meets their dietary needs and food preferences for an active and healthy life" (FAO 1996b). Food insecurity is viewed

as a problem that needs to be addressed and monitored in all its many dimensions. For example, energy rich foods could be micronutrient poor and *vice versa*. Hence, an improvement in dietary calorie supply as reported in the FAO's State of Food Insecurity in the World (SOFI) series may not necessarily imply an improvement in micronutrient supply. The assessment of the micronutrient supply and adequacy is therefore one of the recommended areas that needs to be studied (CFS 2011, FAO *et al.* 2013) to obtain a comprehensive picture of the extent of malnutrition and to seek resolutions to the problems.

1.2 Hidden hunger

Malnutrition from under-nutrition can manifest as stunting, underweight, wasting and micronutrient deficiencies (Horton *et al.* 2009); and over-nutrition manifested as overweight and obesity (de Onis and Blössner 2000). While the consequences of under- and over-nutrition of macronutrients are often visible, chronic micronutrient deficiencies (MNDs) are often invisible, hence known as *hidden hunger*. *Hidden hunger* is the consequence of one or more dietary MNDs. Micronutrients are substances that are required in small quantities by the human body and comprise vitamins and minerals (WHO and FAO 2004). It was estimated that 2 - 3.5 billion people are affected by one or more MNDs (FAO 2013b, Kumssa *et al.* 2015a). Besides, MNDs lead to an estimated loss of 44 million Disability Adjusted Life Years (DALYs) annually (Stein 2014) with variation across regions. For example, in SSA, MNDs are responsible for 1.5-12 % of the DALYs lost per 100,000 population (Muthayya *et al.* 2013).

1.3 Some essential micronutrient minerals and consequences of deficiencies

Essential micronutrient minerals are chemical elements that are required by human body for normal physiological functioning. They cannot be synthesized in the body and are obtained primarily from food. Inadequate intakes of mineral nutrients can result from poor dietary choices, or socioeconomic bottlenecks to gain access to a balanced diet (FAO and WHO 2001, Miller *et al.* 2016). Mineral MNDs can occur due to inadequate intakes, failure of the human body to adequately utilize ingested mineral nutrients due to infection (WFP 2015), low bioavailability[2] (Wei *et al.* 2013, Fekadu *et al.* 2014, Petry 2014, Petry *et al.* 2014, Salunke *et al.* 2014, Li *et al.* 2015), and nutrient interactions where a nutrient and/or anti-nutrient antagonize the utilization of the other (WHO *et al.* 2004). In this thesis, calcium (Ca), copper (Cu), iodine (I), iron (Fe), magnesium (Mg), selenium (Se) and zinc (Zn) are discussed under various circumstances in relation to human nutrition. Some roles of these mineral elements in the human body, major dietary sources, Reference Nutrient Intake (RNI)[3] and the consequences of deficiencies are presented in (Table 1.1). Estimates of dietary supply or consumption, deficiency risks, and interactions among the mineral nutrients and anti-nutrient will be discussed in the subsequent chapters of this thesis.

[2] "Bioavailability is the proportion of an ingested nutrient that is absorbed and utilized for some essential metabolic function." (Miller and Welch 2013)

[3] Reference Nutrient Intake (RNI) is a daily nutrient intake level that is sufficient to fulfil the requirements of 97-98 % of healthy individuals in a population.

Table 1.1. The roles of some essential mineral nutrients in human body, dietary sources, consequences of deficiency, and reference nutrient intake (RNI) for a healthy adult man aged 19 – 65 yrs.

Mineral nutrient	RNI (mg d^{-1})	Roles in human body	Main dietary sources	Some consequences of deficiency	Reference
Calcium	1000	Provide rigidity to skeleton and teeth, metabolism, blood clotting, nerve and muscle functions	Dairy products, whole grains, dark green leafy vegetables	Fracture of bones, osteoporosis, defective mineralization of bones (osteomalacia)	(FAO *et al.* 2001, IOM 2006)
Copper	0.9	Component of metalloenzymes (e.g., Diamine oxidase, Ferroxidase, etc.)	Organ meats, seafood, nuts whole grains	Hypochromic anaemia, leukopenia, osteoporosis in copper deficient infants and children	(IOM 2006)
Iodine	0.15	Component of thyroid hormone (thyroxine (T4) and triiodothyronine (T3))	Seafood, iodized salt	Goitre, hypothyroidism, mental retardation, abnormal growth and development	(IOM 2006)
Iron	8	Component cytochromes, haemoglobin, myoglobin and enzymes	Haem iron (Meat, poultry, fish), and non-haem iron (cereals, pulses, legumes, fruits and vegetables)	Iron deficiency anaemia	(FAO *et al.* 2001, IOM 2006)
Magnesium	420	Component of >300 enzymes and bone, nerve and muscle functions, metabolism	Green leafy vegetables, whole grains, nuts	Muscle cramps, hypertension coronary and cerebral vasospasms	(IOM 2006)
Selenium	0.055	Component of selenoproteins, regulate thyroid hormone, protects body against oxidative stress	Meat, seafood, grains, dairy products, fruits, vegetables	Keshan and Kashin-Beck disease	(IOM 2006)
Zinc	11	Growth & development, metabolism, components of enzymes, gene expression	Red meat, seafood, whole grains	Growth retardation, alopecia, diarrhoea, delayed sexual maturation and impotence, eye and skin lesions	(IOM 2006)

1.4 Estimating dietary mineral nutrient supply and deficiency risks

Assessing dietary mineral nutrient supply/consumption and deficiency risks (*hidden hunger*) in a given population is essential for making an informed policy decision in public nutrition, health and agriculture. Human mineral nutritional deficiency risks can be estimated using tissue biomarkers (Hambidge 2003, Ong *et al.* 2014), dietary recall surveys (Treiber *et al.* 1990, Nicklas *et al.* 1991, Olendzki *et al.* 1999, Mircescu *et al.* 2006, Park *et al.* 2009, Bueno and Czepielewski 2010, Alemayehu *et al.* 2011), and food balance sheets (FBS) available in the public domain combined with food composition tables (IOM 2000b, Wessells *et al.* 2012b, FAO 2014, Joy *et al.* 2014, Kumssa *et al.* 2015a). The advantages and disadvantages of using the various methods of assessing mineral nutrient deficiency, and among different population sizes, are discussed elsewhere (Hambidge 2003, IOM 2003, 2006, Joy *et al.* 2014).

When a tissue biomarker is used to assess deficiency risk of a mineral nutrient, often the concentration of the nutrient in the tissue is compared with certain established threshold ranges (Hambidge 2003). On the other hand, when dietary recalls and Food Balance Sheets (FBS) are used to assess mineral nutrient deficiency risk, mineral nutrient intake is estimated using food supply/consumption data and food composition tables, and compared with published Dietary Reference Intakes (DRI) (IOM 2003, WHO *et al.* 2004, IOM 2006, Wessells *et al.* 2012b, Joy *et al.* 2014, Kumssa *et al.* 2015a). Dietary Reference Intakes are a set of estimates of nutrient intake levels that are used to assess and plan the diets of healthy population (IOM 2006). The DRI values for the different gender groups vary due to the differences

in requirements for nutrients as a consequence of differences in human physiology, for example, pregnancy, lactation, etc. (IOM 2000b, WHO *et al.* 2004). These include the Tolerable Lower Limit (LL)[4], Estimated Average Requirement (EAR)[5], Adequate Intake (AI)[6], Reference Nutrient Intake (RNI)[7], and Tolerable Upper Limit (UL)[8] (IOM 2003, Bates *et al.* 2014).

1.5 Dietary diversification using underutilized crops

Since the 1960s, to ensure food security, agriculture has been intensified on few crop and animal species with the main objective of supplying enough carbohydrate, fat, and protein (i.e., macronutrients) to fulfil the dietary requirements of the ever increasing global population which is projected to reach 8.1 billion by 2025 and 9.6 billion by 2050 (UN-DESA 2014). Reliance on few food crop species, for example, led to agricultural "simplification" which in turn resulted in agro-biodiversity decline and human dietary simplification (Welch and Graham 1999, Khoury *et al.* 2014). In addition, the existing mono-crop intensive agricultural practices are feared to be vulnerable to predicted climatic and environmental changes (Lane and Jarvis 2007, Elbehri *et al.* 2015, McKersie 2015). From ecological, economic and social

[4] Tolerable Lower Intake (LL), also known as Lower Reference Nutrient Intake (LRNI), is a daily nutrient intake level that is sufficient to fulfil the requirements of 2.5% of a healthy individuals in a population.

[5] Estimated Average Requirement (EAR) is a daily nutrient intake level that is sufficient to fulfil the requirements of 50% of the healthy individuals in a population.

[6] Adequate Intake (AI) is a daily nutrient intake level that is sufficient to meet the requirement of all healthy individuals in a population and it is used when it is not possible to derive RNI for a given nutrient.

[7] Reference/Recommended Nutrient Intake (RNI), also known as Recommended Daily Allowance (RDA) is a daily nutrient intake level that is sufficient to fulfil the requirements of 97-98 % of a healthy individuals in a population.

[8] Upper Tolerable Limit (UL), also known as Upper Reference Nutrient Intake (URNI) is a maximum daily nutrient intake level that is considered not to cause adverse health effects.

perspectives, interest is rising to diversify cropping systems to make agriculture sustainable and profitable (Gunasena 2001). Crop diversification improves food security by diversifying human diet (Frison *et al.* 2011) and has positive impact on agrobiodiversity. Alternative underutilized food crops (UFC) need to be brought into production to increase agrobiodiversity thereby enhancing the resilience from forecasted and unforeseen environmental changes, and improve the dietary micronutrient supplies to human beings through dietary diversification.

Underutilized food crops are plant species that are cultivated for food but have been neglected due to various constraints; for instance, adaptation to diverse growing conditions, ease of cultivation, storage, nutritional properties and taste, and social taboos (Padulosi *et al.* 2002, Virchow 2008, Mayes *et al.* 2012, Cernansky 2015). Despite the inherent characteristics that made UFCs underutilized, they can play a vital role in alleviating essential micronutrient nutritional insecurity (Vuong 2000, Gupta *et al.* 2005, Khoo *et al.* 2008, Padulosi *et al.* 2008, Arivalagan *et al.* 2012, Ebert 2014, Galluzzi and Noriega 2014). For instance, in Vietnam, Vuong (2000) reported that the gac fruit produced by an indigenous underutilized perennial vine (*Momordica cochinchinensis*) can provide 17–35 mg of β-carotene per 100 g of edible portion which is a good source of pro-vitamin A. Similarly, in Malaysia, Khoo *et al.* (2008) reported that the β-carotene content of *Baccaurea reticulata (*mafai*), Durio kutejensis* (Durian), *Garcinia prainiana* (button mangosteen), and *Baccaurea polyneura* fruits ranged between 11–18 mg per 100 g of edible portions. For comparison, the mean vitamin A requirement for an adult male of 19-65 yrs old is 0.3 mg d^{-1} (WHO *et al.* 2004). Arivalagan *et al.* (2012) argued that underutilized indigenous cereals, pulses, fruits and vegetables can supply the Fe required to maintain the requirements of the Indian population. For example, 100 g edible

portions of *Psophocarpus tetragonolobus* (winged bean), *Lablab purpureus* (hyacinth bean), *Vigna angularis* (adzuki bean), *Echinochloa esculenta* (barnyard millet) provide 52, 63, 72, and 81 % of the RNI for Fe (assuming 18.77 mg), respectively. However, they emphasized that policy leverage from the Government is necessary to promote these UFCs so that the population incorporate them in their day to day dishes to fight Fe deficiency anaemia.

In addition to fruit and grain producing annual and perennial UFCs, underutilized leafy vegetables with a potential to enhance food security are not only limited to annual plants but also perennial shrubs/trees. For example, *Moringa* spp. are known to be used in the diets of communities in Africa, Asia and the Americas (NRC 2006, TFLI 2014, Stevens *et al.* 2015, Gopalakrishnan *et al.* 2016). Among the *Moringa* spp., *M. oleifera* (MO) (commonly known as drumstick tree in English, and mlonge in Swahili) and *M. stenopetala* (MS) (commonly known as cabbage tree in English, and Haleko in Walayita and Gamo languges in southern Ethiopia) are known to produce edible foliage, immature pods and seeds that are rich in essential amino acids, vitamins and minerals (Jahn 1991b, Debela and Tolera 2013, Moyo *et al.* 2013, Popoola and Obembe 2013). For instance, 100 g of fresh leaves of *M. oleifera* can supply 414, 9.66, 0.002, 135, 0.018 and 0.418 mg of Ca, Fe, I, Mg, Se and Zn respectively in Malawi (Joy *et al.* 2015a). Making use of indigenous underutilized crops/trees where the local people are accustomed to the taste and the ways of cultivation will support efforts to alleviate hunger and malnutrition (Kalia *et al.* 2007). Concerted efforts are required to popularize and improve the crop characteristics (for instance, storage, packaging, yield, taste, etc.) that made them neglected from cultivation and utilization (Padulosi *et al.* 2002). Thence, developing diversified human dietary sources can assist in alleviating malnutrition

in its various forms and UFCs, e.g., *Moringa spp*, can play a central role to ensure nutritional security.

1.6 Main objectives

1) To estimate the dietary mineral nutrient supplies and the prevalence of deficiency risks at national level, and how these have changed since 1992. Chapters 2, 3.
2) To estimate dietary mineral nutrient consumption and prevalence of deficiency risks at subnational level in the Malawian population. Chapter 4.
3) To assess the role MO and MS edible parts can play in alleviating mineral nutrient deficiencies, and to determine the association between mineral nutrient concentration in edible parts of MO and MS and soil chemical properties. Chapter 5
4) To explore the challenges and opportunities of MO and MS growers with emphasis on dietary utilization. Chapter 6.

1.7 Thesis structure

In line with the objectives outlined above, in the first part of this thesis, national level dietary mineral nutrient supplies and prevalence of deficiency risks are assessed globally on an annual basis between 1992 and 2011 by integrating food supply data from the FAO FBS (FAO 2014) for Ca, Zn and Mg (Kumssa *et al.* 2015a, Kumssa *et al.* 2015b), food composition data from the United States Department of Agriculture (USDA) nutrient database for standard reference 26 [USDA-SR26] (USDA 2013), the EAR for the various minerals (WHO *et al.* 2004), and temporal national demographic data (UN 2013b). These data are presented in Chapters 2 and 3.

In the second part of this thesis, sub-national 7 d household dietary recall based estimates of mineral nutrient deficiency risks were determined in Malawi (NSO 2014). This case study in Malawi was conducted due to a readily available food consumption and composition data to compare estimates of MNDs from the low resolution national level FAO food supply data in the year 2011. Local food composition data was used to calculate the adult male equivalent per capita Ca, Cu, I, Fe, Mg, Se and Zn, and dietary energy intakes from the household level reported food consumption data. These estimated dietary mineral and energy intakes were compared with corresponding population weighted EARs (WHO *et al.* 2004) to determine deficiency risks at household level (Joy *et al.* 2015b). These data are presented in Chapter 4.

Sub-Saharan Africa is one of the regions where MNDs are highly prevalent (Joy *et al.* 2014, Joy *et al.* 2015b, Kumssa *et al.* 2015a). But, favourably, the range of distribution of MO and MS overlap with this MND hotspot to assist in tackling mineral nutrient undernutrition. In the third part of this thesis, the roles MO and MS can play in alleviating the widespread mineral deficiency risks in SSA is explored. Data on mineral nutrient concentration in edible parts of MO and MS, chemical properties of soil underneath the trees, and ethnobotany of these species with focus on the dietary usage was collected from various locations in two SSA countries, Ethiopia and Kenya. From these case studies, the variation in mineral nutrient concentrations of the edible parts because of differences in soil chemical properties at various locations, the role of MO and MS edible parts in alleviating human mineral MND is presented in Chapter 5. Data on the dietary-focused ethnobotany of these species is presented in Chapter 6.

In the last part of the thesis, the overall human dietary mineral micronutrient intake and deficiency risk estimation process are concisely discussed. Besides, as a household or community vegetable crop, the role MO can play in alleviating Se deficiency in the Malawian population is presented.

CHAPTER 2. DIETARY CALCIUM AND ZINC DEFICIENCY RISKS ARE DECREASING BUT REMAIN PREVALENT

2.1 Authors contribution

DBK[9], MRB, ELA and EJMJ designed the study. **DBK** collated the data, designed and developed the database, carried out data analyses and produced the initial draft of the manuscript. MJW, SDY and SW contributed to drafting the manuscript.

2.2 Abstract

Globally, more than 800 million people are undernourished while >2 billion people have one or more chronic micronutrient deficiencies (MNDs). More than 6% of global mortality and morbidity burdens are associated with undernourishment and MNDs. Here we show that, in 2011, 3.5 and 1.1 billion people were at risk of calcium (Ca) and zinc (Zn) deficiency respectively due to inadequate dietary supply. The global mean dietary supply of Ca and Zn in 2011 was 684 ± 211 and 16 ± 3 mg capita^{-1} d^{-1} (±S.D.) respectively. Between 1992 and 2011, global risk of deficiency of Ca and Zn decreased from 76 to 51%, and 22 to 16%, respectively. Approximately 90% of those at risk of Ca and Zn deficiency in 2011 were in Africa and Asia. To our knowledge, these are the first global estimates of dietary Ca deficiency risks based on food supply. We conclude that continuing to reduce Ca and Zn deficiency risks through dietary diversification and food and agricultural interventions including fortification, crop breeding and use of micronutrient fertilisers will remain a significant challenge.

[9] Diriba Bekere Kumssa (**DBK**) is the author of this thesis

2.3 Background

Food security is essential to human wellbeing (FAO 1996a, FAO *et al.* 2013, Lim *et al.* 2013, Stein 2014) and a central component of the Millennium Development Goals (MDGs) of the United Nations (UN) (UN 2013a). Food security exists when "all people, at all times, have physical, social and economic access to sufficient safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life" FAO (FAO 1996a) and therefore includes consideration of the macro and micro-nutrient components of the diet. For example, >800 million people are undernourished (UN 2013a), and >2 billion people are likely to be at risk of one or more micronutrient deficiencies (MNDs), notably the minerals Ca, iodine (I), iron (Fe), selenium (Se), Zn, and vitamins (e.g. vitamin A) (Wuehler *et al.* 2005, Fairweather-Tait *et al.* 2011, Wessells and Brown 2012a, Lim *et al.* 2013, Zimmermann 2013, Stein 2014). Micronutrients have pivotal roles in human health and MNDs can retard growth and cognitive development, impair immunological functioning and increase the risks of non-communicable diseases including skeletal, cardiovascular and metabolic disorders (WHO and FAO 2003, Fairweather-Tait *et al.* 2011). It has been estimated that Fe and Zn deficiency reduce the Gross Domestic Product (GDP) of developing countries by 2–5% (Stein 2014). Although the estimated prevalence of inadequate dietary energy supply decreased from 18.7% to 11.3% at global and 23.4% to 13.5% for developing countries between 1990/2 and 2011/14 (FAO *et al.* 2014), trends in MNDs for all essential mineral nutrients at various geospatial scales have not been quantified over this period.

Determining the prevalence of MNDs is challenging (Joy *et al.* 2014). For some micronutrients (e.g. Fe, I, Se, Zn), body tissues or urine can be analyzed directly for

micronutrients and micronutrient-responsive enzymes (Gibson 2005, Fairweather-Tait *et al.* 2011, Zimmermann 2013). Where reliable tissue biomarkers are lacking and for larger population sizes, MND risks can be quantified from dietary analyses or surveys incorporating food composition data (Ecker and Qaim 2011). However, many countries lack nationally-representative surveys and food composition tables. Data from surveys are also affected by behavioral change and systematic misreporting (Archer *et al.* 2013). The Food and Agriculture Organization (FAO) of the United Nations (UN) uses national food balance sheets (FBSs) as a proxy for food consumption to estimate the global prevalence of undernourishment (de Haen *et al.* 2011). The FBSs of the FAO are currently available from 1961–2011 (FAO 2014)and represent net per capita food supply calculated from national production, trade, transport losses, storage, non-food uses, livestock feed, etc., but with no adjustment for household waste or inter- and intra-household variation in access to food (FAO 2001). Per capita micronutrient supply can be estimated by multiplying the edible portion of each food item (derived from energy values provided by the FAO in kcal $capita^{-1}$ d^{-1}) (Wessells *et al.* 2012a) by its micronutrient concentration compiled in food composition tables (Lukmanji *et al.* 2008, USDA 2013). The per capita supply of a mineral is then compared to a demographically-weighted requirement threshold for a population, termed the WtdEAR, which represents the daily intake that meets the requirements of half the healthy individuals in that population (IOM 2000b, WHO *et al.* 2004). FBS and food composition data were used to estimate a global prevalence of dietary Zn deficiency of 17% in 2003–07 (Wessells *et al.* 2012a), however, the prevalence of dietary Ca deficiency has not previously been reported.

2.4 Materials and Methods

Food supply, food composition, Estimated Average Requirements (EARs) for Ca and Zn, and demographic data were compiled to assess global dietary Ca and Zn supplies and deficiency risks between 1992 and 2011. A total of 145 countries with population >1 million were included in this study. The EAR "cut-point" (EAR-CP) method was used to assess the prevalence of Ca and Zn deficiency risks.

2.4.1 Data sources

The four major datasets required for this research were food supply, food composition, EARs for Ca and Zn, and demographic data for each country. Food supply data from 1992 to 2011 were obtained from FAOSTAT (FAO 2014). Food composition data for Ca and Zn were obtained from the United States Department of Agriculture National Nutrient Database for Standard Reference 26 (USDA SR26) which was released in 2013 (USDA 2013), and the phytate composition of foods were obtained from the Tanzanian food composition table (TFC) (Lukmanji *et al.* 2008). The EAR for Ca and Zn were obtained from the FAO/WHO Human Vitamin and Mineral Requirements (WHO *et al.* 2004). Demographic data were obtained from the United Nations, Department of Economic and Social Affairs Population Division, Population Estimates and Projection web page (UN 2013b). These data were compiled and integrated in to MS Access relational database management system to assess global dietary Ca and Zn supplies and deficiency risks.

2.4.2 Calcium and zinc supply

The 94 food items from FAOSTAT (FAO 2013a) food supply data, reported in g *capita*$^{-1}$ d^{-1}, were matched (Stadlmayr *et al.* 2011) with the food commodities in the nutrient composition data in the USDA SR26 food composition database (see Supplementary Table S2 online[10]). Nutrient composition data were assumed to not change between 1992 and 2011. Per capita Ca and Zn supply was estimated by multiplying the edible portion of each food item (derived from energy values provided by the FAO in kcal *capita*$^{-1}$ d^{-1}) (FAO 2001, Wessells *et al.* 2012a). Calcium and Zn supply from each food commodity was summed to obtain the per capita nutrient supply (PCNS) per day for every reference year and country.

2.4.3 Calcium and zinc intakes and requirements

Calcium and Zn intakes were estimated to be the per capita Ca and Zn supply with a coefficient of variation of 25% (Yang *et al.* 2007, Wessells *et al.* 2012a, Umaretiya *et al.* 2013). The EAR was estimated and available for a given age and gender groups (WHO *et al.* 2004). As supply data were available for the whole population at national level, the EAR was converted to single WtdEAR (Equation 2.1) based on the population size in each age and gender group for each country and year (UN 2013b). The EAR for a given age/gender group was assumed to remain unchanged while the WtdEAR varied with the population composition and size. The WtdEAR for Ca and Zn is a per capita intake level that fulfils the Ca and Zn

[10] All supplementary information is available at http://www.nature.com/article-assets/npg/srep/2015/150622/srep10974/extref/srep10974-s1.pdf.

needs of half of the healthy individuals of the population in a given country in a specific year.

2.4.4 Estimated average requirement "cut-point" (EAR-CP)

Historical Ca and Zn deficiency risks were assessed using the EAR-CP[11] as described and used by Carriquiry (1999), Joy *et al.* (2014) and Wuehler *et al.* (2005). The EAR-CP method provides an estimate of the number of people in a given country and year with intakes of Ca and Zn below the WtdEAR, which is termed as the deficiency risk in this paper. The EAR-CP method has been applied with the following underlying assumptions: little correlation between requirement and intake, the distribution of requirement is symmetrical around the EAR, and variability in intake is greater than the variability in requirement (Carriquiry 1999, IOM 2000b, Murphy and Poos 2002).

2.4.5 Calculation of molar ratio of phytate:Zn

The dietary phytate composition of the food items was obtained from the TFCT (Lukmanji *et al.* 2008) and the phytate supply for each country across the years was estimated by applying similar methods as for Ca and Zn supplies. Thence, the daily molar ratio of PA:Zn was calculated by dividing the molar intake of phytate

[11] *The NORM.DIST function in Microsoft EXCEL was used to calculate the deficiency risk*

The syntax is $NORM.DIST(x, mean, standard\ deviation, cumulative)$,

$Where\ \ x$ is the WtdEAR (Ca or Zn),

$mean$ is the nutrient (Ca or Zn) supply,

$standard\ deviation$ is the $supply \times 0.25$.

The figure 25% (0.25) is the assumed coefficient of variation in the nutrient supply

(molecular weight = 660 g mol^{-1}) by the molar intake of Zn (molecular weight = 65.4 g mol^{-1}).

2.4.6 Data analyses and visualisation

Descriptive statistics calculations were conducted in MS Excel. Visualizations were carried out in Tableau Software for desktop version 8.2, and ArcGIS 10.2.1. Correlation analyses was carried out using IBM SPSS Statistics version 21. The EAR, and aggregations of results (mean and standard deviations) at global, regional, and continental levels were weighted by the population sizes of the member countries (Equation 2.1 and 2.2).

Equation 2.1. Derivation of the WtdEAR for Ca and Zn.

$$WtdEAR = \frac{\sum(EARgroup \times GroupPop)}{TotalPop},$$

Where, $WtdEAR$ is the weighted EAR for Ca or Zn,

$EARgroup\ is$ the EAR for either Ca or Zn of a given age/gender group,

$GroupPop$ is the population size of a given age/gender group, and

$TotalPop$ is the totalpopulation in a given year for a given country

Equation 2.2. Aggregation of mean (a) and standard deviation (b) of supply, WtdEAR, and deficiency risk of Ca at regional level as an example.

a) Derivation of the mean of aggregated information at regional level.

$$WtdMeanCaSup_i = \frac{\sum(CaSup_j * PCountry_j)}{\sum_i Population}$$

Where, $WtdMeanCaSup_i$ is the weighted mean Ca supply in region i;

$CaSup_j$ is Casupply in country j;

$PCountry_j$ is the population in country j

$\sum_i Population$ is the total population in region i

$$WtdCaWtdMeanEAR_i = \frac{\sum(CaWtdEAR_j * PCountry_j)}{\sum_i Population}$$

Where, $WtdCaWtdMeanEAR_i$ is the weighted mean Ca WtdEAR in region i;

$CaWtdEAR_j$ is the Ca WtdEAR in country j

$$WtdCaMeanDefRisk_i = \frac{\sum(CaDefRisk_j * PCountry_j)}{\sum_i Population}$$

Where, $WtdCaMeanDefRisk_i$ is the weighted mean Ca deficiency risk in region i;

$CaDefRisk_j$ is the Ca deficiency risk in country j

b) Derivation of the standard deviation of aggregated information at regional level.

$$SD^{i}{}_{CaSupPerFood} = \sqrt{\frac{\sum_{j}^{i}\left(CaPerFood_j - WtdMeanCaPerFood_i\right)^2 * \left(PCountry_j\right)}{\sum_{i} Population}}$$

Where, $SD^{i}{}_{CaSupPerFood}$ is the standard deviation of Ca supply per food item in region *i;*

$CaPerFood_j$is the Ca composition of a food item in country *j;* and

$WtdMeanCaPerFood_i$ is the weighted mean of Ca composition per food item in a given region *i;*

$$SD^{i}{}_{CaSup} = \sqrt{\frac{\sum_{j}^{i}\left(CaSup_j - WtdMeanCaSup_i\right)^2 * \left(PCountry_j\right)}{\sum_{i} Population}}$$

Where, $SD^{i}{}_{CaSup}$ is the standard deviation of Ca supply in region *i*

$$SD^{i}{}_{CaWtdEAR} = \sqrt{\frac{\sum_{j}^{i}\left(CaWtdEAR_j - WtdCaMeanWtdEAR_i\right)^2 * \left(PCountry_j\right)}{\sum \ Population}}$$

Where, $SD^{i}{}_{CaWtdEAR}$ is the standard deviation of Ca WtdEAR in region *i*

$$SD^{i}{}_{CaDefRisk} = \sqrt{\frac{\sum_{j}^{i}\left(CaDefRisk_j - WtdCaWtdMeanDefRisk_i\right)^2 * \left(PCountry_j\right)}{\sum_{i} Population}}$$

Where, $SD^{i}{}_{CaDefRisk}$ is the standard deviation of Ca deficiency risk in region *i*

2.5 Results and discussion

The supplies and deficiency risks (means ± S.D. unless stated) of Ca and Zn are presented at various spatial (global, continental, regional and country), and temporal (from 1992 to 2011) scales.

2.5.1 Calcium supply and deficiency risk

At a global scale, in 2011, Ca supply was 684 ± 211 mg *capita*$^{-1}$ d^{-1} and Ca deficiency risk was 51 ± 32% (3.5 billion people). In 1992, Ca supply was 547 ± 230 mg *capita*$^{-1}$ d^{-1} and Ca deficiency risk was 76 ± 23% (4.1 billion people) (see Supplementary Table S7 online). These reflect an overall increase in global food supply between 1992 and 2011 (Porkka *et al.* 2013, Remans *et al.* 2014). In 2011, the WtdEAR for Ca at a global level was 644 ± 3 mg d^{-1}. At a continental scale, Ca supply in 2011 was 474 ± 188, 858 ± 234, 639 ± 223, 982 ± 130, and 936 ± 50 mg *capita*$^{-1}$ d^{-1} for Africa, Americas, Asia, Europe, and Oceania respectively (see Supplementary Table S8 online). The mean Ca deficiency risk in 2011 was 80 ± 31, 29 ± 27, 57 ± 36, 11 ± 7 and 11 ± 4% for Africa, Americas, Asia, Europe and Oceania respectively (see Supplementary Table S8 online). Regionally, Ca supply in 2011 ranged from 356 ± 295 mg *capita*$^{-1}$ d^{-1} in South-Eastern Asia to 1126 ± 269 mg *capita*$^{-1}$ d^{-1} in Northern America, representing Ca deficiency risks of 98 ± 3 and 4 ± 1% (Fig 2.1 and 2.2, and see Supplementary Table S9 online). At a country level, Ca supply ranged from 157 mg *capita*$^{-1}$ d^{-1} in Mozambique in 1992 to 1640 mg *capita*$^{-1}$ d^{-1} in the United States of America in 1994 (Supplementary Table S6). In 1992, 86 out of 137 countries had Ca deficiency risk >50%, which decreased to 69 out of 145 countries in 2011 (Fig 2.1, and see Supplementary Table S6 online).

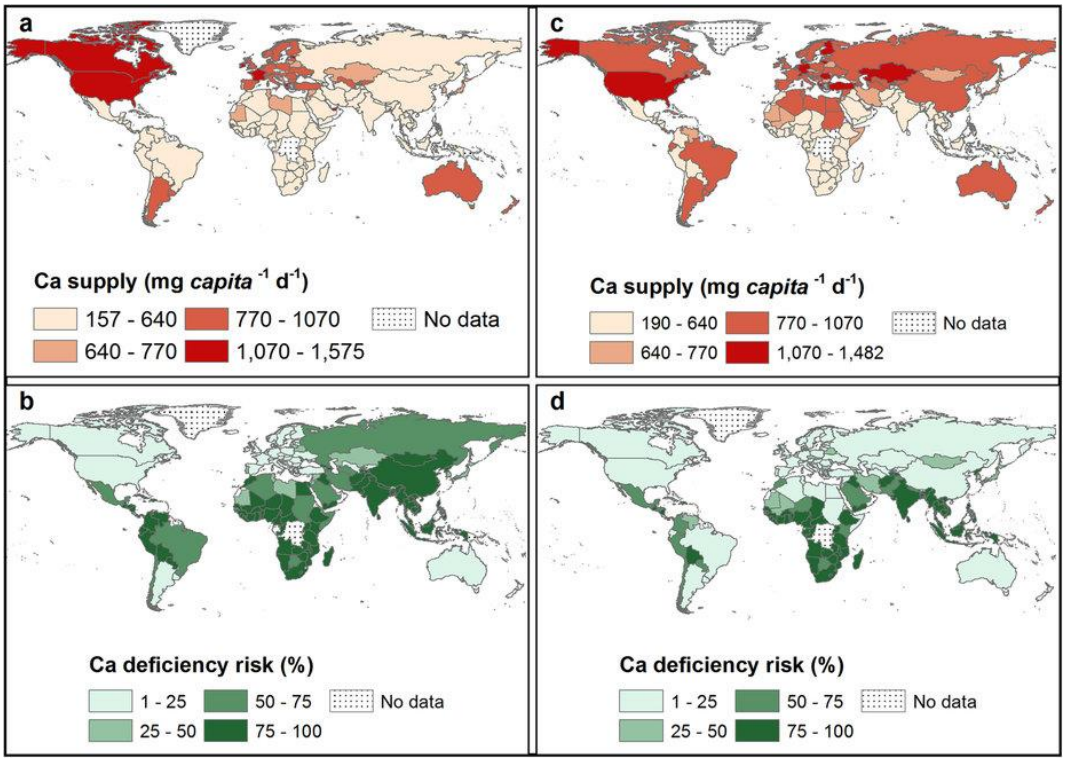


Fig 2.1. Calcium supply in 1992 (a) and 2011 (c). Calcium deficiency risk in 1992 (b) and 2011 (d). Country boundaries were downloaded from the GADM Global Administrative Areas database (http://gadm.org/, Version 2, January 2012). Thematic mapping of Ca supply and deficiency risk was carried out in ArcGIS 10.2.1.

To our knowledge, these are the first global estimates of dietary Ca deficiency risks based on data for food supply, food composition, demography and EAR. In a study conducted in Africa by Joy *et al.* (2014), the deficiency risk of Ca was estimated to be 54%, compared to our estimate of 82% in 2009. This disparity can be attributed to the different food composition table used by Joy *et al.* (2014) because similar food supply, demographic and EAR data were used. However, there is general agreement about the existence of high Ca deficiency risk in Africa between the two studies. Interestingly, in a recent analysis of all age- sex- and country-specific groups from 187 countries (4862 observations), the median Ca intake was 611 (third-quintile range 553-658) mg *capita* d^{-1} (Imamura *et al.* 2015). These data are

based on dietary recall surveys of milk consumption, as a proxy for Ca intake, but are remarkably consistent with our estimates of mean Ca intake based on food supply (Imamura *et al.* 2015).

Animal products were the major sources of dietary Ca, but there is variation between regions (see Supplementary Fig. S1 online). For example, in Central, North and South America, Central Asia, Europe, and Oceania, 50–70% of dietary Ca supply was from animal products. Fruits and vegetables contributed 10–40% of dietary Ca supply, but had a higher contribution in some regions (e.g., >50% in Eastern Asia, see Supplementary Fig. S1 online). Cereals contributed little Ca to the diet although there is enormous difference in Ca concentrations in various cereals. For example, concentrations of Ca in whole grain maize and sorghum from Kenya is 51.2 and 103 mg kg^{-1} dw, respecitvely (Kumssa *et al.* 2017), and whole grain wheat flour from Northern America is 340 mg kg^{-1} (USDA 2013). The changes in the proportional dietary sources of Ca between 1992 and 2011 was small in most of the regions, reflecting food production and supply systems that are generally consistent over the time period.

Given the high prevalence of Ca deficiency risks, an important question is whether these translate into detrimental health outcomes such as rickets, which can be caused by vitamin D and/or Ca deficiency, or osteoporosis. A review by Pettifor (2008) indicated that nutritional rickets was more prevalent in infants and children in developing countries where the diet is based on cereal staples and the phytate content is high. In mid-latitude countries, where there is adequate sunlight to enable the production of vitamin D in the skin, rickets is mainly attributed to Ca deficiency unless exposure to sun is limited due to religious and cultural reasons (Gupta 2014).

Norhaizan and Nor Faizadatul Ain (2009) reported 2.4 million cases of osteoporosis due to Ca deficiency in Malaysia in 2009, where Ca deficiency is likely to be prevalent based on Ca supply. It is noteworthy that when Ca intakes are low, the efficiency of Ca homeostasis can increase through reduced Ca excretion, thereby enabling populations with low intake of Ca to maintain a healthy skeleton and teeth (Nordin 1997). Conversely, in populations with high intakes of animal products with high calcium and protein, and high sodium intakes, urinary Ca excretion increases balancing the plasma Ca (Nordin 1997, Umaretiya *et al.* 2013). Hence the manifestations of Ca deficiency risk in the form of rickets and osteoporosis may not be as conspicuous as the observed Ca deficiency risks. Clearly, there are many complex issues surrounding dietary Ca deficiency risk, which warrant much further investigation.

2.5.2 Zinc supply and deficiency risk

At a global scale, in 2011, Zn supply was 16 ± 3 mg *capita*$^{-1}$ d^{-1}and Zn deficiency risk was 16 ± 14% (1.1 billion people). In 1992, Zn supply was 15 ± 3 mg *capita*$^{-1}$ d^{-1} and Zn deficiency risk was 22 ± 19% (1.2 billion people) (see Supplementary Table S7 online). As seen for Ca, these data reflect an overall increase in global food supply between 1992 and 2011 (Porkka *et al.* 2013, Remans *et al.* 2014). In 2011, the WtdEAR for Zn at a global level was 10.3 ± 0.1 mg *capita*$^{-1}$ d^{-1}. At a continental scale, Zn supply in 2011 was 14 ± 4, 18 ± 3, 15 ± 4, 19 ± 2, and 20 ± 0 mg *capita*$^{-1}$ d^{-1} for Africa, Americas, Asia, Europe, and Oceania respectively (see Supplementary Table S8 online).

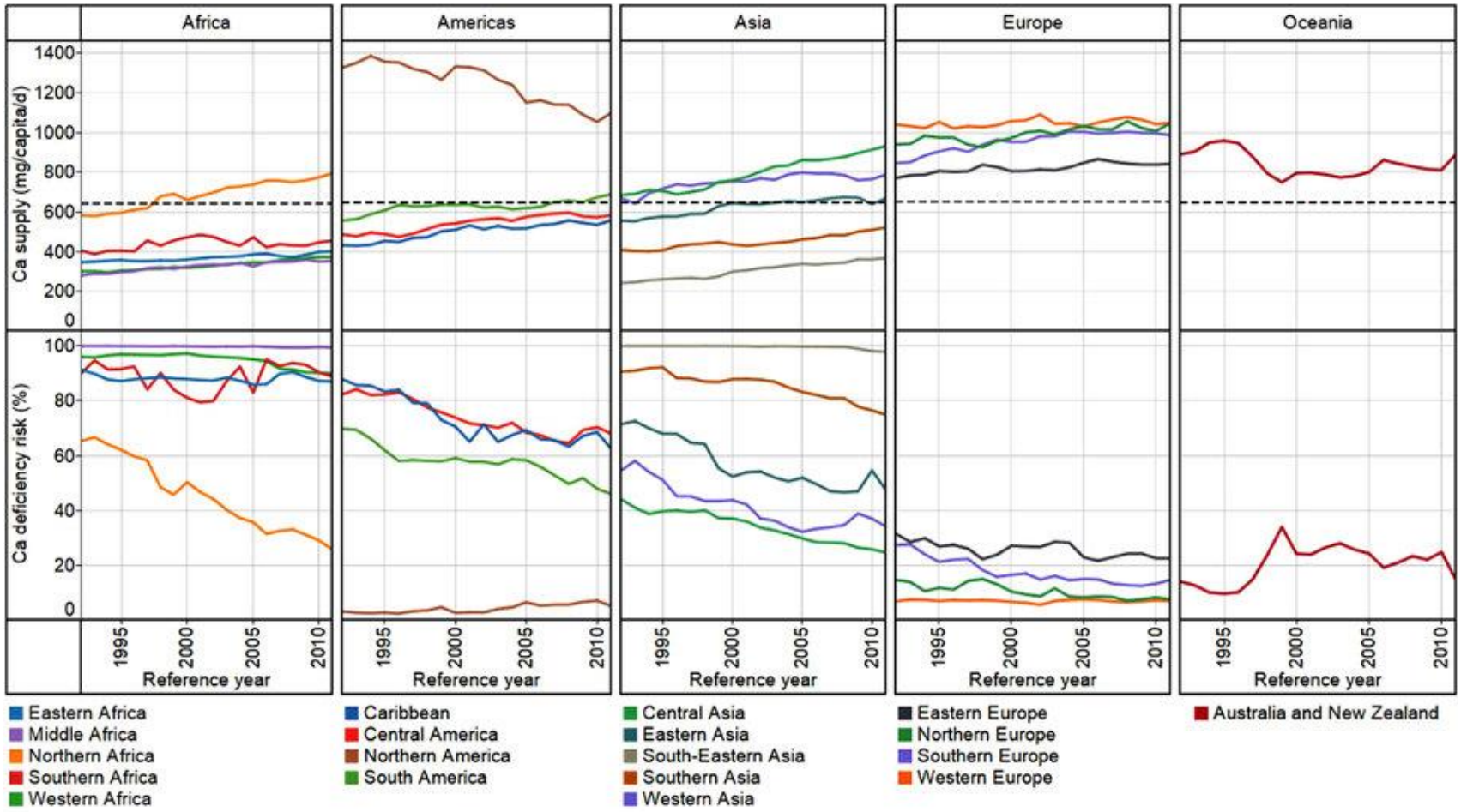
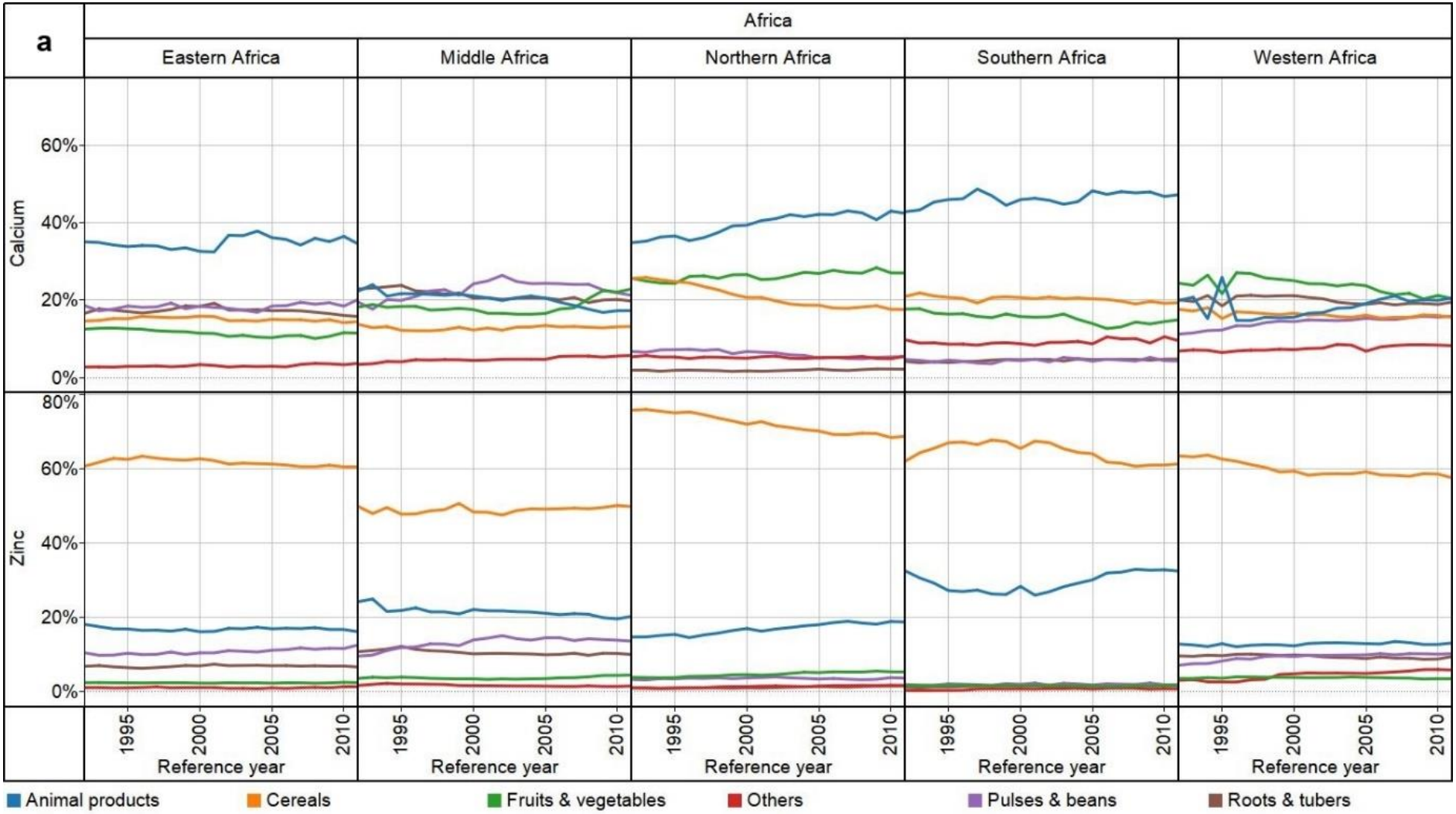


Fig 2.2. Regional temporal trends in population-weighted mean Ca supply (mg capita-1 d-1) and deficiency risk (%) between 1992 and 2011. The horizontal broken lines represent the average WtdEAR for Ca. Line graphs were drawn using Tableau Software for Desktop version 8.2.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Africa
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Middle Africa
Northern Africa
Southern Africa
Western Africa
Calcium
Zinc
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40%
20%
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1995
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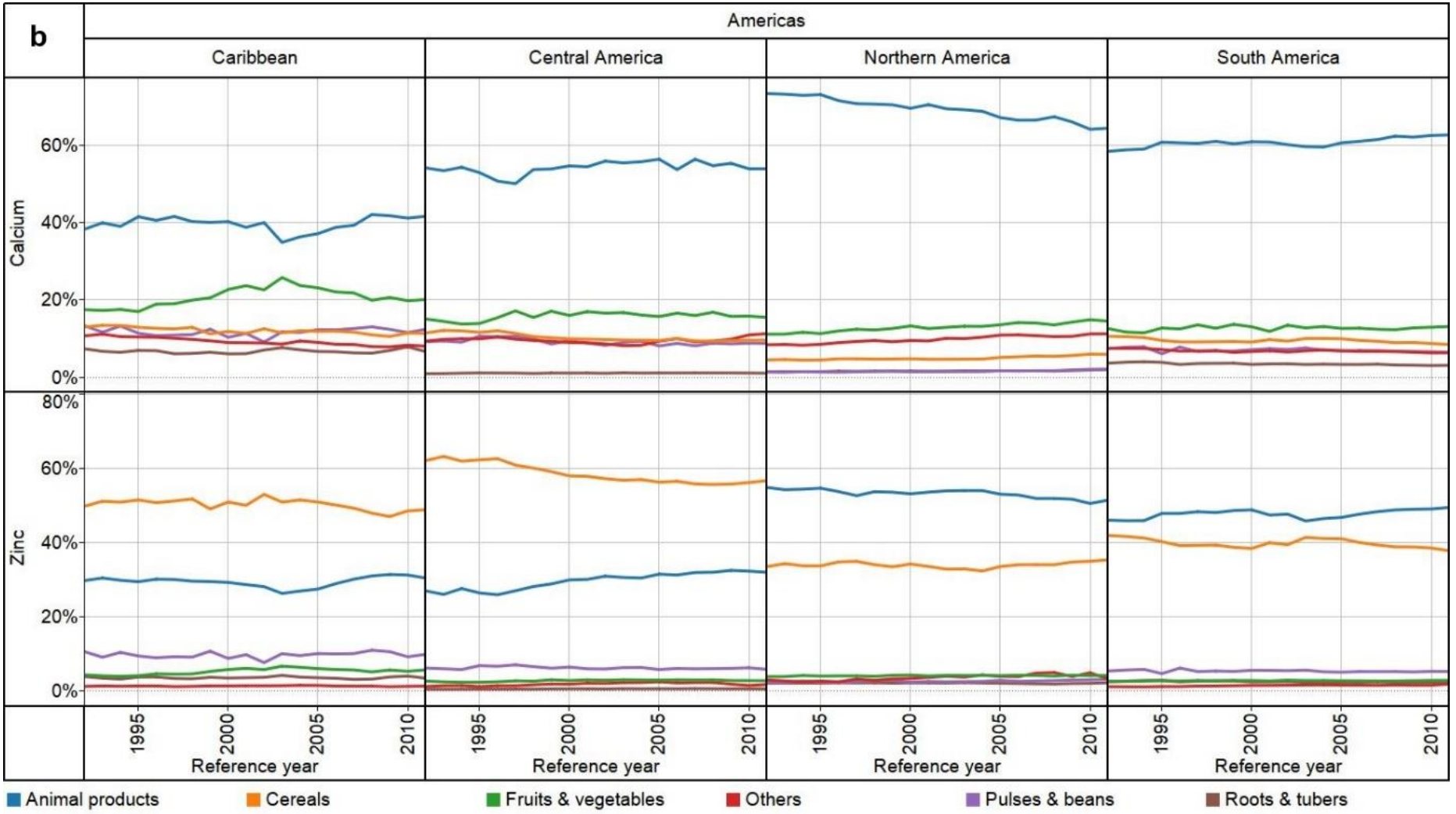
2010
Reference year
Animal products
Cereals
Fruits & vegetables
Others
Pulses & beans
Roots & tubers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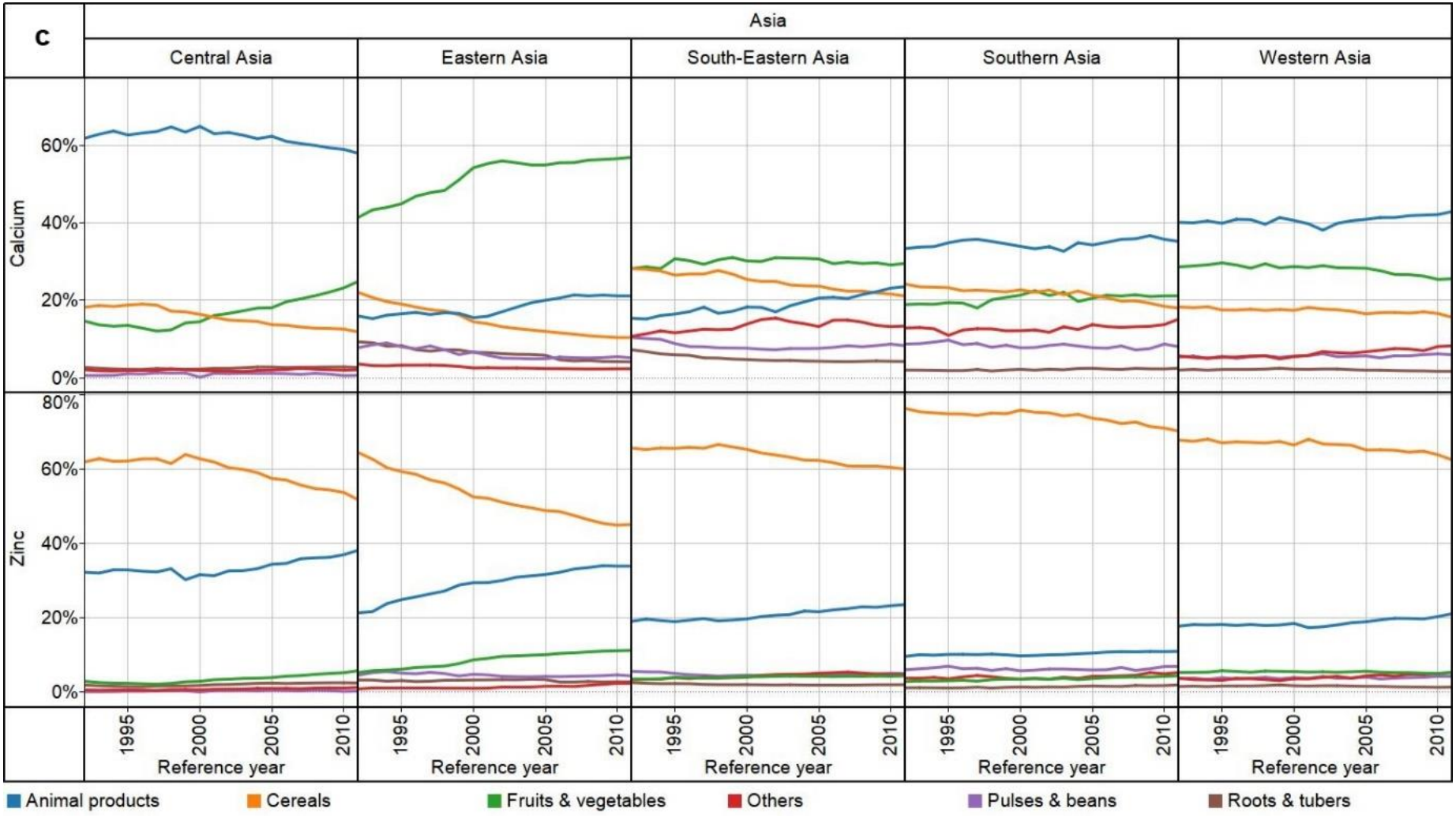
Roots & tubers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Central Asia
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Animal products
Cereals
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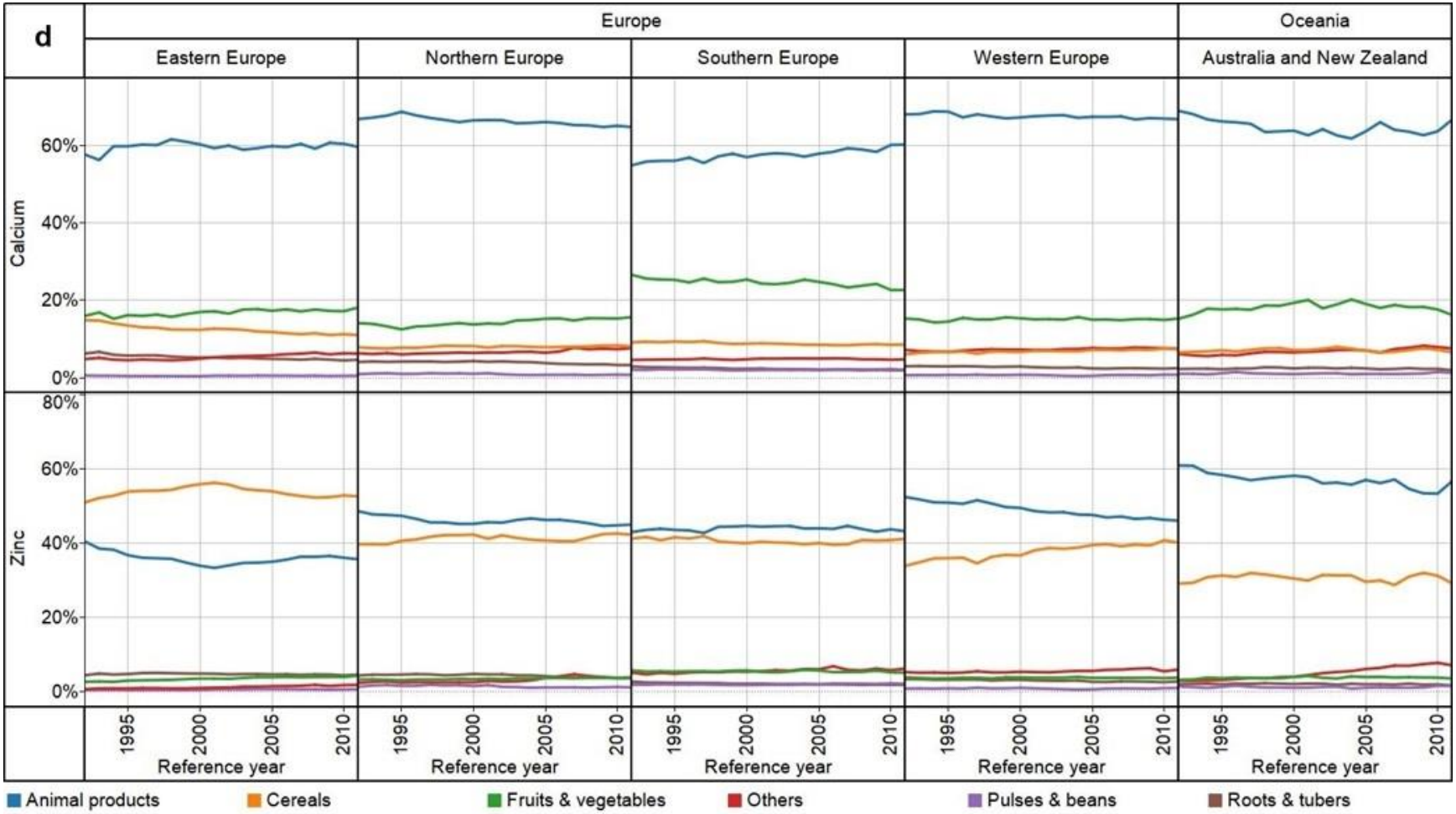


Fig 2.3. Trends in the percentage contribution of food groups to Ca and Zn supply between 1992 And 2011. Africa (a), Americas (b), Asia (c), and Europe and Oceania (d). National-level data were weighted by population size in each country.

The mean Zn deficiency risk in 2011 was 25 ± 20%, 7 ± 10%, 17 ± 14%, 3 ± 2%, and 2 ± 0% for Africa, Americas, Asia, Europe and Oceania respectively. Regionally, Zn supply in 2011 ranged from 12 ± 6 mg *capita*$^{-1}$ d^{-1} in Caribbean to 22 ± 7 mg*capita*$^{-1}$d^{-1} in Central Asia, Zn deficiency risks ranged from 2 ± 0% in Australia and New Zealand to 36 ± 38% in Caribbean (Fig 2.4 and 2.5,and see Supplementary Table S9 online). At country level, Zn supply ranged from 6 mg *capita*$^{-1}$ d^{-1} in Rwanda in 1998 to 28 mg *capita*$^{-1}$ d^{-1} in Uruguay in 1994, representing Zn deficiency risks of ~100% and 1% respectively (see Supplementary Table S6 online). In 1992, 48 out of 137 countries had Zn deficiency risk >25%, which decreased to 39 out of 145 countries in 2011 (Fig 2.4 and see Supplementary Table S6 online).

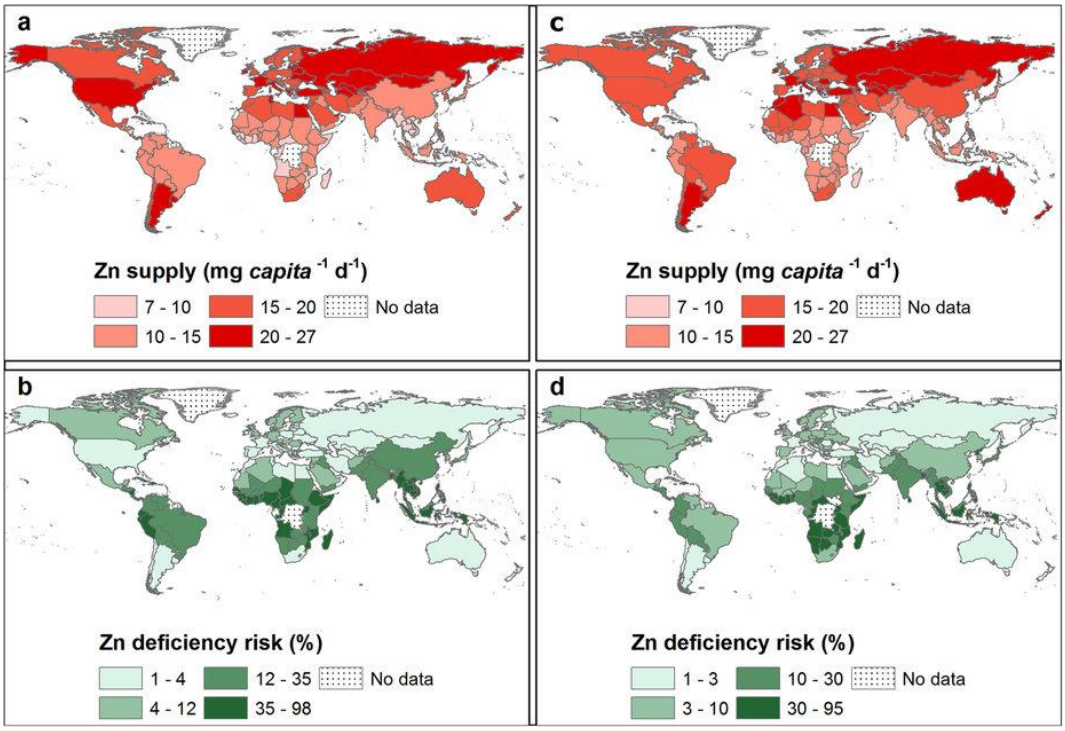


Fig 2.4. Zinc supply in 1992 (**a**) and 2011 (**c**). Zinc deficiency risk in 1992 (**b**) and 2011 (**d**). Country boundaries were downloaded from the GADM Global Administrative Areas database (http://gadm.org/, Version 2, January 2012). Thematic mapping of Zn supply and deficiency risk was carried out in ArcGIS 10.2.1.

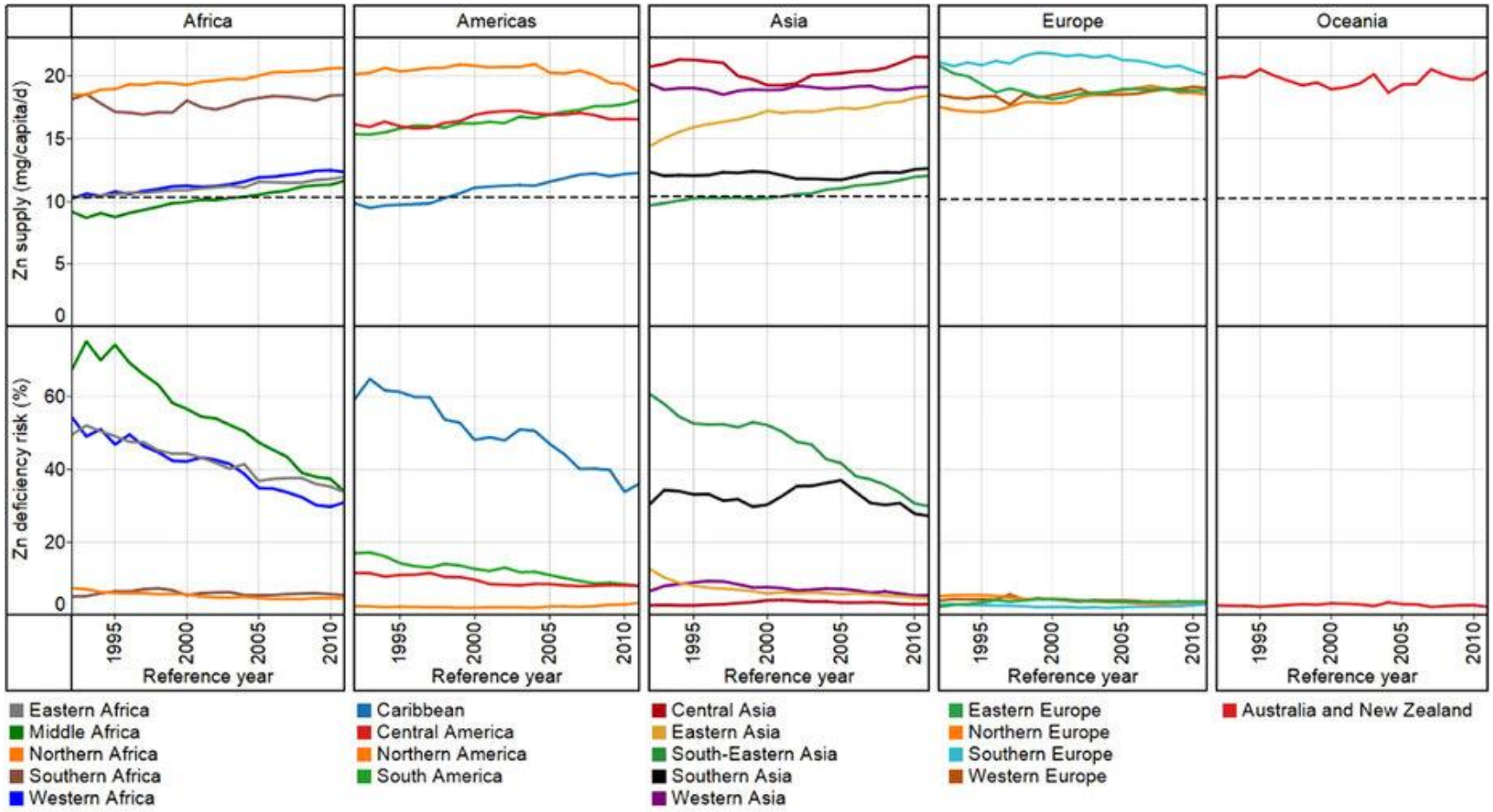


Fig 2.5. Regional temporal trends in population-weighted mean Zn supply (mg *capita*$^{-1}$ d^{-1}) and deficiency risk (%) between 1992 and 2011. The horizontal broken lines represent the average WtdEAR for Zn. Line graphs were drawn using Tableau Software for Desktop version 8.2.

Our estimate of Zn deficiency risk (20%) is comparable to (Wessells *et al.* 2012a, Wessells *et al.* 2012b) global estimate of 17.3% in 2003-07 where they accounted for the impact of various factors to Zn absorption in their model. Joy *et al.* (2014) estimated the Zn deficiency risk for Africa in 2009 to be 40% as compared to our estimate of 26%. This discrepancy, as for Ca, is due to the different food composition data used.

Dietary sources of Zn varied across regions. Animal products were the major sources of Zn (>40%) in North Africa; North and South America; North, South and West Europe; and Australia and New Zealand. In other regions, the major source of Zn was cereals (Fig 2.3). The prevalence of deficiency risk and utilization of Zn is influenced by the quantity of Zn intake, and the overall dietary composition that either promotes or antagonises Zn bioavailability (Gibson 2012, Wessells *et al.* 2012a, Wessells *et al.* 2012b). Among the anti-nutrient factors that reduce the absorption of Zn is the phytate (myoinositol hexakisphosphate). Phytate chelates with mono and divalent cations such as Zn. Due to the absence of intestinal digestive phytases, humans cannot digest phytate and hence chelated Zn cannot be utilized (Ma *et al.* 2007, Kumar *et al.* 2010). In 2011, dietary phytate supply ranged from 997 in Ecuador to 4179 mg $capita^{-1}$ d^{-1} in Niger and 25-80% of the Zn supply in all regions originated from cereals which have high phytate content (Fig 2.3, and see **Supplementary Table S6** online). Phytate:Zn (PA:Zn) ratios ranged between 5 and 27 (Fig 2.6). Phytate:Zn ratio for most countries in this study were >15, the critical threshold level beyond which Zn absorption is considered to be inhibited (Bindra *et al.* 1986, Ellis *et al.* 1987, Adams *et al.* 2002, Ma *et al.* 2007). This will compound the Zn deficiency risk of the population due to lower bioavailability (Gibson 2005, 2006, 2012). Populations relying on plant-sourced foods with low

Ca and Zn are also vulnerable to toxic metals that have similar properties to Ca and Zn, for example cadmium (Cd) (Reeves and Chaney 2004). Deficiencies in Ca and Zn may lead to organ accumulation and retention of dietary Cd (Lalor 2008).

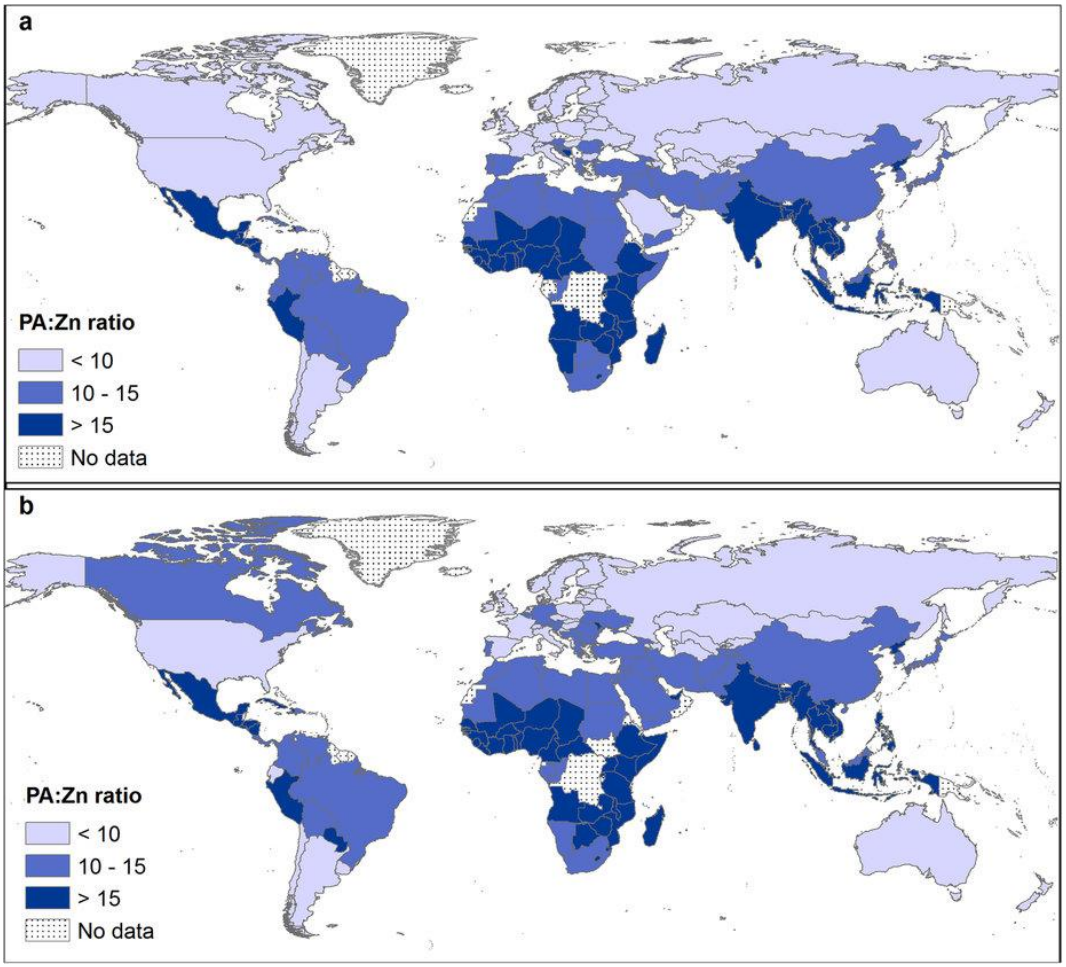


Fig 2.6. Phytate:Zn (PA:Zn) molar ratio in 1992 (a) and 2011 (b), based on per capita PA and Zn supplies. Country boundaries were downloaded from the GADM Global Administrative Areas database (**http://gadm.org/**, Version 2, January 2012). Thematic mapping of PA:Zn ratio was carried out in ArcGIS 10.2.1.

2.5.3 Per capita income, Ca and Zn supplies, and deficiency risks

Calcium and Zn supplies were highly positively correlated with per capita Gross National Income based on purchasing power parity (GNI-PPP), across all countries and years (Table 2.1). Countries with lower GNI-PPP had higher Ca and Zn

deficiency risks than those with high per capita GNI-PPP (see **Supplementary Table S10** online), with strong positive correlations observed between GNI-PPP and Ca and Zn supplies (Table 2.1). These relationships imply that those living in countries with higher GNI-PPP purchase or produce more animal products, fruits, legumes and vegetables than those with lower GNI-PPP, which is consistent with observations from household surveys (Ecker *et al.* 2011). The green revolution of the 1960s was supported by high rates of investment in research on staple foods (mainly wheat, rice and maize) (Khush 2001, Pingali 2012). Lower-yielding cereal and legume landraces that were potentially better sources of essential micronutrients (e.g., Fe, Zn, Ca, and potassium) have generally been replaced with higher yielding cereal varieties (Welch *et al.* 1999, Khoury *et al.* 2014). This general decline in agro-biodiversity might therefore have compounded MNDs (Pingali 2012).

Table 2.1. Relationship between Ca, Zn and energy supply, phytate:Zn molar ratio, and GNI-PPP. Based on 2655 data points from 1992-2011 (see Supplementary Table S10 online). **Spearman's Rank Correlation is significant at $p \leq 0.01$. GNI-PPP is gross national income converted to international dollars using purchasing power parity rates. An international dollar has the same purchasing power as a US Dollar in the US.

	Ca supply	Zn supply	Phytate: Zn	GNI-PPP
Zn supply	0.817**			
Phytate:Zn	−0.775**	−0.714**		
GNI-PPP	0.755**	0.604**	−0.682**	
Energy supply	0.832**	0.770**	−0.641**	0.825**

2.5.4 Calcium and Zn deficiency risks and Millennium Development Goal 1

At a global scale, if the Millennium Development Goal 1 (MDG1) (UN 2013a) target to halve hunger by 2015 is framed in terms of Ca deficiency risks, the 76%

risk observed in 1992 should have decreased to 46% in 2011. However, the Ca deficiency risk was 51% in 2011. For Zn, the 22% risk in 1992 should have decreased to 13% in 2011 but was 16%. National level reductions in Ca and Zn deficiency risks were uneven. By 2011, 101 and 86 countries were not on target to halve Ca and Zn deficiency risks, respectively, by 2015 (see Supplementary Table S11 online). Out of these; 34, 19, 26, 20, and 2 countries fell short of halving Ca deficiency risks and 22, 14, 22, 26, and 2 countries fell short of halving Zn deficiency risks, from Africa, Americas, Asia, Europe and Oceania respectively.

2.5.5 Conclusion and limitations

In 2011, 3.5 and 1.1 billion people were at risk of Ca and Zn deficiency respectively. To our knowledge, these are the first global estimates of dietary Ca deficiency risks based on food supply. Our estimates of Zn deficiency risks are consistent with recent studies (Wessells *et al.* 2012a, Wessells *et al.* 2012b). Supplies and deficiency risks of Ca and Zn differed geographically with countries in Asia and Africa accounting for >90% of the estimated Ca and Zn deficiency risks. At higher spatial resolution, differences in Ca and Zn supplies and deficiency risks between individual countries become pronounced.

This study uses data from several sources, each of which has its limitations. For example, food supply data are aggregated at national level with a fixed coefficient of variation in intake. Such data do not capture community and household level socio-economic factors which systematically affect intake, nor food wastage at a household level, nor seasonal variation in the type and quantity of food supply. All of these factors will, of course, affect deficiency risks for Ca and Zn. In addition, the rise in awareness of the roles that mineral micronutrients play in human health

has led to increased research and development to biofortify food crops with micronutrients (Yip 1997, Yang *et al.* 2007, Cakmak 2008, Broadley *et al.* 2009, White and Broadley 2009, Zhao and McGrath 2009, Bouis and Welch 2010, Rahman *et al.* 2013, Joy *et al.* 2015c). This may lead to change in the food composition in addition to future increases in food supply due to improvement in crop yields. However, the impact of changes in food composition on the reduction of deficiency risk of Ca and Zn due to fortification either through agronomic interventions or breeding could not be captured by this study mainly due to a lack of temporal, spatial, and varietal food composition data. Similarly, the effect of Ca fortification in cereals and milk is not addressed in this study due to lack of data. Therefore, whilst the information presented here can inform policy in a general sense, such analyses would become more useful at higher geospatial resolution (for example, at subnational levels). Further research could include assessing the health, and nutritional status of various age/gender/socioeconomic groups through biochemical, clinical and anthropometric measurements in countries with high deficiency risks of Ca and Zn (WHO *et al.* 2004). Development of localized food composition tables and updating existing ones with information on new/under-utilised food crop varieties is crucial to improve the accuracy of estimating deficiency risks of Ca and Zn.

Possible solutions to Ca and Zn deficiency include: supplementation, direct fortification (Yip 1997, Zimmermann 2013), fertiliser application (Broadley *et al.* 2009, White *et al.* 2009, Joy *et al.* 2015c) and plant breeding (Cakmak 2008). Supplementation is crucial in situations that require short term actions with high impact, for example, for pregnant women. However, supplementation and direct fortification of foodstuffs with Ca and Zn may not be economically feasible and

cannot reach the majority of the population in developing countries who produce their own food (Pingali 2012). Under such circumstances, agronomic intervention by applying Zn fertiliser either through foliar routes or to the soil can help increase the composition of Zn in food crops (Joy *et al.* 2015c). Similarly, breeding interventions through developing food crop varieties with the ability to absorb and accumulate more Zn and Ca from the soil and translocate them to the edible parts, or with lower phytate composition (Raboy 2009), can potentially be pursued to increase the bioavailability of these nutrients. In addition, the production and provision of affordable animal products, and education on how to reduce the impact of phytate in plant source foods on Zn bioavailability (for example, soaking, germination, and fermentation) are essential (IOM 2000b). We conclude that continuing to reduce Ca and Zn deficiency risks through dietary diversification (Pingali 2012) and food and agricultural interventions including fortification (Zimmermann 2013), crop breeding (Cakmak 2008) and use of micronutrient fertilisers (Rahman *et al.* 2013, Joy *et al.* 2015c) will remain a significant challenge in the post-Millennium Development Goals (MDGs) era.

CHAPTER 3. GLOBAL MAGNESIUM SUPPLY IN THE FOOD CHAIN

3.1 Authors contribution

DBK, MRB, ELA and EJMJ designed the study. **DBK** collated the data, designed and developed the database, carried out data analyses and produced the initial draft of the manuscript. MJW, SDY, SW and MRB contributed to drafting the manuscript.

3.2 Abstract

Magnesium (Mg) is an essential mineral micronutrient in humans. Risks of dietary Mg deficiency are affected by the quantity of Mg ingested and its bioavailability, which is influenced by the consumption of other nutrients and 'antinutrients'. Here, we assess global dietary Mg supplies and risks of dietary deficiency, including the influence of other nutrients. Food supply and food composition data were used to derive the amount of Mg available per capita at national levels. Supplies of Mg were compared with estimated national per capita average requirement 'cut points'. In 2011, global weighted mean Mg supply was 613 ± 69 mg person^{-1} day^{-1} compared with a weighted estimated average requirement for Mg of 173 mg person^{-1} day^{-1}. This indicates a low risk of dietary Mg deficiency of 0.26% based on supply. This contrasts with published data from national individual-level dietary surveys, which indicate greater Mg deficiency risks. However, individuals in high-income countries are likely to under-report food consumption, which could lead to overestimation of deficiency risks. Furthermore, estimates of deficiency risk based on supply do not account for potential inhibitors of Mg absorption, including calcium, phytic acid and oxalate, and do not consider household food wastage.

3.3 Introduction

Magnesium (Mg) is an essential mineral micronutrient in humans, required for a variety of physiological functions. The recommended nutrient intake for men 19–65 years old is 260 mg day^{-1} (WHO *et al.* 2004). A healthy adult contains ~24 g Mg, mainly in bone, muscle and soft tissues (Ebel and Günther 1980, Elin 1987, Vormann 2003, WHO *et al.* 2004). Magnesium is a cofactor in >350 enzymatic reactions, with roles including protection from oxidative stress, and metabolism of calcium (Ca), vitamin D and potassium (Ebel *et al.* 1980, Elin 1987, WHO *et al.* 2004, Atkinson *et al.* 2009, Broadley *et al.* 2012, Deng *et al.* 2013, Das 2014, Dibaba *et al.* 2014, Rodriguez-Moran and Guerrero-Romero 2014).Deficiency in Mg can manifest as metabolic syndrome (Gartside and Glueck 1995, Hata *et al.* 2013, Rosanoff and Plesset 2013, Cosaro *et al.* 2014, Ju *et al.* 2014, Panhwar *et al.* 2014), lower bone-mineral density (Orchard *et al.* 2014), premenstrual syndrome (Elin 1987), and attention deficit hyperactivity disorder (Blaszczyk and Duda-Chodak 2013).

Magnesium is obtained primarily from food sources, although drinking and cooking water can make important contributions. depending on its hardness and the volume of water consumed (Ong *et al.* 2009). Median dissolved Mg concentrations of North American spring, mineral, and groundwater from various regions ranged from 0 to 130 mg L^{-1} (Azoulay *et al.* 2001). Magnesium contents of some commercially available bottled waters in Europe were, for example, 36, 110, and 128 mg L^{-1} for Abbey Well from the UK, Vichy Nouvelle from Finland, and Robacher from Germany, respectively (Azoulay *et al.* 2001). However, unrefined cereals, legumes and green leafy vegetables are the primary dietary sources of Mg (White *et al.* 2009,

Blaszczyk *et al.* 2013). The bioavailability and absorption of ingested Mg is affected by other nutrients and 'anti-nutrients'. For example, high concentrations of phytate in cereal and legume seeds, and oxalate in some leafy vegetables, can reduce Mg absorption through chelation in the gut (Brink and Beynen 1991, Bohn *et al.* 2004a). Addition of 1.5 mmol of phytic acid (PA, in dodecasodium salt hydrate form) to white bread reduced Mg absorption from 33% to 13% in human feeding studies (Bohn *et al.* 2004b). Similarly, Bohn *et al.* (2004a) reported that Mg absorption from a meal containing oxalate-rich spinach (*Spinacia oleracea* L.) was 27%, compared with 37% from a meal containing kale (*Brassica oleracea* L.), which has a lower oxalate concentration. Fractional Mg absorption of 44% (Sabatier *et al.* 2003) and 35% (Marshall *et al.* 1976) has been reported in typical Western diets. A study on rats showed that increased Ca intake led to reduced intestinal absorption and renal re-absorption of Mg (Bertinato *et al.* 2014), although Palacios *et al.* (2013) reported no effects of Ca intake on urinary Mg excretion in females aged 11–15 years. Nonetheless, absorption of Mg is under homeostatic control and can increase when there is deficiency of Mg in the human body (Hansen *et al.* 2014).

Dietary Mg intake and the prevalence of deficiency risks can be estimated from tissue biomarkers, food recall or food balance sheets (FBSs) (Ford and Mokdad 2003, Broadley *et al.* 2012, Joy *et al.* 2012, Joy *et al.* 2014, Ju *et al.* 2014, Rodriguez-Moran *et al.* 2014). However, the accuracy of estimates of the prevalence of Mg deficiency risks, using tissue biomarkers, suffers from the lack of a reliable index (Reinhart 1988, Hansen *et al.* 2014, Ong *et al.* 2014). Estimates based on dietary intakes are preferred, particularly for wide-scale assessment. Dietary recall studies suggest high risks of Mg deficiency. For example, ~60% of the USA population were reported to consume Mg below an estimated average

requirement (EAR) of 330 mg person^{-1} day^{-1} for men aged 19–30 based on the National Health and Nutrition Examination Survey (NHANES) 24-h dietary recall in 1999 and 2000 (Ford *et al.* 2003, Dibaba *et al.* 2014). The EAR is the daily nutrient intake estimated to meet the requirements of half of the healthy individuals in a given age and sex-specific population (IOM 2000b). During the 2001–02 NHANES, 64% and 67% of men and women 19 years of age, respectively, had Mg intake less than the EAR (Moshfegh *et al.* (2005) cited in (Rosanoff 2010). Similarly, in the UK, the National Diet and Nutrition Survey (NDNS) from 2008–09 to 2011–12 reported that 53% of females aged 11–18 years, 14% of adults aged 19–64 years and 19% of males 65 years had dietary Mg intakes below their lower reference nutrient intake (LRNI), as measured using a 4-day diary dietary record (Bates *et al.* 2014). The LRNI is an intake level sufficient for <2.5% of the age- and sex-specific population group and is 190 mg person^{-1} day^{-1} for all people aged 15–18 years and adult males. However, dietary-recall or diary methods are known to be affected by misreporting, especially under-reporting in developed countries, and behavioural change (Bingham *et al.* 1994, IOM 2000b, Rennie *et al.* 2004, Rennie *et al.* 2005, Mirmiran *et al.* 2006, Rennie *et al.* 2007, Liberato *et al.* 2009, Archer *et al.* 2013, Bates *et al.* 2014, Winkler 2014). In addition, dietary survey data are lacking in many developing countries (Gibson 2005), as well as site specific and relevant food composition data to determine the Mg concentrations of foods consumed (Joy *et al.* 2014, Kumssa *et al.* 2015a).

Global-scale estimates of Mg supply and deficiency risks have not been reported. However, estimates of mean global Ca supply in 2011 of 684 ± 211 mg person^{-1} day^{-1} by Kumssa *et al.* (2015a) based on FBSs were similar to those of Imamura *et al.* (2015), who estimated median Ca intakes of 611 mg person^{-1} day^{-1} (third

quintile range 553–658) from a large meta-analysis of milk consumption as a proxy for Ca intake, based primarily on dietary recall data. The global risk of zinc (Zn) deficiency has been estimated, based on FBS supply, to be 16% in 2011 by Kumssa *et al.* (2015a) and 17% in 2003–07 by Wessells *et al.* (2012a). In Africa, a mean continental Mg supply of 678 mg person^{-1} day^{-1} was estimated from FBSs and African food composition data, with a 0.7% prevalence of deficiency risk (Joy *et al.* 2014). The aims of the present study were (*i*) to estimate the global risk of dietary Mg deficiency based on food supply, composition and demographics; and (*ii*) to assess the potential impact of the supply of other components of human diet that might affect the bioavailability of Mg.

3.4 Materials and methods

Methods are identical to those described previously for estimating the risks of dietary Ca and Zn deficiencies (Kumssa *et al.* 2015a). Briefly, secondary data for food supply, food composition, demography and EAR for Mg were integrated for 145 countries with populations >1 million by using food-supply and demographic data from 1992 to 2011. The EAR 'cut-point' (EAR-CP) method was used to assess the prevalence of Mg deficiency risks.

3.4.1 Data sources

The four types of datasets required for this study were food supply, food composition, the EAR for Mg, and national demographic data. Per capita food supply data for 94 food items were obtained from the Food and Agriculture Organisation of the United Nations (FAO) Statistics Division (FAOSTAT) website for the years 1992–2011 (FAO 2014). Food composition data were obtained from the United States Department of Agriculture (USDA) National Nutrient Database

for Standard Reference 26 (USDA SR26), which was released in 2013 (USDA 2013). The EARs for Mg were obtained from the World Health Organisation (WHO) and FAO vitamin and mineral requirements (WHO *et al.* 2004). Demographic data were obtained from the United Nations Department of Economic and Social Affairs Population Division, Population Estimates and Projection (UN 2013b). Spatial aggregation of countries was made based on FAO regional and continental classification (http://faostat.fao.org/site/371/default.aspx). Income level aggregation was obtained from the World Data Bank, World Development Indicators in February 2015, and countries are kept within the same group from 1992–2011 (http://databank.worldbank.org/data/reports.aspx?source=World-Development-Indicators).

3.4.2 Magnesium supply

The 94 food items from the FAOSTAT food supply (g person^{-1} day^{-1}) data (see Supplementary Materials table S1, available on the Journal's website) were matched *sensu* (Stadlmayr *et al.* 2011) with the Mg composition of fresh and/or uncooked food commodities in the nutrient composition data. The nutrient composition of food items was assumed not to change with time or location. Per capita Mg supply from each food item in each country was calculated by multiplying the per capita food supply by its nutrient concentration. Magnesium supply from each food commodity was summed within country to obtain the per capita nutrient supply at a national level. Magnesium supplies from fortification and supplements, and drinking and cooking water were not accounted for in this study.

3.4.3 Magnesium intakes and requirements

Magnesium intakes were estimated as the mean per capita Mg supply at a national level, with an inter-individual coefficient of variation of 25% (Joy *et al.* 2012, Wessells *et al.* 2012a). The EAR for Mg is available according to age (~5-year groupings) and gender classes (WHO *et al.* 2004). Thus, a national weighted EAR was calculated (WtdEAR) following the procedure in Equation 2.1 for Ca and Zn, using the population size in each age and gender group for each country and year. For a given age or gender group, the EAR was assumed to remain unchanged whereas the WtdEAR varied with the population structure, which in turn varied between countries and years. The WtdEAR for Mg is hence assumed to approximate the per capita intake that fulfils the Mg requirements of half of the healthy individuals in a population of a given country in a specific year.

3.4.4 Estimated average requirement 'cut-point'

The prevalence of Mg-deficiency risk was assessed using the EAR-CP as described and used by Carriquiry (1999), Wuehler *et al.* (2005), Joy *et al.* (2014) and Kumssa *et al.* (2015a). The EAR-CP method yields an estimate of the number of people in a given country and year with intakes of Mg below the WtdEAR, which is termed hereafter as the 'deficiency risk'. The EAR-CP method has been applied with the following underlying assumptions: (*i*) little correlation between requirement and intake; (*ii*) the distribution of requirement is symmetrical around the EAR; and (*iii*) variability in intake is greater than the variability in requirement (IOM 2000b).

3.4.5 Nutritional ratio

Dietary Ca and phytate, which represents the mixed salts of PA, or *myo*-inositol hexakisphosphate, were calculated in a similar manner to Mg. The PA and Ca data are those presented previously (Kumssa *et al.* 2015a). The Ca : Mg ratio was calculated on a gravimetric basis (Rosanoff 2010), whereas the Mg : PA ratio was derived from the molar weights (Mg = 24.3 g mol^{-1}, PA = 660 g mol^{-1}) (Cheryan *et al.* 1983).

3.4.6 Aggregating information

Spatial aggregation (i.e. regional, continental, global) and income level aggregations (i.e. low income, lower middle income, upper middle income, high income) of the mean and standard deviation (s.d.) of Mg supply, WtdEAR, and deficiency risk, and Ca : Mg and Mg : PA ratios, were weighted by the national population size. (see example Equation 2.2) Aggregated information is presented as mean ± s.d. unless specified.

3.4.7 Data analyses and visualisation

Datasets were compiled using Microsoft Excel 2013 and exported to Microsoft Access 2013 (Microsoft Corp., Redmond, WA, USA) to make a relational database. The database was queried to extract the per capita Mg supply, and the WtdEAR for Mg. The risk of Mg deficiency during the 20-year period was then calculated in Microsoft Excel. Visualisations and calculations of descriptive statistics were carried out in Tableau Software for desktop version 8.3 (Tableau Software, Seattle, WA, USA), GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA) and ArcGIS 10.2.1 (ESRI, Redlands, CA, USA). Country boundaries for thematic

mapping were obtained from the GADM Global Administrative Areas database (http://gadm.org/, Version 2; accessed January 2014).

3.5 Results

3.5.1 Magnesium supply and deficiency risk

Globally, the weighted mean Mg supplies were 558 ± 61 person^{-1} day^{-1} in 1992 and 613 ± 69 mg person^{-1} day^{-1} in 2011, and the respective weighted mean WtdEARs were 166 ± 3 and 173 ± 3 mg person^{-1} day^{-1}. Consequently, 0.37% and 0.26% of the population were likely at risk of dietary Mg deficiency in 1992 and 2011, respectively. Globally in 2011, the number of people likely to be at risk of Mg deficiency was ~14 million based on supply data (Fig 3.1 and Supplementary table S2[12]).

At a continental level in 2011, the supplies of Mg in Africa, the Americas, Asia, Europe and Oceania, respectively, were 653 ± 95, 556 ± 45, 615 ± 69, 627 ± 54 and 552 ± 4 mg person^{-1} day^{-1}; the WtdEARs for Mg were 159 ± 3, 174 ± 3, 174 ± 3, 180 ± 1 and 178 ± 1 mg person^{-1} day^{-1}; and the risks of Mg deficiency were 0.19%, 0.33%, 0.26%, 0.24% and 0.33% (Supplementary table S3). In Africa, the Americas, Asia, Europe and Oceania, respectively, the number of people at risk of Mg deficiency in 2011 was 1.2, 2.8, 8.6, 1.6 and 0.1 million (Supplementary table S3). Regionally in 2011, Mg supplies ranged from 492 ± 34 person^{-1} day^{-1} for Southeast Asia to 848 ± 114 mg person^{-1} day^{-1} for Northern Africa. The risk of Mg deficiency in 2011 ranged from 0.08% in Northern Africa to 0.64% in Caribbean

[12] Supplementary information are available at http://www.publish.csiro.au/CP/Fulltext/CP15096

(Fig 3.2 and Supplementary table S4). At a country level, the supply of Mg in 2011 ranged from 340 to 944 mg person^{-1} day^{-1} (Fig 3.3 and Supplementary table S5). In 1992, the supplies of Mg ranged from 460 ± 93 mg person^{-1} day^{-1} in low-income countries to 594 ± 57 mg person^{-1} day^{-1} in high-income countries, with respective deficiency risks of 0.75% and 0.27%. In 2011, the supplies of Mg ranged from 549 ± 103 mg person^{-1} day^{-1} in low-income countries to 679 ± 107 mg person^{-1} day^{-1} in upper middle-income countries, with respective Mg deficiency risks of 0.35% and 0.21% (Fig 3.4 and Supplementary table S6).

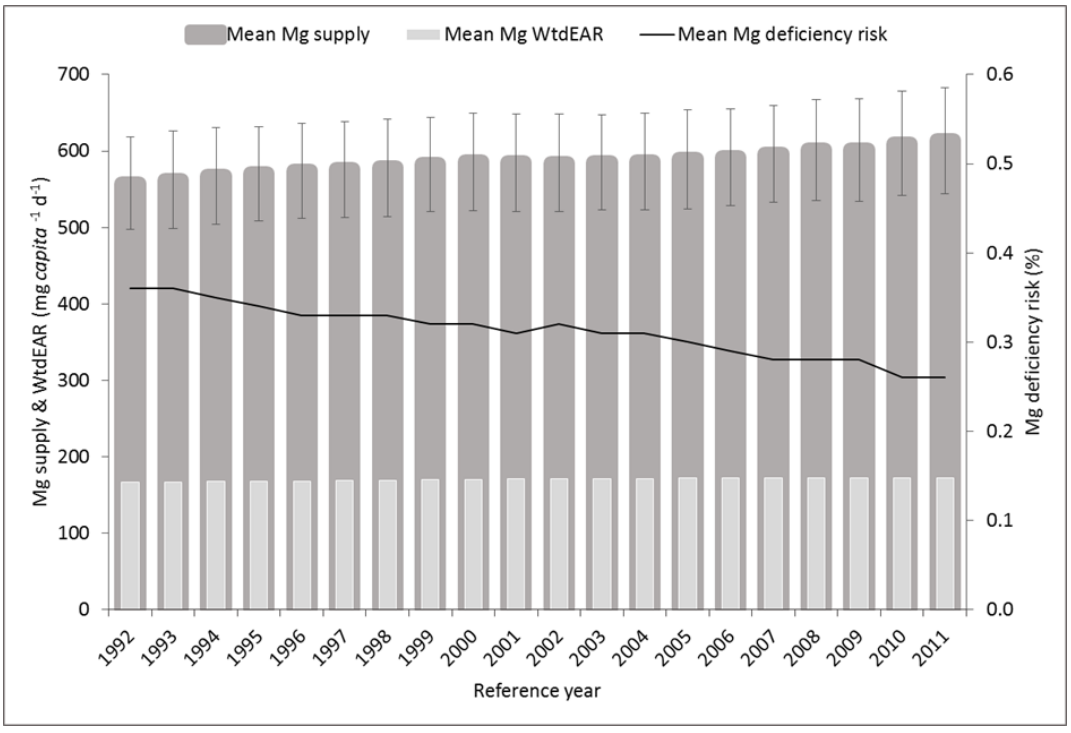


Fig 3.1. Global weighted mean magnesium (Mg) supply, weighted estimated average requirement (WtdEAR) and deficiency risk between 1992 and 2011. Capped lines are ± standard deviation.

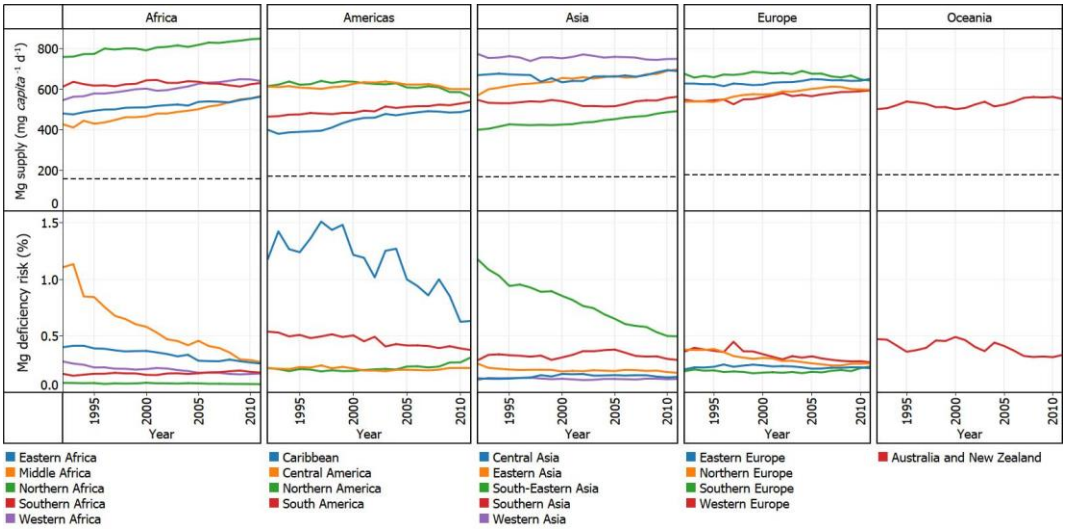


Fig 3.2. Regional population-weighted mean magnesium (Mg) supply and deficiency risk between 1992 and 2011. Horizontal broken lines represent the population-weighted mean weighted estimated average requirement.

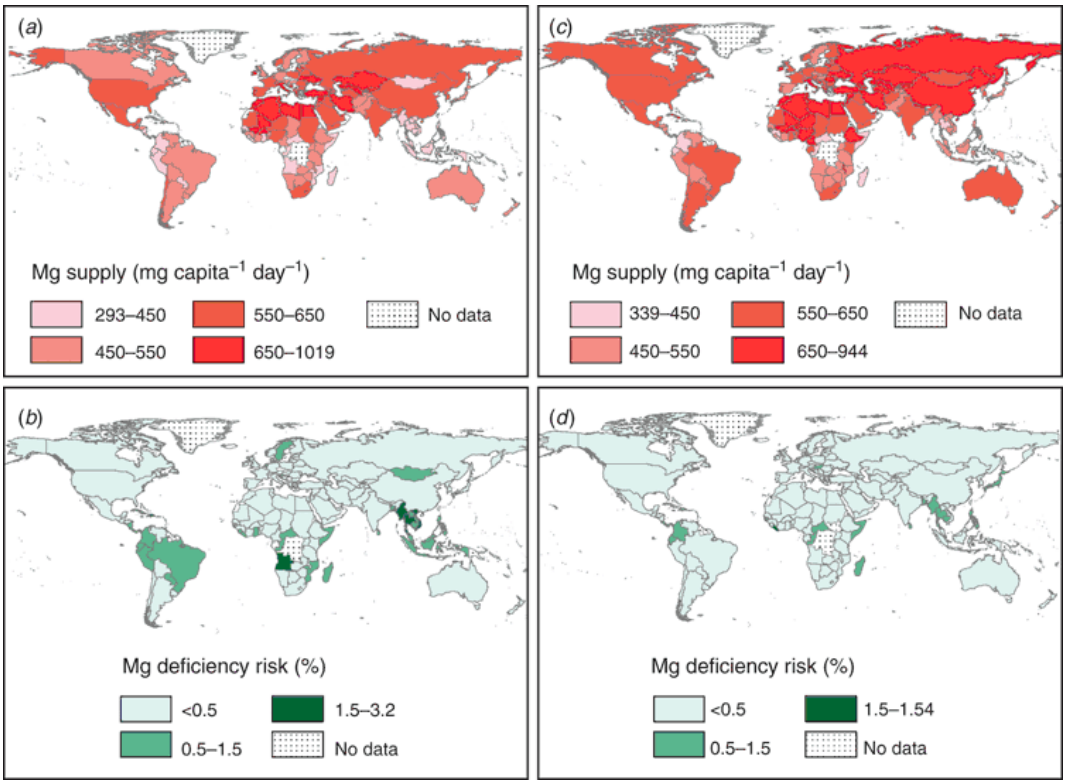


Fig 3.3. National magnesium (Mg) supplies in (*a*) 1992 and (*c*) 2011, and Mg deficiency risks in (*b*) 1992 and (*d*) 2011.

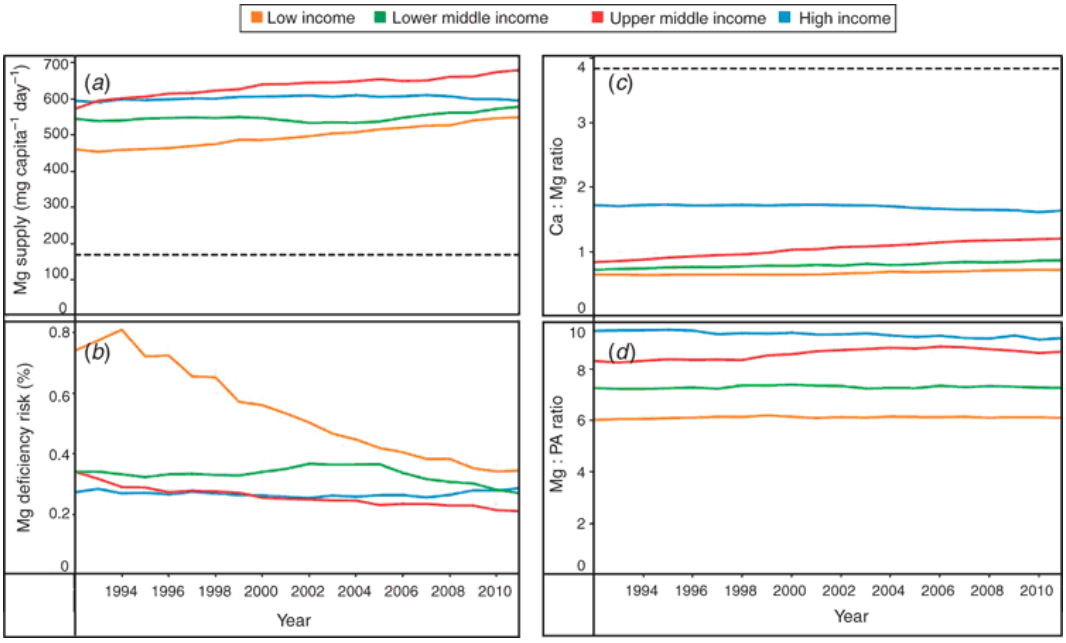


Fig 3.4. Population-weighted mean (*a*) magnesium (Mg) supply (broken horizontal line represents weighted estimated average requirement); (*b*) deficiency risk; (*c*) calcium (Ca) : Mg ratio (broken horizontal line is optimum Ca : Mg ratio); and (*d*) Mg : phytic acid (PA) ratio between 1992 and 2011 according to income.

3.5.2 Sources of dietary magnesium

Typically, 40–80% of dietary Mg in all regions and years originated from cereals (Fig 3.5). For example, in 2011, 79% of dietary Mg in Afghanistan originated from wheat, 64% in Bangladesh from rice, and 63% in Zambia from maize (Supplementary table S7). In high-income countries, wheat provided 43% of dietary Mg, while aquatic plants, nuts, potatoes and vegetables contributed 6% each to dietary Mg (Fig 3.6).

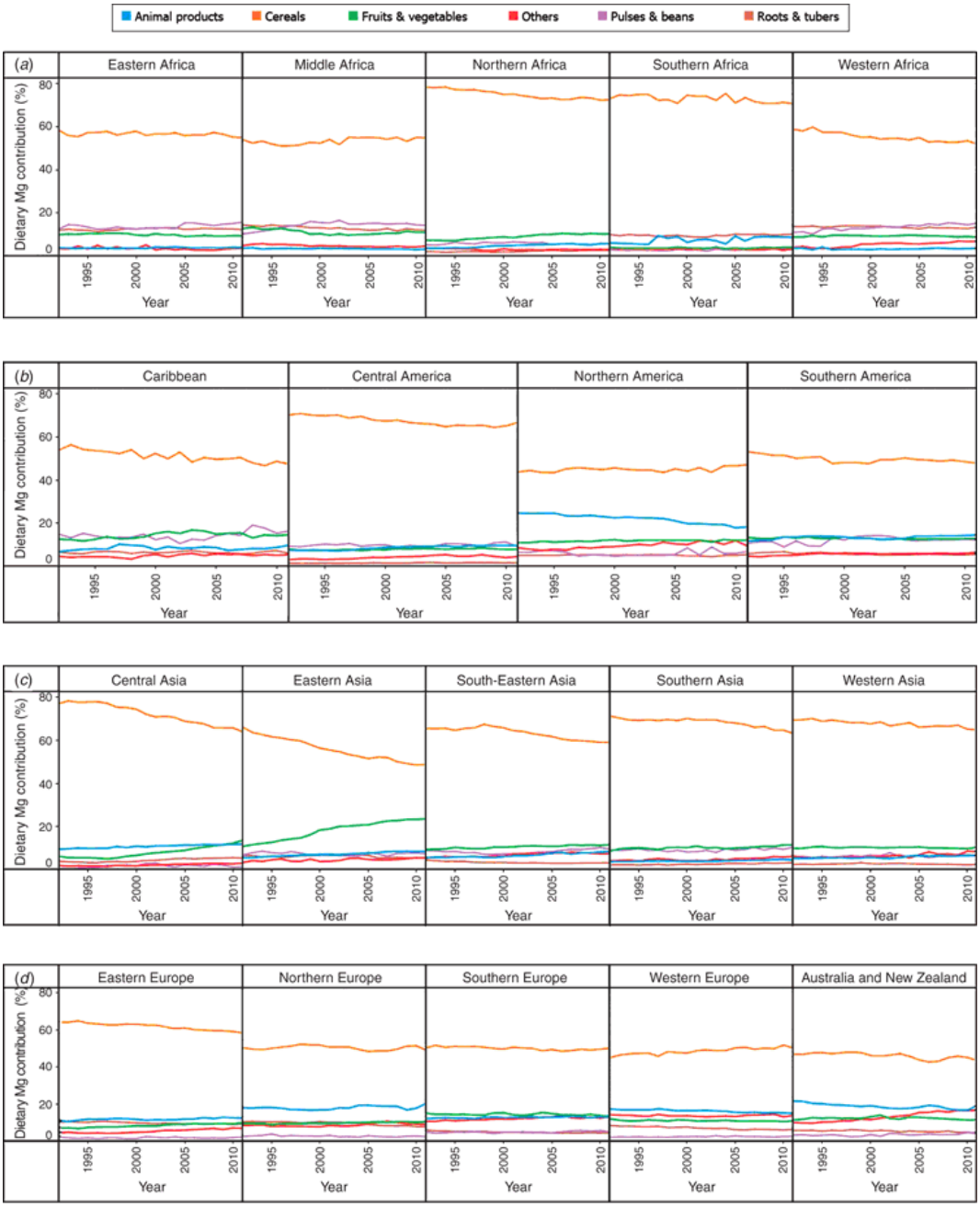


Fig 3.5. Regional temporal trends in the percentage contribution of food groups to magnesium (Mg) supplies between 1992 and 2011 Data are shown for (*a*) Africa, (*b*) the Americas, (*c*) Asia, (*d*) Europe and Oceania.

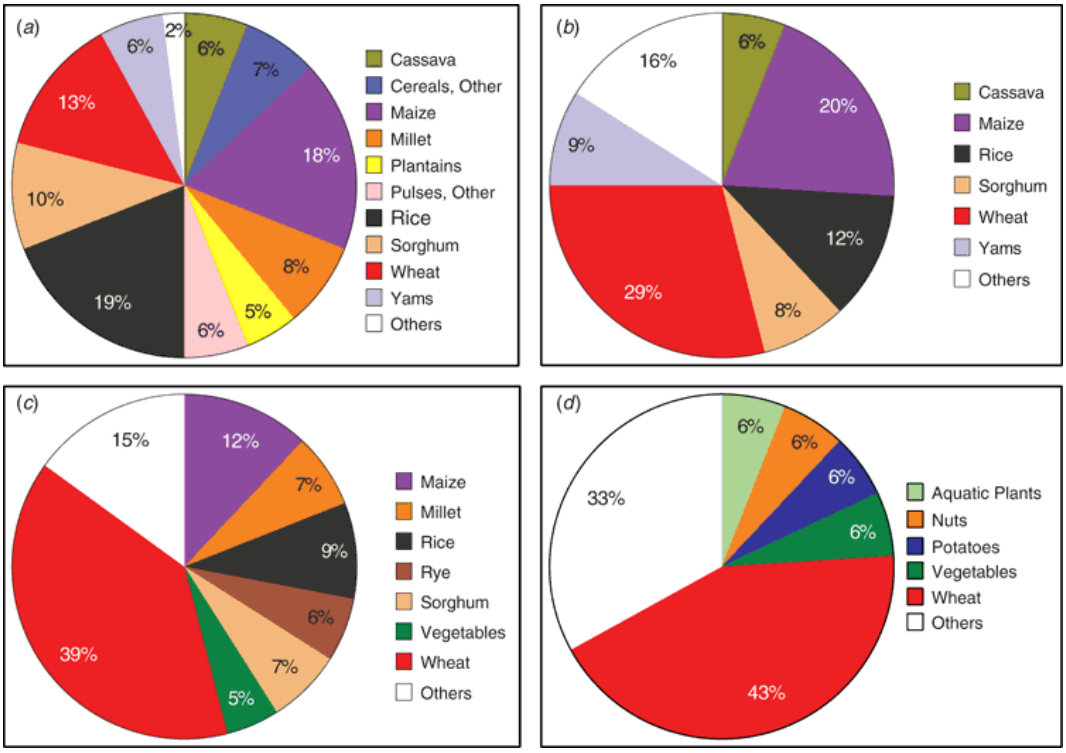


Fig 3.6. Percentage contribution of food items to total dietary magnesium (Mg) supplies in (*a*) low-income, (*b*) lower middle-income, (*c*) upper middle-income, and (*d*) high-income countries. Others represents all food commodities that individually contribute <5% to total dietary Mg in 2011.

3.5.3 Nutritional ratios

Globally, the Ca : Mg supply ratios were 0.96 ± 0.49 in 1992 and 1.11 ± 0.38 in 2011 (Supplementary table S2). In 2011 at a continental level, the Ca : Mg ratios were 0.72 ± 0.24 in Africa, 1.55 ± 0.41 in the Americas, 1.01 ± 0.23 in Asia, 1.57 ± 0.21 in Europe and 1.69 ± 0.08 in Oceania (Supplementary table S3). In 2011, regionally, the Ca : Mg ratios ranged from 0.61 ± 0.1 in Western Africa to 2.00 ± 0.08 in Northern America (Supplementary table S4), and at a country level from 0.36 to 2.15 (Fig 3.7 and Supplementary table S5). For low and high income countries, respectively, Ca : Mg ratios ranged from 0.64 ± 0.20 to 1.72 ± 0.55 in

1992 and from 0.71 ± 0.23 to 1.64 ± 0.32 in 2011 (Fig 3.4 and Supplementary table S6).

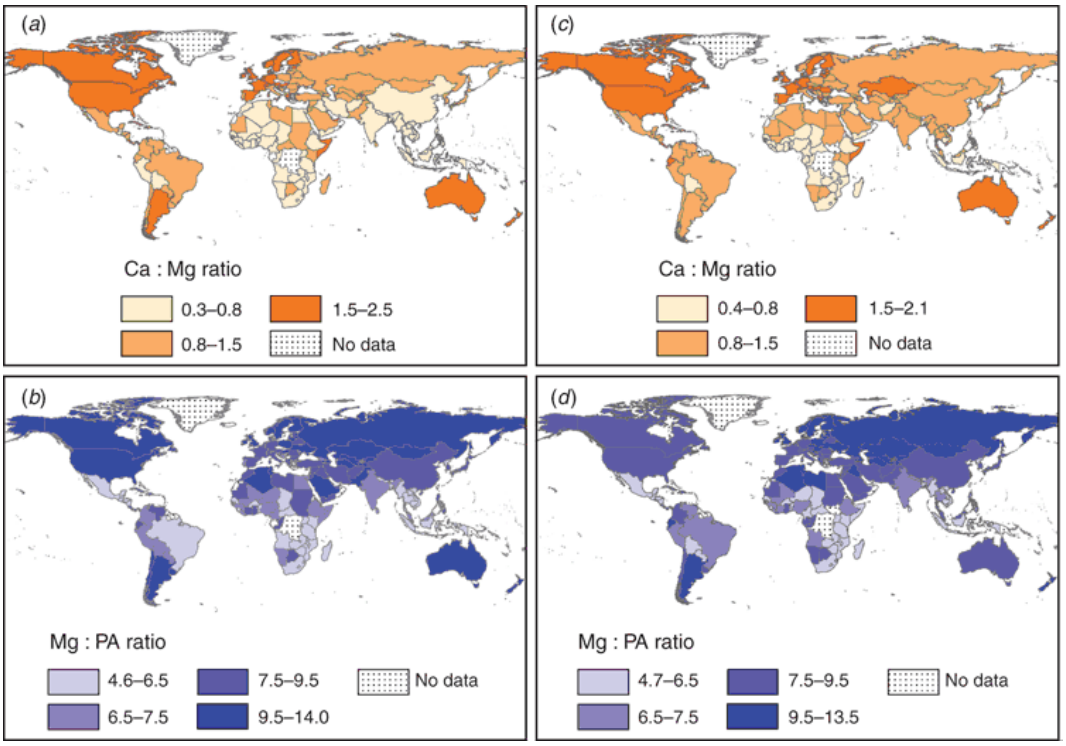


Fig 3.7. National gravimetric ratios of calcium (Ca) : magnesium (Mg) in (*a*) 1992 and (*c*) 2011, and molar ratios of Mg : phytic acid (PA) in (*b*) 1992 and (*d*) 2011 based on per capita Mg, Ca and PA supply.

Global Mg : PA ratios were 8.00 ± 1.53 in 1992 and 7.99 ± 1.48 in 2011 (Supplementary table S2). At a continental level, the Mg : PA ratios in 2011 were 6.81 ± 1.26 in Africa, 7.97 ± 1.59 in the Americas, 8.00 ± 1.33 in Asia, 9.49 ± 0.94 in Europe and 8.91 ± 0.68 in Oceania (Supplementary table S3). Regionally, the Mg : PA ratios in 2011 ranged from 5.65 ± 0.49 in Central America to 11.18 ± 0.65 in Central Asia (Supplementary table S4). At a country level, the Mg : PA ratios ranged from 4.67 to 13.49 in 2011 (Fig 3.7 and Supplementary table S5). For low and high income countries, respectively, the Mg : PA ratios ranged from 6.02 ± 0.8

to 9.49 ± 1.03 in 1992 and from 6.11 ± 0.96 to 9.21 ± 0.87 in 2011 (Fig 3.4 and Supplementary table S6).

3.6 Discussion

The global prevalence of dietary Mg-deficiency risk, based on food supply data, was <1% during 1992–2011 and decreased over this period. In 2011, 14 million people globally were likely at risk of dietary Mg deficiency, based on these data. The decreasing trend in the risk of dietary Mg deficiency is likely due to the overall increase in global food production, especially cereals, which are the major sources of dietary Mg (Welch *et al.* 1999, Pingali 2012, FAO *et al.* 2014). This is in agreement with published estimates of dietary Mg deficiency risks for Africa (Broadley *et al.* 2012, Joy *et al.* 2012, Joy *et al.* 2014). The risk of dietary Mg deficiency is greater in low-income countries.

Estimates of the risk of dietary Mg deficiency based on dietary recalls are much greater than the above estimates (14–53%, UK NDNS data Bates *et al.* (2014); 64–67%, NHANES data, Moshfegh *et al.* (2005) cited in (Rosanoff 2010). Those reports contrast markedly with the results presented here, which suggest that the risks of dietary Mg deficiency for USA and UK in 2011 were 0.32% and 0.25%, respectively. This discrepancy might be attributed in part to misreporting of dietary intakes by respondents participating in dietary-recall surveys (Bingham *et al.* 1994, IOM 2000b, Rennie *et al.* 2004, Rennie *et al.* 2005, Mirmiran *et al.* 2006, Rennie *et al.* 2007, Liberato *et al.* 2009, Archer *et al.* 2013, Bates *et al.* 2014, Winkler 2014). For example, energy intake was under-reported from 24-h dietary recall by 15% in Brazil (Avelino *et al.* 2014), >25% in the UK (Rennie *et al.* 2007), and 67% for men and 59% for women in the USA (Archer *et al.* 2013). Galan *et al.* (2002)

reported dietary Mg intake in France for adult female (35–60 years) and male (45–60 years) tap water drinkers of 284 and 377 mg day^{-1}, respectively, using 24-h recall surveys. Charlton *et al.* (2005) reported dietary Mg intakes in Cape Town, South Africa, of 228, 261, and 285 mg $person^{-1}$ day^{-1} for mixed ancestry, black and white ethnic groups, respectively, using 24-h recall surveys of men and women aged 20–65 years. These reported Mg intakes are less than half of our estimates of 600 and 637 mg $person^{-1}$ day^{-1} of Mg supply in 2011 for France and South Africa, respectively. By contrast, Ca supply calculated from FBS (Kumssa *et al.* 2015a) was only 15% greater than that estimated from dietary-recall data using milk as a proxy (Imamura *et al.* 2015).

Other caveats in this study include the lack of spatial and temporal resolution in food composition data. Thus, Mg concentration data for foods were sourced from the USDA-SR26 food composition database, which is centred on foods grown in North America (USDA 2013). Thus, the impact of different crop varieties, soil types and agronomy (White *et al.* 2009) between and within countries and over time cannot be accounted for in the present study (Davis 2009, White *et al.* 2009). Therefore, the estimated risks of dietary Mg deficiency will be compromised by the absence of relevant, reliable and up-to-date food composition data, which require more detailed local study. For example, in a recent analysis of dietary duplicates in Malawi from a single day (Hurst *et al.* 2013), mean and median Mg intakes were 418 and 353 mg $person^{-1}$ day^{-1}(n = 114). This is in broad agreement with the low estimated risk of Mg deficiency based on FBS supply data, but is still lower than the Mg supply estimate of 530 mg $person^{-1}$ day^{-1} in the present study. However, large differences were observed in Mg intake from those living in Zombwe Extension Planning Area (predominantly non-calcareous soil; n = 56) and

Mikalango Extension Planning Area (predominantly calcareous soil; n = 58). Median Mg intake in Zombwe was 267 mg person^{-1} day^{-1}, compared with 538 mg person^{-1} day^{-1} in Mikalango (unpublished data collected during the study of (Hurst *et al.* 2013)). These differences were due primarily to differences in cereal Mg concentrations between soil types (Broadley *et al.* 2012, Joy *et al.* 2015a) and to dietary choices. For example, sorghum grain had a higher Mg concentration than maize grain and it was consumed more often in Mikalango.

Other methodological weaknesses in determining Mg deficiency risks from food-supply data include effects of food processing and food waste at the household level. In terms of food processing, the USDA food composition table (USDA 2013) shows that enriched white bread-wheat flour (25 mg 100 g^{-1}) contains much less Mg than whole-grain wheat flour (137 mg 100 g^{-1}). Thus, if food processing is not captured accurately by FBS data, then further discrepancies in estimates of Mg deficiency risks could arise from supply-based methods *v.* dietary recall. Food balance sheets also do not capture waste at the household level and will therefore overestimate consumption (FAO 2001). In developed countries, household food wastage occurs from unplanned purchases, behaviour and 'best-before-dates' (Parfitt *et al.* 2010, Gustavsson *et al.* 2011, Eriksson *et al.* 2012). It was estimated that food waste in Europe and North America was 95–115 kg person^{-1} year^{-1}, compared with 6–11 kg person^{-1}year^{-1} in sub-Saharan Africa, and South and Southeast Asia. For cereals, 2–25% of the initial production is wasted at household level (Gustavsson *et al.* 2011). Given that cereals are the major source of dietary Mg (Fig 3.6), quantifying deficiency based on FBSs is likely to systemically underestimate Mg deficiency risk. Drinking and cooking water can also have an important contribution to Mg nutrition, where water Mg concentrations are

sufficiently elevated (Marier 1982, Rosanoff 2013, Kanadhia *et al.* 2014), but this was not assessed in this study.

The risk of Mg deficiency is determined not only by Mg intake but also by the proportion of other nutrients and anti-nutrients (e.g. Ca, PA, oxalate, fibre, saturated fat, etc.) in the gut that affect its bioavailability (Vitale *et al.* 1957, Seeling 1964, Reinhold *et al.* 1976, Cheryan *et al.* 1983, Pallauf *et al.* 1998, Coudray *et al.* 2003, Bohn *et al.* 2004a, b). The dietary Ca : Mg ratios based on dietary recall were 2.9 in France (Galan *et al.* 2002) and 1.9 in South Africa (Charlton *et al.* 2005), compared with our estimates of 1.69 and 0.65, respectively, in 2011. Dai *et al.* (2007) reported that a Ca : Mg ratio >2.8 may affect Mg absorption. In our study, the Ca : Mg ratio from food supply was generally <2; however, processing of cereals is likely to result in larger reductions in intake of Mg than of Ca, thereby increasing Ca : Mg ratios at the intake level. For example, the concentration of Mg in whole grain wheat was reduced by 82%, whereas Ca was reduced by 56% when processed into un-enriched bread flour (USDA 2013). Thus, in countries where Ca : Mg supply ratio approaches or exceeds ~2, the impact of Ca and other nutrients on Mg bioavailability needs to be investigated further. Interestingly Seeling (2006) has argued that the rise in recommended Ca intake could affect Mg absorption if there is not a concurrent increase in Mg. High concentrations of PA in cereals and legumes, and oxalates in some green leafy vegetables, can also reduce Mg absorption in the gut because of chelation (Brink *et al.* 1991, Bohn *et al.* 2004a). In high-income countries, aquatic plants provided 6% of the total dietary Mg (Fig 3.6), indicating the important potential role of underutilized crops in human dietary Mg nutrition. The estimated Mg : PA molar ratio in all countries was 5–14, which is in the range observed to affect the absorption of Mg (Cheryan *et al.* 1983). At a global

scale, our results indicate that while Mg supply from agricultural production is likely to be sufficient to meet the requirements of the population, the prevalence of high Mg : PA ratios in diets around the world requires further study to determine the extent to which Mg absorption might be impaired.

CHAPTER 4. DIETARY MINERAL SUPPLIES IN MALAWI: SPATIAL AND SOCIOECONOMIC ASSESSMENT

4.1 Authors contribution

Edward J. M. Joy and **Diriba B. Kumssa** contributed equally to this work. EJMJ, ELA, MRB and **DBK** conceived the study. EJMJ and **DBK** compiled the food supply data. EJMJ, MJW, ELA, SDY and ADCC generated the food composition data. EJMJ, **DBK** and ELA integrated the datasets. EJMJ and **DBK** drafted the manuscript and figures/tables, with input from all authors. All authors read and approved the final manuscript.

4.2 Abstract

Dietary mineral deficiencies are widespread globally causing a large disease burden. However, estimates of deficiency prevalence are often only available at national scales or for small population sub-groups with limited relevance for policy makers. This study combines food supply data from the Third Integrated Household Survey of Malawi with locally-generated food crop composition data to derive estimates of dietary mineral supplies and prevalence of inadequate intakes in Malawi. We estimate that >50% of households in Malawi are at risk of energy, calcium (Ca), selenium (Se) and/or zinc (Zn) deficiencies due to inadequate dietary supplies, but supplies of iron (Fe), copper (Cu) and magnesium (Mg) are adequate for >80% of households. Adequacy of iodine (I) is contingent on the use of iodised salt with <1% of households getting adequate I supply from food alone. *Hidden hunger* is likely to be widespread: among households with adequate energy supply, 30, 56 and 27% had inadequate supplies of Ca, Se and Zn, respectively. Over 80%

of the poorest households had inadequate dietary supplies of Ca and Zn compared to <30% of the wealthiest households; >80% of rural households living on low-pH soils had inadequate dietary Se supplies compared to 55% on calcareous soils; concurrent inadequate supplies of Ca, Se and Zn were observed in >80% of the poorest rural households living in areas with non-calcareous soils. Prevalence of inadequate dietary supplies was greater in rural than urban households for all nutrients except Fe. Interventions to address dietary mineral deficiencies were assessed. For example, an agronomic biofortification strategy could reduce the prevalence of inadequate dietary Se supplies from 82 to 14% of households living in areas with low-pH soils, including from 95 to 21% for the poorest subset of those households. If currently-used fertiliser alone were enriched with Se then the prevalence of inadequate supplies would fall from 82 to 57% with a cost per alleviated case of dietary Se deficiency of ~ US$ 0.36 $year^{-1}$. Household surveys can provide useful insights into the prevalence and underlying causes of dietary mineral deficiencies, allowing disaggregation by spatial and socioeconomic criteria. Furthermore, impacts of potential interventions can be modelled.

4.3 Background

Food security is defined as having access to sufficient, safe and nutritious food to meet the needs of an active and healthy life (FAO *et al.* 2013). Food insecurity can manifest as 'hunger' due to inadequate dietary energy intake, or *hidden hunger*, due to deficiencies of vitamins and mineral elements. *Hidden hunger* is widespread globally with an estimated two billion people at risk of vitamin A, iron (Fe), iodine (I) and zinc (Zn) deficiencies, causing a considerable social and economic burden particularly in low-income countries including sub-Saharan Africa (WHO 2008, 2009, Stein 2010, Andersson *et al.* 2012, Lim *et al.* 2012, Wessells *et al.* 2012a, Muthayya *et al.* 2013, Joy *et al.* 2014, Kumssa *et al.* 2015a). Deficiencies of other vitamins and elements are also likely to be widespread globally, including selenium (Se) which shows significant spatial variation due to environmental factors (Combs 2001, Lyons *et al.* 2005, Fairweather-Tait *et al.* 2011, Broadley *et al.* 2012).

The prevalence of vitamin or element deficiencies can be quantified through analysis of their concentrations in blood plasma, other tissues or urine; however, conducting wide-scale surveys can be expensive and logistically challenging. In addition, some biomarkers might not be sufficiently accurate or sensitive indicators of deficiency, particularly for mild deficiencies, e.g. for Zn (Gibson *et al.* 2008). In certain cases, health outcomes can be a useful proxy to measure prevalence of dietary nutrient deficiencies, for example stunting as an indicator of Zn deficiency (Stein 2014). However, such relationships can be confounded by environmental factors or multiple causes of the same health outcome and surveys of health outcomes remain expensive and logistically challenging to conduct. Thus, dietary assessment can be a useful approach whereby the mass of an element consumed or

supplied in the diet is quantified, either through direct analysis of composite diets or through matching of food intake records and relevant composition data. Following conventional terminology, 'inadequate dietary supply' of a micronutrient puts an individual 'at risk of deficiency' where deficiency causes negative health outcomes. There are a number of factors that may confound the relationship between 'dietary intakes' and 'nutritional sufficiency', such as nutrient-nutrient interactions, impaired gut absorption or increased losses of vitamins and elements due to infection. For example, phytic acid (PA) is the principal form of phosphorus in cereal grains and inhibits the absorption of Fe, Mg and Zn in the human gut (Sandström 1989).

Previously, the prevalence of micronutrient deficiencies or inadequate dietary supplies in Malawi have been reported using different methods (*c.f.* Table 1 of Joy *et al.* (2015a) for a summary). Anthropometric measures and dietary recall matched to local or regional food composition data were used to quantify dietary supplies and deficiency prevalence of micronutrients in sub-national populations (Ferguson *et al.* 1989, Gibson and Huddle 1998, Eick *et al.* 2009, Hurst *et al.* 2013, Siyame *et al.* 2013, Dickinson *et al.* 2014, Gibson *et al.* 2015). Global, regional or national estimates of deficiency risks were generated using food supply data captured in Food Balance Sheets (FBSs) published by the United Nations Food and Agriculture Organization (FAO 2001, Chilimba *et al.* 2011, Broadley *et al.* 2012, Hurst *et al.* 2013, Joy *et al.* 2014, Joy *et al.* 2015a), or consumption data captured in national Household Surveys (Ecker *et al.* 2011, World-Bank 2012, Verduzco-Gallo *et al.* 2014). These studies have shown or estimated that deficiencies of calcium (Ca), I and Zn are likely to be widespread in Malawi due to inadequate dietary supplies whereas dietary supplies of copper (Cu) and magnesium (Mg) appear to be adequate

for those with sufficient dietary energy intake. Some studies report generally adequate dietary supplies of Fe and low prevalence of Fe-deficiency anaemia (Joy *et al.* 2014, Gibson *et al.* 2015, Joy *et al.* 2015a), while others report generally inadequate dietary Fe supplies and high prevalence of Fe-deficiency anaemia (Dickinson *et al.* 2014, Verduzco-Gallo *et al.* 2014), and this requires further study. Spatial variation in crop composition due to soil type is an important determinant of dietary supplies of some elements; for example, a high prevalence of Se deficiency is likely among populations living on low-pH soils but not on calcareous soils with pH >6.5 (Hurst *et al.* 2013).

The present study considers both environmental and socioeconomic determinants of dietary element supplies in Malawi. Household dietary energy, PA, Ca, Cu, Fe, I, Mg, Se and Zn supplies were quantified by integrating datasets for food consumption, food composition and nutrient requirements. Results were aggregated by defining household characteristics, e.g. urban/rural location, at national and Extension Planning Area (EPA) levels. The EPA is an administrative unit of the Ministry of Agriculture and Food Security. There are 186 EPAs in Malawi with mean and median land areas of 49,600 and 38,900 ha, respectively. Typically, an EPA office will have a good working knowledge of the local area and maintain contact with a high proportion of member households. Thus, EPAs provide an effective network through which agriculture-nutrition interventions can be implemented, especially given that 85% of the population are involved in agriculture, predominantly subsistence production (NSO 2012b).

4.4 Methods

4.4.1 Food consumption

Household food consumption and socioeconomic data were derived from the Third Malawi Integrated Household Survey (IHS3) in which a nationally-representative sample of 12,271 households were interviewed during March 2010-March 2011 (World-Bank 2012). These data were obtained by the authors as fully anonymised secondary data from the World Bank open-data repository (World-Bank 2012). Author use of this open-data archive is compliant with requirements of World Bank, as specified upon data retrieval through the data portal (World-Bank 2012). In the first stage of household selection, Enumeration Areas (EAs) were chosen at random to represent Districts; the probability of EA selection was proportional to the number of member households. In the second stage of selection, 16 households were selected at random to represent each EA with five replacement households in case of failure to complete the interview process. On average, EPAs were represented by 4.3 EAs (range 1–28) (NSO 2012a).

In the food consumption module, interviewees were asked to recall the food consumed in the household during the past 7 days from a list of 112 food items (e.g. 'Maize *ufa* refined (fine flour)', 'Dried fish', etc.). Enumerators recorded the source of the food item (i.e. 'own production', 'bought' or 'gift') and the amount consumed. Interviewees could choose from a selection of units that included standard metric measures (grams, litres etc.) and local units (small plate, large plate, small bucket, large bucket, basin etc.) to assist in estimating the quantity consumed.

There were some inexplicit food categories (e.g. 'Other cultivated green leafy vegetables'); these items were matched to a generic crop in this study (e.g. 'Cabbage

leaf') unless enumerators provided a more specific description (e.g. 'Other cultivated green leafy vegetables – *Bonongwe*, meaning leaf of *Amaranthus* spp.), in which case new item codes were assigned to match more appropriate crop composition data. In total, >99% of food records were decipherable and were included. To calculate mass consumed, local units were converted into metric units; this was done on an item-specific basis because of the variation in density of food items. The IHS3 team measured the mass of local units of common food items from 48 retail markets in Malawi and report a conversion table by region (north, central and south). However, there are still many data gaps in the published conversion table. In addition, some of the conversion factors given vary widely between regions with no apparent explanation; for example, the mass of unit 'Pail (small)' for food item 'Maize *ufa* refined (fine flour)' is reported as 1.83, 5.02 and 3.11 kg in north, central and south regions, respectively. Therefore, the conversion table was re-constructed using author judgement and was applied independent of region (Additional file 1: Table S1). The inedible portions of food items (e.g. banana skin, maize cob) were estimated using author judgement (Additional file 1: Table S2). The unit/mass conversion table, inedible proportion and moisture content data (see below) were used to estimate daily household (hh) consumption of the edible portion (EP) of food items on a dry-weight (DW) basis (i.e. kg hh^{-1} d^{-1}, DW EP).

Inspection of the raw survey data revealed some implausible entries. For example, 145 households were recorded as consuming >1 kg $capita^{-1}$ d^{-1} of the food item 'Maize *ufa* refined (fine flour)'. To mitigate such potential entry errors, a maximum plausible daily consumption of each food item was imposed; this affected just 1459 (<1%) out of a total of >197,000 food entries (Additional file 1: Table S3).

4.4.2 Nutrient composition

Food items in the IHS3 were matched to food crop samples from a previous national survey of plant elemental concentrations (Joy *et al.* 2015a). Survey samples were pooled by species and tissue, for example 'Mango_fruit', 'Cassava_leaf' or 'Cassava_root', and were assigned to one of three composition tables according to the soil type at sampling location: 'calcareous', 'non-calcareous' and 'undifferentiated' (i.e. independent of soil type; Fig. 1; Additional file 1: Table S4 and Additional file 1: Table S5) (Joy *et al.* 2015a). Further food composition data generated since the publication of earlier findings are included in this study and are highlighted in Additional file 1: Table S5. These include nine fish samples collected at lakeshore and inland markets, comprising both small fish (*usipa* and *kapenta*) which are typically eaten whole, and larger fish (*matemba, chambo, utake* and *mbalule*) which are typically gutted before cooking with the meat, small bones and head all consumed but larger bones discarded. The consumption of whole fish including bones is likely to be a significant source of dietary Ca intake, yet the IHS3 questionnaire did not specify the size of fish consumed so mineral composition data for all fish samples were combined into one category which was not stratified by soil class. Rice samples were also not stratified by soil class as, unlike other grains or fresh crops, rice is mainly grown along the lakeshore and in the Shire River basin and is frequently traded over long distances in Malawi. For example, among rural households, 73, 15 and 12% of rice consumption entries in IHS3 came from purchases, own production and gifts, respectively, compared to 38, 57 and 5%, respectively, for refined maize flour. Thus for rice, the soil type at household, market or mill sampling site is unlikely to be a good predictor of the soil type on which it was grown.

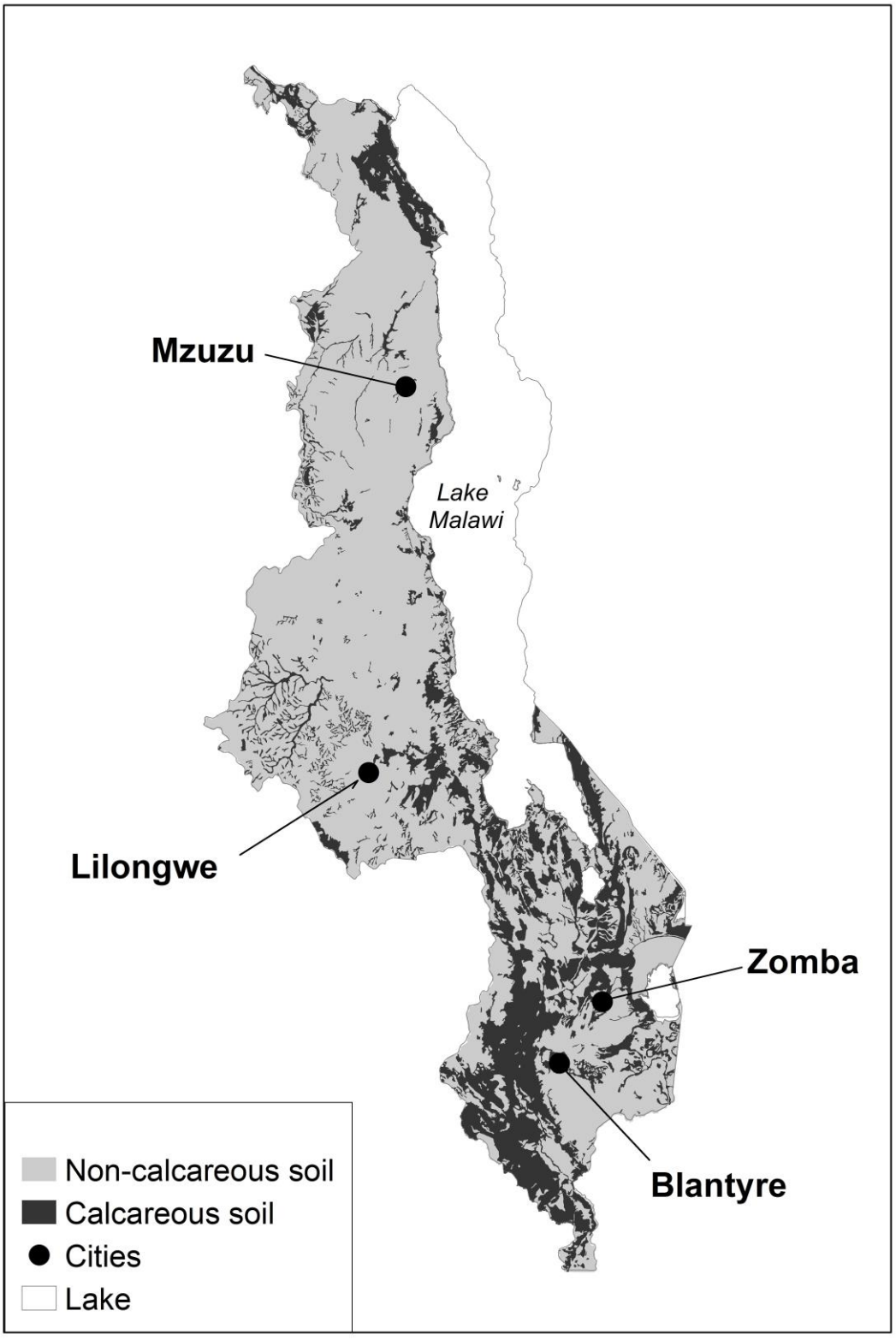


Fig 4.1. Soils of Malawi. Soils are classified as calcareous or non-calcareous. Soil series mapping from (Green and Nanthambwe 1992).

The influence of outlying composition data was minimised by using median concentrations of plant/tissue groupings; however, as some sample sizes were very small (e.g. three samples of 'Pumpkin_fruit' from calcareous soils), data were screened for evidence of extraneous contamination that might make composition data unrepresentative of what is eaten. Plausible maximal elemental concentrations of plant tissues were derived from the plant nutrition literature, e.g. 500 and 100 mg kg^{-1} of Cu in leaf and seed, and 100 mg kg^{-1} of Zn in seed and fruit (Yruela 2005, Broadley *et al.* 2007). Thus, seven and four Cu and Zn data points, respectively, were excluded. Concentrations of aluminium, titanium and vanadium in these samples did not show evidence of contamination with soil dust so elevated Cu and Zn concentrations were likely due to sample preparation methods including cutting and blending samples with metal blades and were deemed unlikely to be representative of foods eaten in Malawi (Additional file 1: Table S5). One sample of tomato fruit had a high Cu concentration (sample number 13380–0002, 190.2 mg kg^{-1} DW) but was not excluded as Cu-based fungicides are commonly used on tomato plants in Malawi, especially during the rainy season when this sample was collected, and this Cu concentration may be representative of what is eaten.

The staple dish *nsima* is prepared using maize flour (*ufa*), using either refined or whole grain. To make refined flour, grain is winnowed, de-hulled, soaked in water for ~3 d, dried in the sun and then milled. Of the 12,271 households with food consumption records, ~10,000 reported consuming maize flour of which ~6000 consumed 'Maize *ufa* refined (fine flour)' with mean consumption of 276 g $capita^{-1}$ d^{-1}. However, inadequate samples of refined flour were collected during the survey of plant crop elemental concentrations to provide reliable composition data for this important food item. Therefore, composition data for whole maize grain was used,

adjusted by a standard ratio for the effects of processing on elemental concentrations. The standard ratio was calculated from paired whole grain and refined maize flour samples collected at maize mills (Additional file 1: Table S6). Consumption of whole grain maize flour, i.e. the food item 'Maize *ufa mgaiwa* (normal flour)', was recorded by ~6000 households with mean consumption of 249 g *capita*$^{-1}$ d^{-1}; maize grain composition data without any conversion factor was matched to this food item.

Where relevant composition data were not available (including for most animal products and for energy and PA), suitable matches were established using published food composition tables, primarily Tanzania (Lukmanji *et al.* 2008) and the USA (USDA 2013) (Additional file 1: Table S4). Composition data from published sources were converted to DW concentrations using matched moisture content data (Additional file 1: Table S4). The concentration of PA is greater in the bran than the endosperm of maize grain and milling and processing typically reduces PA concentrations, e.g. from 880 down to 234 mg 100 g^{-1} DW (Ferguson *et al.* 1989). Thus, the reported PA concentration of 800 mg 100 g^{-1} edible portion of maize flour (Lukmanji *et al.* 2008) is likely to represent whole grain flour. To avoid over-estimating the supply of PA from food items made with refined cereal flours, a 75% reduction in PA concentration was assumed due to milling and processing (Additional file 1: Table S7).

4.4.3 Nutrient requirements

Household demographic information was used to calculate the dietary nutrient requirements of households. For calorie requirements, Dietary Energy Requirements (DERs) recommended by the FAO were used (FAO *et al.* 2004). All

individuals were assumed to lead a moderately active to active lifestyle with physical activity level (PAL) of 1.9 and mean body mass for adult males and females was assumed to be 70 and 65 kg, respectively (this is revisited in the Discussion). Estimated Average Requirements (EARs) and Recommended Nutrient Intakes (RNIs) for Ca, Fe, I, Mg and Zn were obtained from the World Health Organization (WHO) (WHO *et al.* 2004) as these data are likely to be suitable for non-U.S. population groups. The EAR and RNI define intake levels adequate for 50 and 97.5%, respectively, of healthy individuals in an age and sex-specific population group. The WHO does not provide requirement data for Cu, so these were obtained from the Institute of Medicine (IOM 2001). The US/IOM values were also used for Se, e.g. adult EAR = 45 μg d^{-1} (IOM 2000a), because WHO recommendations for Se intake, e.g. adult male EAR = 28 μg d^{-1} (WHO *et al.* 2004), are probably too low based on recent evidence (Fairweather-Tait *et al.* 2011).

Module D of the IHS3 provides the opportunity for pregnancy of household members to be recorded under the section 'illness or injury'. Only 21 instances of pregnancy were recorded from 12,842 women aged 15–49 included in the survey. Conversely, the total fertility rate in Malawi during 2005–10 was 5.83 (UN 2013b) which would translate to ~1650 instances of pregnancy; thus data capture appears to be incomplete. Lactation status was also not captured, so fertility rates by five-year age group for 2010 were used to estimate the proportion of women who were pregnant or lactating and to make an adjustment to nutrient requirements to account for their greater requirements. For example, the EARs of Zn for women aged 15–19 years are 12.0, 14.0 and 15.0 mg $d^{-1}$for non-pregnant, pregnant and lactating individuals, respectively (WHO *et al.* 2004). In Malawi in 2010, 11 and 14% of women aged 15–19 were estimated to be pregnant and lactating, respectively.

Therefore, the adopted EAR for all 15–19 year old women in the IHS3 was 12.6 mg d^{-1}.

4.4.4 Data integration

Data were integrated in a database (Microsoft Access 2010, Microsoft Corporation, Redmond, WA, USA). Statistical analyses were carried out using MINITAB (Version 15, Minitab Corporation, Pennsylvania, USA), 'R' (Version 3.0.2, R Foundation for Statistical Computing, Vienna, Austria) and GenStat (Version 17, VSN International, Hemel Hempstead, UK). Spatial data management and analyses were conducted using ArcGIS (Version 10.2.1, ESRI, Redlands, CA, USA).

Food composition tables developed for 'calcareous', 'non-calcareous' or 'undifferentiated' soil types were applied to households depending on their location. Exact Geographical Position System (GPS) locations of the households are not available in the public domain to protect confidentiality of the respondents (NSO 2014). The data field which provides the greatest spatial resolution is the administrative unit Enumeration Area (EA). The IHS3 sampled 768 EAs with mean and median land areas of 1748 and 745 ha, respectively. A single GPS point is reported in the survey to represent all households within an EA. The point was formed by taking the average latitude and longitude of all households in an EA followed by a displacement (for data protection reasons) of 0–2 km in urban EAs and 0–10 km in rural area EAs to create a modified location within the original EA (NSO 2014). The modified EA point locations were overlaid with the EA polygons as per the 1998 Malawi population census (latest publicly available GIS data) to provide polygon spatial data for each EA; this was spatially overlaid with a soil map

of Malawi (Green *et al.* 1992) using the intersection function in ArcGIS to extract the proportion of each EA covered by calcareous and non-calcareous soils.

Food consumption of households in rural areas was matched to either 'calcareous' or 'non-calcareous' food composition data depending on the soils in the EA; EA soil class was assigned as 'calcareous' or 'non-calcareous' if more than two-thirds of the area was covered by one of these soil classes (Additional file 1: Table S8). Some soils are likely to be unsuitable for agricultural production, for example mountainous terrain with minimal or zero soil depth or marsh land that frequently floods, and these were omitted from the calculation of area by soil class. If there was no dominant soil class then the 'undifferentiated' food composition table was adopted. A total of 13 EAs in the survey had a land area ≥10,000 ha. These large EAs included National Parks and other sparsely populated areas and a more accurate prediction of the soil type on which households were located was determined by laying a 5 km buffer around the aggregated household EA GPS point. The soil class was then determined by calculating the proportion of calcareous and non-calcareous soils within the buffer (example map provided in Additional file 2: Figure S1). For urban EAs, the 'undifferentiated' food composition table was adopted as household location is unlikely to be a good predictor of the soil type on which their food was grown.

Households were eliminated if energy consumption was implausible, defined as >8000 or <400 kcal per Adult Male Equivalent (AME) d^{-1}, thus eliminating 154 out of 12,271 households to leave 12,117 households. The AME is a unit based on the ratio of energy requirement between an individual and the benchmark of an adult male aged 18–30 with a PAL of 1.75, i.e. 2800 kcal (FAO *et al.* 2004, Weisell and

Dop 2012). For example, a household with one adult male, one adult female, a 4-year-old daughter and a 1-year-old son would have an AME value of ~3. The effect of more stringent exclusion criteria is explored in the Discussion.

4.4.5 Estimates of nutrient supplies and prevalence of inadequate intakes

Food consumption (kg^{-1} hh^{-1} d^{-1}, DW EP) and food composition data (mg kg^{-1}, DW EP) were combined to calculate the supply of each nutrient at the household level. While the unit of analysis remains the household, supply per capita was calculated for each household as this provides a tangible metric that allows comparison with previously published estimates. In addition, household demographic composition was used to calculate supply per AME for each household. Supply per AME is a preferable metric when comparing households with different age or gender compositions and is used for comparing household supplies within the present study, e.g. between poorer and wealthier households.

The contribution of food groups to nutrient supplies was quantified by assigning food items to the following groups: 'Animal products', 'Cereals', 'Fats and oils', 'Fish', 'Fruits', 'Legumes', 'Milk products', 'Roots and tubers', 'Vegetables' and 'Others' (Additional file 1: Table S4). Nutrient supplies from each food group were summed for a defined set of households, and were divided by the sum of nutrient supplies from all food groups.

The prevalence of inadequate intakes was estimated at the household level by comparing dietary element supplies with the combined EAR or RNI of all household members. Adequacy of household Zn supply was further characterised

by the dietary PA:Zn molar ratio, where a value >15 is considered to provide inadequate bioavailable Zn (Sandström 1989).

4.5 Results

4.5.1 Household characteristics

Food consumption and household characteristics were recorded for 12,271 households in the IHS3 with a combined occupancy of ~56,000 individuals. A total of 154 households were found to consume unrealistic amounts of energy and were excluded from further analysis. A summary of the socioeconomic and environmental characteristics of the remaining 12,117 households is provided in Additional file 1: Table S9, with the relationships between characteristics provided in Additional file 1: Table S10 and Additional file 1: Table S11. More rural households (9944) were interviewed than urban (2173) while the number of households interviewed by expenditure quintile ranged from 1840 to 3191 in quintiles 1 (poorest) and 5 (wealthiest). Expenditure quintiles were delimited based on per capita consumption expenditure. Mean household size was 5.8, 5.2, 4.8, 4.3 and 3.6 for expenditure quintiles 1 to 5, respectively. Thus, although the number of interviewed households varied between expenditure quintiles, the number of individuals covered in each expenditure quintile was equivalent. The number of rural households located on non-calcareous and calcareous soils was 6523 and 2047, respectively, while 1374 were not assigned to a particular soil type. The 179 EPAs were represented by varying numbers of households, e.g. from 15 in Chileka to 431 in Ntonda, with varying socioeconomic and environmental characteristics, e.g. median expenditure quintile 1 in Dolo to 5 in seven of the EPAs (Additional file 1: Table S12).

4.5.2 Foods consumed

Interviewees were asked to recall foods consumed over the past 7 days. Maize is the dominant staple crop of Malawi and 11,704 households (97%) consumed either refined or whole grain maize flour while 3815 (31%) consumed rice (Additional file 1: Table S13). Median consumption of maize flour per AME was 320 g DW d^{-1}. Only 408 households consumed sorghum and 264 consumed either pearl or finger millet. 5263 households (43%) consumed cassava (either as a boiled root or flour), 4664 consumed sweet potato (orange or white) and 2018 consumed potato (Additional file 1: Table S13).

A total of 10,529 households (87%) consumed some form of animal product (meat, fish, eggs or milk) during the 7 day recall period, with 9286 (79%) consuming fish. Dried fish were particularly popular with 7935 households consuming this item and median consumption per AME of 17 g DW d^{-1} (Additional file 1: Table S13). Fish consumption (yes/no) was related to household expenditure quintile (Pearson Chi-square = 652, df = 4, p <0.001; Fig 4.2), with greater consumption in wealthier and urban households (Additional file 1: Table S14).

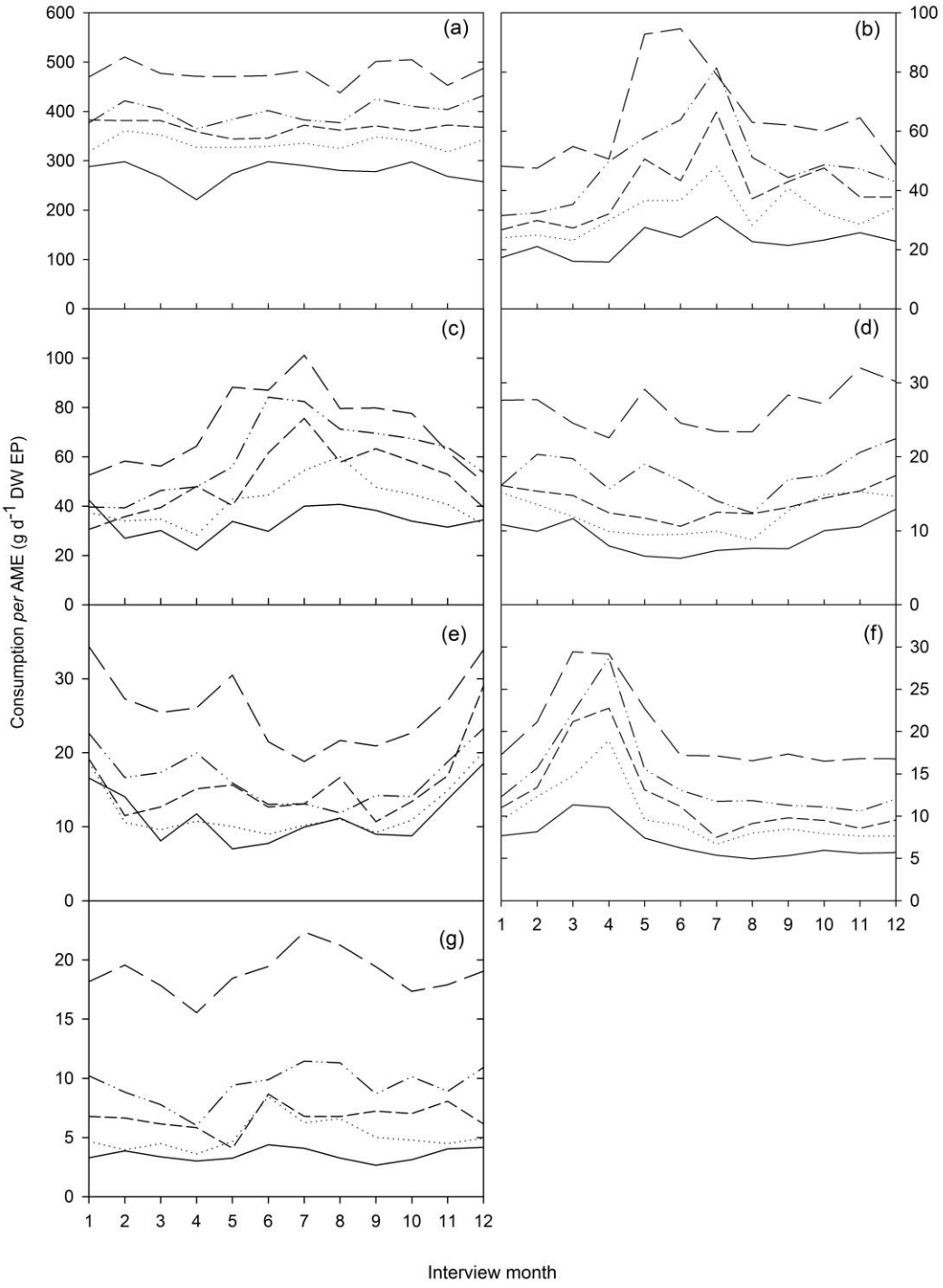


Fig 4.2. Seasonal supply of food groups. Median daily supply of the food groups cereals (**a)**, legumes (**b)**, roots and tubers (**c)**, fish (**d)**, fruits (e), vegetables (**f**) and animal (**g)** products (not including fish) per Adult Male Equivalent, dry-weight edible portion (AME, DW EP) by expenditure quintile and interview month (1 - January to 12 - December). Quintiles are 1 (poorest, continuous), 2 (dot), 3 (short-dash), 4 (dash-dot-dot) and 5 (wealthiest, long-dash).

Interviews were conducted from March 2010 to March 2011 inclusive, each month was represented by ≥527 interviews. The climate of Malawi is characterised by one rainy season, generally lasting from November to February in the Southern region and December to April in the Central and Northern regions. The mid-rainy season is sometimes referred to as the 'hunger gap' as cereal crops have not matured and last season's household stores are depleted. Over 75% of households reported consuming the item 'Green Maize' (i.e. fresh cob, boiled) in March, but <15% in May-December. Overall, cereal consumption showed no marked seasonal variation suggesting that stocks from the 2009 and 2010 harvests were adequate for most households or were covered by increased purchases or gifts (Fig 4.2; Additional file 1: Table S15). The 2009/10 growing season was favourable in Northern and Central regions and produced a national surplus of maize (FEWS-NET 2010). However, there were prolonged dry spells in parts of the Southern region which reduced crop yields (FEWS-NET 2010). Despite this, there was no evidence of seasonal variation in cereal consumption in Southern region households covered by the IHS3 (data not shown).

Conversely, other crops showed marked seasonal variation. For example, mean consumption per AME of 'Legumes' was >87 g d^{-1} DW in May-July but <55 g d^{-1} DW in October-March. Consumption of 'Roots and tubers' peaked during May-September, 'Vegetables' during March and April and 'Fruit' during January and February coinciding with the availability of mangoes; there were 3226 records of mango consumption of which 79% were in November-January. Fish is a particularly important component of dietary micronutrient supply and there was no

marked seasonal variation in consumption nationally (Fig 4.2; Additional file 1: Table S15). Of relevance to food fortification schemes, 11,950 (99%), 7756 (64%) and 7611 (63%) of households reported consuming salt, sugar and cooking oil, respectively.

4.5.3 Nutrient supplies and prevalence of inadequate intakes at household levels

4.5.3.1 Energy

Nationally, median energy supplies per capita and per AME were 2114 and 2463 kcal d^{-1}, respectively, but were lower in rural areas (Table 4.1 and 4.2; Additional file 1: Table S16 and Additional file 1: Table S17). For comparison, the DER of an adult man with body mass 70 kg and physical activity level 1.9 is 3300 kcal d^{-1} (FAO *et al.* 2004). Median household supply of energy as a proportion of sum of member DERs was 0.92 (Additional file 1: Table S18 and Additional file 1: Table S19). Overall, 57% of households reported consumption of insufficient calories to meet the sum of member DERs (Additional file 1: Table S20 and Additional file 1: Table S21). Among EPAs, median energy supply per AME ranged from 1127 kcal d^{-1} in Kalumba (number of households, $n = 16$) to 3817 kcal d^{-1}in Nkhunga ($n = 125$) and was <2000 kcal d^{-1} or >4000 kcal d^{-1} in 33 of 179 EPAs (Additional file 1: Table S22 and Additional file 1: Table S23). Thus, despite data cleaning as described in the Methods and use of median household nutrient supplies during aggregation, there remains an issue with implausible dietary energy (and other nutrient) supplies. This is re-visited in the Discussion.

Table 4.1. Nutrient supplies in rural households. Median energy, calcium (Ca), copper (Cu), iron (Fe), iodine (I), magnesium (Mg), selenium (Se), zinc (Zn) and phytic acid (PA) supplies per Adult Male Equivalent (AME) in rural households by household expenditure quintile (1 = poorest, 5 = highest). 'n' is the number of households. Iodine supply excludes salt.

Expenditure quintile	**n**	**Median dietary supply (AME^{-1} d^{-1})**									
		Energy (kcal)	Ca (mg)	Cu (mg)	Fe (mg)	I (µg)	Mg (mg)	Se (µg)	Zn (mg)	PA (mg)	PA:Zn (molar ratio)
1	1783	1479	307	0.98	11.5	5.5	303	12.6	6.1	1976	35.2
2	2038	1976	510	1.40	16.0	9.3	397	18.6	7.9	2401	32.3
3	2103	2423	636	1.78	19.5	12.1	464	22.5	9.3	2680	30.7
4	2140	2870	830	2.23	23.6	16.8	548	28.8	11.5	2951	28.2
5	1880	3828	1304	3.28	30.8	26.9	741	43.3	16.1	3846	24.8
All	9944	2384	649	1.80	19.5	12.6	469	23.5	9.6	2764	30.2

Table 4.2. Nutrient supplies in urban households. Median energy, calcium (Ca), copper (Cu), iron (Fe), iodine (I), magnesium (Mg), selenium (Se), zinc (Zn) and phytic acid (PA) supplies per Adult Male Equivalent (AME) in urban households by household expenditure quintile (1 = poorest, 5 = highest). 'n' is the number of households. Iodine supply excludes salt.

Expenditure quintile	**n**	**Median dietary supply (AME-1 d-1)**									
		Energy (kcal)	Ca (mg)	Cu (mg)	Fe (mg)	I (µg)	Mg (mg)	Se (µg)	Zn (mg)	PA (mg)	PA:Zn (molar ratio)
1	57	1343	425	1.09	10.4	7.0	291	15.7	6.1	1951	32.2
2	135	1784	645	1.49	12.1	11.5	327	19.7	7.7	1772	29.0
3	228	2123	751	1.60	14.4	14.2	344	21.3	8.2	1814	25.4
4	442	2404	860	1.91	16.1	18.1	390	26.1	9.6	2049	23.5
5	1311	3325	1257	2.95	21.5	31.3	540	39.4	13.9	2416	18.4
All	2173	2830	1021	2.30	18.2	24.1	465	32.2	11.5	2261	20.4

The food groups 'Cereals', 'Legumes' and 'Roots and tubers' contributed 61, 10 and 9%, respectively, of mean national dietary energy supply; other food groups contributed <6% each (Additional file 1: Table S24, Additional file 1: Table S25 and Additional file 1: Table S26).

4.5.3.2 Calcium

Nationally, median Ca supplies per capita and per AME were 602 and 704 mg d^{-1}, respectively, but were lower in rural areas (Table 4.1 and 4.2; Additional file 1: Table S16 and Additional file 1: Table S17). For comparison, the RNI for an adult man is 750 mg d^{-1}(WHO *et al.* 2004). Median household supplies of Ca as a proportion of sum of member EARs and RNIs were 1.0 and 0.9, respectively (Additional file 1: Table S18 and Additional file 1: Table S19). Overall, 49 and 57% of households did not consume enough Ca to meet the sum of member EARs and RNIs, respectively (Table 4.3; Additional file 1: Table S20 and Additional file 1: Table S21). Among EPAs, median Ca supply per AME ranged from 210 mg d^{-1} in Kavukuku ($n = 64$) to 1896 mg d^{-1} in Chiweta ($n = 16$; Additional file 1: Table S22 and Additional file 1: Table S23).

Household dietary Ca supply varied due to household socioeconomic characteristics, soil type and proximity to Lake Malawi (Fig 4.3 and 4.4). For example, 83% of households in quintile 1 had inadequate Ca supply to meet sum of member EARs compared to 22% in quintile 5 (Additional file 1: Table S20). Nationally, median Ca supply as a proportion of energy supply was 291 mg 1000 $kcal^{-1}$ and was 222 and 360 mg 1000 $kcal^{-1}$ in quintiles 1 and 5, respectively, and 368 and 274 mg 1000 $kcal^{-1}$ in urban and rural households, respectively (Additional file 1: Table S27).

Table 4.3. National prevalence (%) of inadequate dietary calcium (Ca), iron (Fe), selenium (Se) or zinc (Zn) supplies at household level. Percentage of households with dietary supply less than sum of household member Estimated Average Requirements, by urban/rural location, expenditure quintile (1 = poorest, 5 = highest) and soil type (1 = calcareous; 2 = non-calcareous; 3 = undifferentiated).

Urban/ rural	Expenditure quintile	Soil type	n	Ca	Fe	Se	Zn
All	All	All	12117	49	18	74	57
All	1	All	1840	83	45	92	88
	2		2173	65	23	86	73
	3		2331	54	14	81	64
	4		2582	38	10	72	50
	5		3191	22	7	51	28
Urban	All	3	2173	30	18	66	49
	1		57	72	58	93	84
	2		135	50	39	90	75
	3		228	45	30	87	76
	4		442	36	19	78	62
	5		1311	22	11	54	35
Rural	All	All	9944	52	18	75	59
	1		1783	83	45	92	88
	2		2038	66	22	86	73
	3		2103	55	12	81	62
	4		2140	39	8	70	48
	5		1880	22	4	49	23
	All	1	2047	51	16	55	49
		2	6523	54	18	82	62
		3	1374	48	21	75	56
	1	1	493	82	37	79	81
	2		450	62	16	67	57
	3		385	51	8	55	44
	4		379	28	5	37	30
	5		340	18	4	25	16
	1	2	1000	85	48	97	92
	2		1297	69	22	93	79
	3		1424	58	13	88	69
	4		1467	43	9	80	54
	5		1335	23	4	55	26
	1	3	290	80	48	93	86
	2		291	61	27	85	72
	3		294	40	14	78	54
	4		294	31	8	67	40
	5		205	18	4	43	18

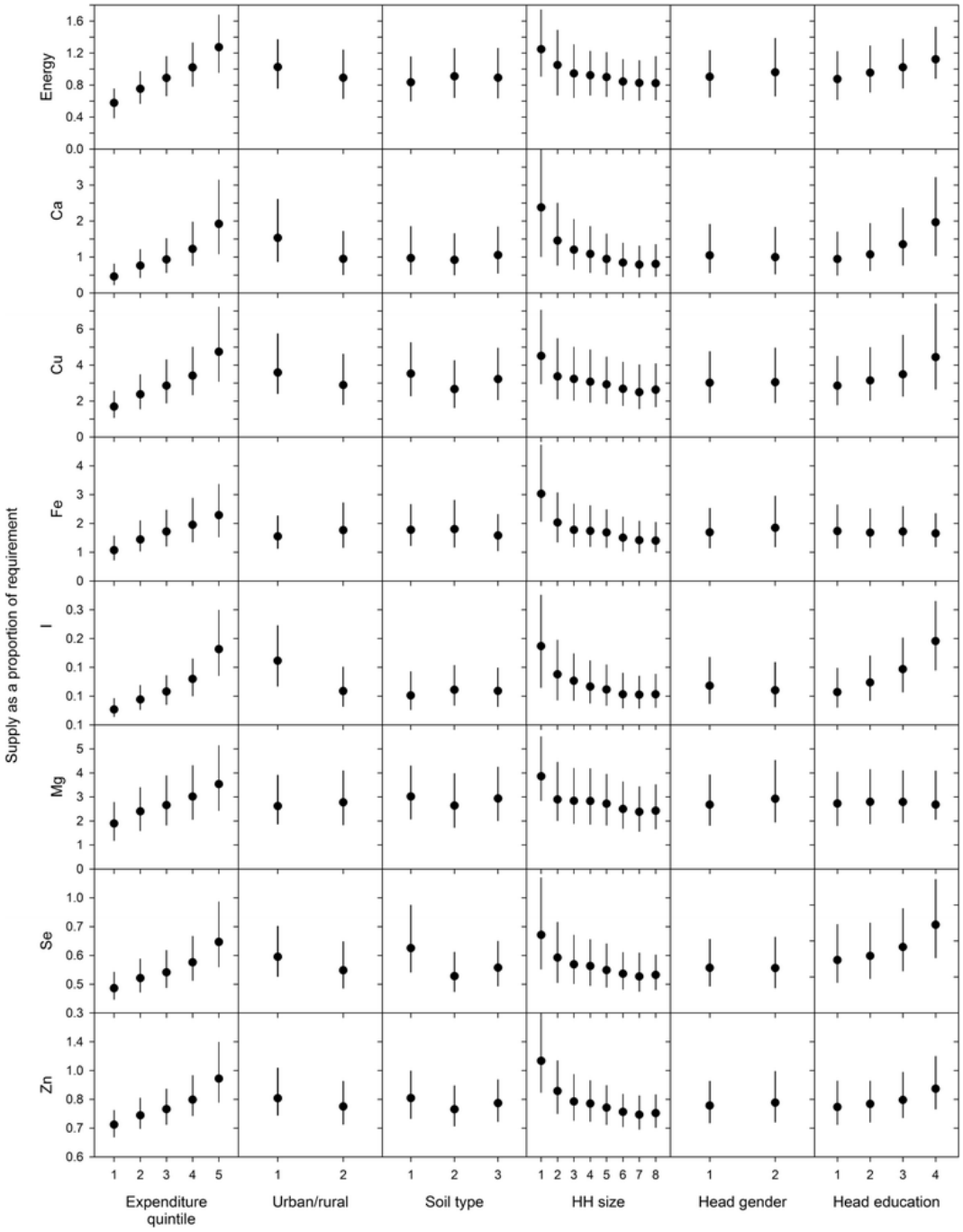


Fig 4.3. Adequacy of household nutrient supplies by household characteristics. Household energy, calcium (Ca), copper (Cu), iron (Fe), iodine (I), magnesium (Mg), selenium (Se) and zinc (Zn) supplies divided by sum of member requirements. Supply of I excludes salt. Households are grouped by expenditure quintile (1 = poorest to 5 = wealthiest), urban/rural location, soil type (1 = calcareous, 2 = non-calcareous, 3 = undifferentiated), household size (capped at 8), head gender (1 = male, 2 = female) and head education (1 = none, 2 = primary school leaving certificate, 3 = junior certificate of education, 4 = Malawi school certificate of education). Points represent median values, bars represent first and third quartiles, respectively.

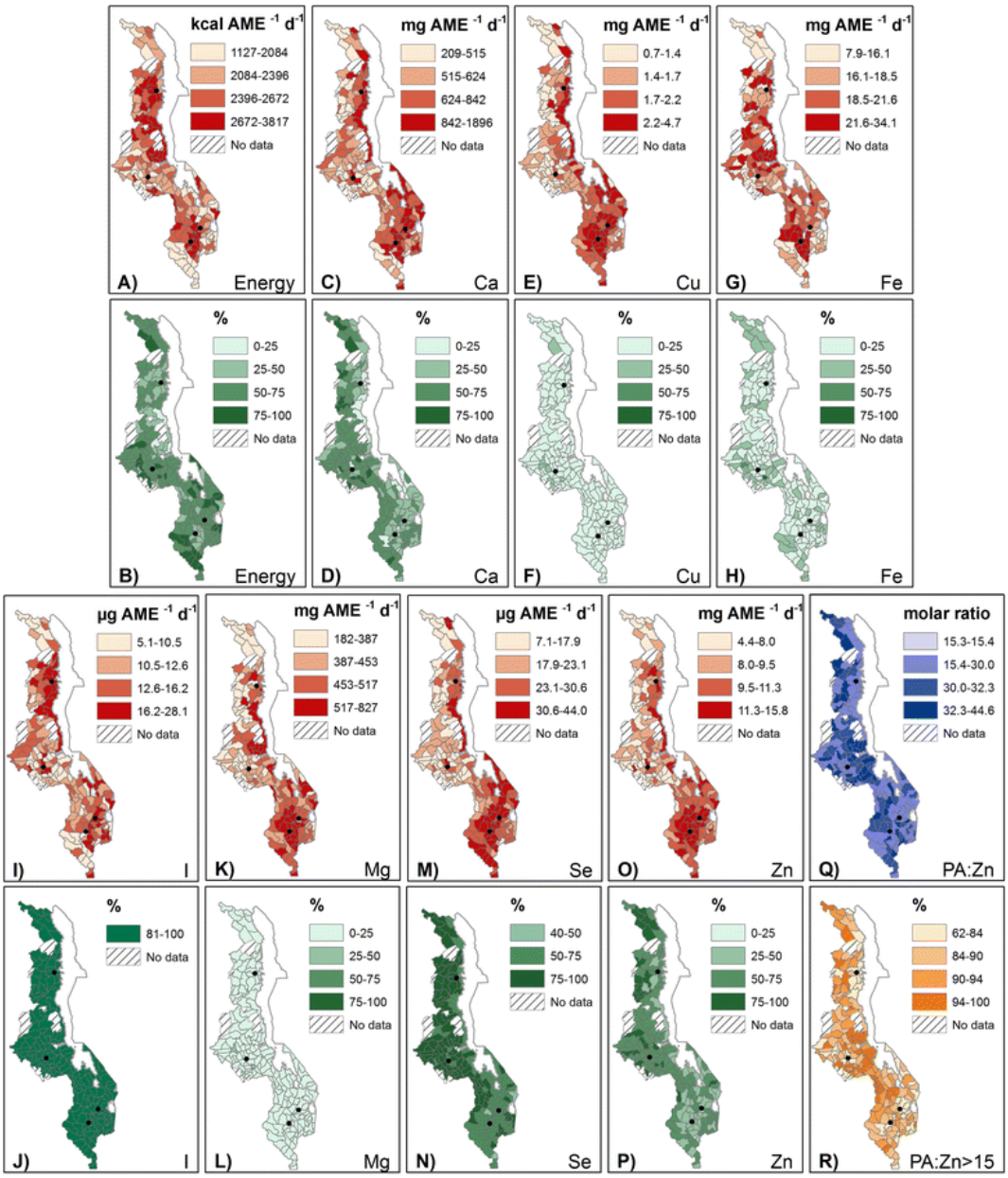


Fig 4.4. Dietary nutrient supplies and deficiencies by Extension Planning Area (EPA). Median household nutrient supplies (mg AME^{-1} d^{-1}) and proportion (%) of households with inadequate dietary supplies to meet sum of member Estimated Average Requirements: **a**, **b** energy, **c**, **d** calcium (Ca), **e**, **f** copper (Cu), **g**, **h** iron (Fe), **i**, **j** iodine (I), **k**, **l** magnesium (Mg), **m**, **n** selenium (Se), **o**, **p** zinc (Zn), **q** median household phytic acid (PA):Zn molar ratio, and **r** proportion of households with PA:Zn molar ratio >15. Underlying data are presented in Additional file 1: Table S21, Additional file 1: Table S22, Additional file 1: Table S29 and Additional file 1: Table S30. Iodine supply excludes salt. EPA boundaries from the Ministry of Agriculture.

The food groups 'Fish' and 'Vegetables' contributed 62 and 15%, respectively, of national annual dietary Ca supply and adequacy of Ca supply was related to consumption of fish; other food groups contributed <7% each (Additional file 1: Table S24, Additional file 1: Table S25 and Additional file 1: Table S26). Dairy products are an important source of dietary Ca in many countries, e.g. 36% of dietary Ca intake in the UK (FEWS-NET 2010), but in the present study they contributed <2% of national Ca supply. Median household supplies of Ca as a proportion of sum of member EARs were 1.32 and 0.37 in households that did and did not consume fish, respectively. Dietary supplies of Ca were greater in lakeshore EPAs (Fig 4.4) due to greater consumption of fish.

4.5.3.3 Cop*per*

Nationally, median Cu supplies per capita and per AME were 1.62 and 1.88 mg d^{-1}, respectively, but were lower in rural areas (Table 4.1 and 4.2; Additional file 1: Table S16 and Additional file 1: Table S17). For comparison, the RNI for an adult man is 0.9 mg d^{-1} (IOM 2001). Median household supplies of Cu as a proportion of sum of member EARs and RNIs were 3.0 and 2.5, respectively (Additional file 1: Table S18 and Additional file 1: Table S19). Overall, 6 and 9% of households were not consuming enough Cu to meet the sum of member EARs and RNIs, respectively (Additional file 1: Table S20 and Additional file 1: Table S21). Among EPAs, median Cu supply per AME ranged from 0.72 mg d^{-1} in Kalumba ($n = 16$) to 4.68 mg d^{-1} in Mbulumbuzi ($n = 47$; Additional file 1: Table S22 and Additional file 1: Table S23).

Household dietary Cu supply was related to household socioeconomic characteristics (Fig 4.3). For example, 22% of households in quintile 1 had

inadequate Cu supply to meet sum of member EARs compared to <1% in quintile 5 (Additional file 1: Table S20). Nationally, median Cu supply as a proportion of energy was 0.79 mg 1000 $kcal^{-1}$ and was 0.74 and 0.87 mg 1000 $kcal^{-1}$ in quintiles 1 and 5, respectively (Additional file 1: Table S27).

The food groups 'Cereals', 'Legumes' and 'Fish' contributed 24, 23 and 22%, respectively, of national annual dietary Cu supply; other food groups contributed <11% each (Additional file 1: Table S24, Additional file 1: Table S25 and Additional file 1: Table S26).

4.5.3.4 Iron

Nationally, median Fe supplies per capita and per AME were 16.6 and 19.2 mg d^{-1}, respectively, and were greater in rural areas (Table 4.1 and 4.2; Additional file 1: Table S16 and Additional file 1: Table S17). For comparison, the RNI for an adult man is 13.7 mg d^{-1} but 49.4 mg d^{-1} for a pregnant woman (WHO *et al.* 2004). Median household supplies of Fe as a proportion of sum of member EARs and RNIs were 1.72 and 1.43, respectively (Additional file 1: Table S18 and Additional file 1: Table S19). Overall, 18 and 27% of households were not consuming enough Fe to meet the sum of member EARs and RNIs, respectively (Additional file 1: Table S20 and Additional file 1: Table S21). Among EPAs, median Fe supply per AME ranged from 7.9 mg d^{-1} in Kalumba ($n = 16$) to 34.1 mg d^{-1} in Mbulumbuzi ($n = 47$; Additional file 1: Table S22 and Additional file 1: Table S23). Nationally, 8% of households had Fe supplies per AME >45 mg d^{-1}, the tolerable upper level of intake for adults (WHO *et al.* 2004).

Household dietary Fe supply was related to socioeconomic characteristics (Fig 4.3). For example, 45% of households in quintile 1 had inadequate Fe supply to meet

sum of member EARs compared to 7% in quintile 5 (Additional file 1: Table S20). Adequacy of household Fe supply varied seasonally according to Fe supply from vegetables, consumption of which peak in February-April (Fig 4.2). Nationally, median Fe supply as a proportion of energy was 7.5 mg 1000 $kcal^{-1}$ and showed little variation between expenditure quintiles, but was greater in rural than urban households, i.e. 7.9 *versus* 6.4 mg 1000 $kcal^{-1}$ (Additional file 1: Table S27).

The food groups 'Cereals', 'Vegetables', 'Legumes' and 'Fish' contributed 34, 29, 12 and 11%, respectively, of national annual dietary Fe supply. Other food groups contributed <5% each (Additional file 1: Table S24, Additional file 1: Table S25 and Additional file 1: Table S26).

4.5.3.5 Iodine

The majority of dietary I supply for most households is likely to come from salt due to mandatory iodisation at >15 mg kg^{-1} in Malawi. Data for household salt consumption were captured in the IHS3 but the concentration of I in salt is highly variable depending on level of iodisation at manufacture and losses due to improper storage (Diosady *et al.* 1998). Not including salt, median supplies per capita and per AME were 12.2 and 14.3 µg d^{-1}, respectively, but were lower in rural areas (Table 4.1 and 4.2; Additional file 1: Table S16 and Additional file 1: Table S17). For comparison, the RNI for an adult man is 150 µg d^{-1} (WHO *et al.* 2004). Median household supplies of I as a proportion of sum of member EARs and RNIs were 0.13 and 0.11, respectively (Additional file 1: Table S18 and Additional file 1: Table S19). Overall, 99 and 100% of households were not consuming enough I through food items other than salt to meet the sum of member EARs and RNIs, respectively (Additional file 1: Table S20 and Additional file 1: Table S21). Among

EPAs, median I supply per AME ranged from 5.1 µg d^{-1} in Dolo ($n = 63$) to 28.1 µg d^{-1} in Ntonda ($n = 431$; Additional file 1: Table S22 and Additional file 1: Table S23).

Nationally, median consumption of salt per AME was 11.2 g d^{-1}, was greater in rural than urban households (median 11.4 *versus* 10.3 g d^{-1}) and was related to expenditure quintile, with median consumption of 8.5, 10.0, 10.9, 12.4 and 15.4 g d^{-1} for quintiles 1 to 5, respectively. If it is assumed that all salt was iodised at 15 mg kg^{-1} and consumed, then 17 and 27% of households would not be consuming enough I to meet the sum of member EARs and RNIs, respectively. Further salt consumption and iodisation scenarios are explored in the Discussion section. Median I supply from foods other than salt as a proportion of energy was 5.80 µg 1000 $kcal^{-1}$ and was 3.7 and 8.1 µg 1000 $kcal^{-1}$ in quintiles 1 and 5, respectively (Additional file 1: Table S27). The food group 'Fish' supplied 49% of I from non-salt food sources (Additional file 1: Table S26).

4.5.3.6 Magnesium

Nationally, median Mg supplies per capita and per AME were 401 and 468 mg d^{-1}, respectively, and were similar in rural and urban areas (Table 4.1 and 4.2; Additional file 1: Table S16 and Additional file 1: Table S17). For comparison, the RNI for an adult man is 260 mg d^{-1}(WHO *et al.* 2004). Median household supplies of Mg as a proportion of sum of member EARs and RNIs were 2.74 and 2.28, respectively (Additional file 1: Table S18 and Additional file 1: Table S19). Overall, 5 and 9% of households were not consuming enough Mg to meet the sum of member EARs and RNIs, respectively (Additional file 1: Table S20 and Additional file 1: Table S21). Among EPAs, median Mg supply per AME ranged

from 182 mg d^{-1} in Kalumba ($n = 16$) to 827 mg d^{-1} in Masambanjati ($n = 32$; Additional file 1: Table S22 and Additional file 1: Table S23).

Household dietary Mg supply was related to household socioeconomic characteristics (Fig 4.3). For example, 17% of households in quintile 1 had inadequate Mg supply to meet sum of member EARs compared to 1% in quintile 5 (Additional file 1: Table S20). Nationally, median Mg supply as a proportion of energy was 207 mg 1000 $kcal^{-1}$ and was 242 and 186 mg 1000 $kcal^{-1}$ in quintiles 1 and 5, respectively (Additional file 1: Table S27).

The food groups 'Cereals', 'Legumes' and 'Vegetables' contributed 45, 19 and 11%, respectively, of national annual dietary Mg supply; other food groups contributed <10% each (Additional file 1: Table S24, Additional file 1: Table S25 and Additional file 1: Table S26).

4.5.3.7 Selenium

Nationally, median Se supplies per capita and per AME were 21.4 and 25.0 μg d^{-1}, respectively, but were lower in rural areas (Table 4.1 and 4.2; Additional file 1: Table S16 and Additional file 1: Table S17). For comparison, the RNI for an adult man is 55 μg d^{-1}(IOM 2000a). Median household supplies of Se as a proportion of sum of member EARs and RNIs were 0.63 and 0.52, respectively (Additional file 1: Table S18 and Additional file 1: Table S19). Overall, 74 and 81% of households were not consuming enough Se to meet the sum of member EARs and RNIs, respectively (Fig 4.5; Additional file 1: Table S20 and Additional file 1: Table S21). Among EPAs, median Se supply per AME ranged from 7.1 μg d^{-1} in Kavukuku ($n = 64$) to 43.9 μg d^{-1} in Nampeya ($n = 47$; Additional file 1: Table S22 and Additional file 1: Table S23).

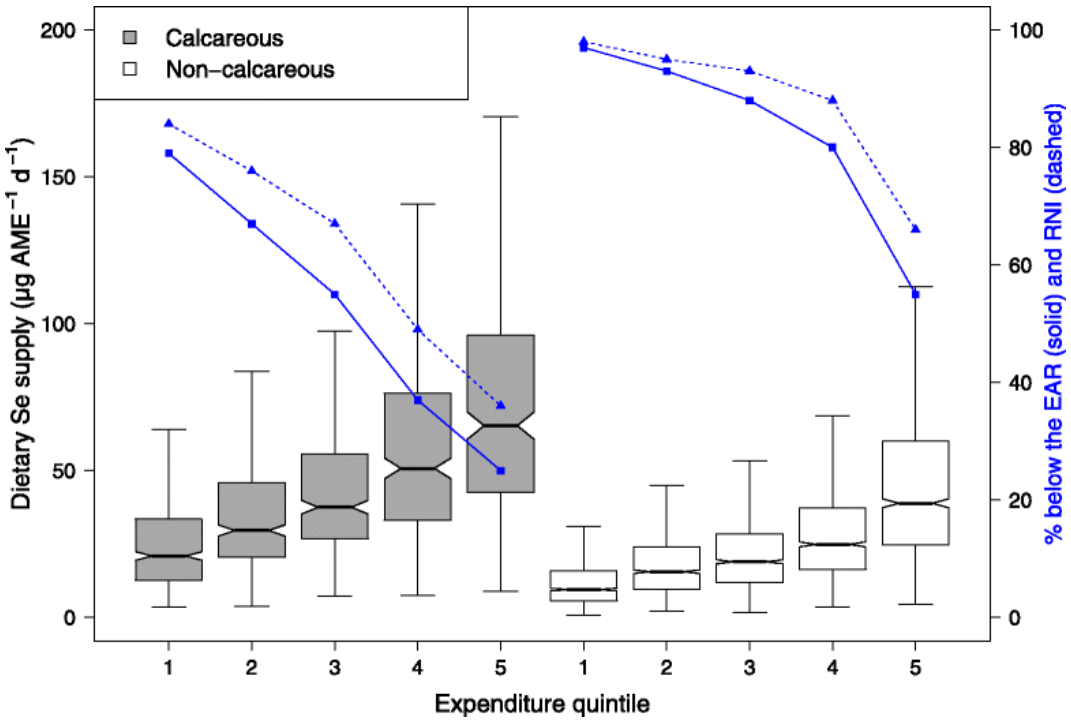


Fig 4.5. Distribution of rural household dietary selenium (Se) supplies and prevalence of inadequacies. Household dietary supplies are quantified per Adult Male Equivalent (AME). Prevalence of inadequate supplies are quantified as percent (%) of households with dietary Se supply less than sum of household member Estimated Average Requirements (EARs) and Reference Nutrient Intakes (RNIs); the EAR and RNI for an adult male are 45 and 55 µg d^{-1}(IOM 2000a). Households are aggregated by soil type (calcareous = dark grey, non-calcareous = white) and expenditure quintile (1 = poorest, 5 = wealthiest). Boxes are median, Q1 and Q3, notches estimate the median's 95% confidence interval, whiskers extend 1.5x Q1 and Q3.

Household dietary Se supply varied due to household socioeconomic characteristics and soil type (Fig 4.3, 4.4 and 4.5). For example, 55 and 82% of households on calcareous and non-calcareous soils, respectively, had inadequate Se supply to meet sum of member EARs and there was a 92 and 51% prevalence of inadequate supplies among households from expenditure quintiles 1 and 5, respectively (Additional file 1: Table S20). Nationally, median Se supply as a proportion of energy was 10.2 µg 1000 $kcal^{-1}$and was 9.0 and 11.7 µg 1000 $kcal^{-1}$ in quintiles 1 and 5, respectively, and 15.6 and 8.4 µg 1000 $kcal^{-1}$ in rural households on calcareous and non-calcareous soils, respectively (Additional file 1: Table S27).

The food groups 'Fish', 'Cereals' and 'Legumes' contributed 47, 21 and 13%, respectively, of national annual dietary Se supply; other food groups contributed <9% each (Additional file 1: Table S24, Additional file 1: Table S25 and Additional file 1: Table S26). 'Cereals' contributed 28 and 19% of dietary Se supply among rural households on calcareous and non-calcareous soils, respectively (Additional file 1: Table S26).

4.5.3.8 Zinc

Nationally, median Zn supplies per capita and per AME were 8.5 and 10.0 mg d^{-1}, respectively, but were lower in rural areas (Table 4.1 and 4.2; Additional file 1: Table S16 and Additional file 1: Table S17). For comparison, the RNI for an adult man is 14 mg d^{-1}(WHO *et al.* 2004). Median household supplies of Zn as a proportion of sum of member EARs and RNIs were 0.90 and 0.75, respectively (Additional file 1: Table S18 and Additional file 1: Table S19). Overall, 57 and 68% of households were not consuming enough Zn to meet the sum of member EARs and RNIs, respectively (Additional file 1: Table S20 and Additional file 1: Table S21). Among EPAs, median Zn supply per AME ranged from 4.4 mg d^{-1} in Kalumba ($n = 16$) to 15.8 mg d^{-1} in Masambanjati ($n = 32$; Additional file 1: Table S22 and Additional file 1: Table S23).

Household dietary Zn supply varied spatially and was related to household socioeconomic characteristics (Fig 4.3 and 4.4). For example, 88% of households in quintile 1 had inadequate Zn supply to meet sum of member EARs compared to 28% in quintile 5 (Additional file 1: Table S20). Nationally, median Zn supply as a proportion of energy was 4.2 mg 1000 $kcal^{-1}$ and was 5.4 and 4.0 mg 1000 $kcal^{-1}$

for households on calcareous and non-calcareous soils, respectively (Additional file 1: Table S27).

The food groups 'Cereals', 'Fish' and 'Legumes' contributed 41, 26 and 13%, respectively, of national annual dietary Zn supply; other food groups contributed <7% each (Additional file 1: Table S24, Additional file 1: Table S25 and Additional file 1: Table S26). Dietary supplies of Zn were greater in lakeshore EPAs due to greater consumption of fish (Fig 4.4).

4.5.3.9 Phytic acid

Nationally, median dietary PA supplies per capita and per AME were 2280 and 2460 mg d^{-1}, respectively and median dietary PA:Zn molar ratio was 29 (Additional file 1: Table S16, Additional file 1: Table S17 and Additional file 1: Table S18). Overall, 87% of households had dietary PA:Zn molar ratios >15.0 (Additional file 1: Table S20). Household dietary PA:Zn molar ratio was related to household socioeconomic characteristics. For example, median PA:Zn molar ratios were 20 and 30 in urban and rural households, respectively, and 35 and 22 in expenditure quintiles 1 and 5, respectively (Table 4.1 and 4.2; Additional file 1: Table S18). Among EPAs, median PA supply per AME ranged from 981 mg d^{-1} in Nthondo ($n = 16$) to 4580 mg d^{-1} in Masambanjati ($n = 32$) while the median PA:Zn molar ratio ranged from 15 in Chiweta ($n = 16$) to 45 in Nakachoka ($n = 32$; Additional file 1: Table S22 and Additional file 1: Table S23).

The food groups 'Cereals' and 'Legumes' contributed 65 and 28%, respectively, of dietary PA supply (Additional file 1: Table S26) and PA:Zn molar ratio was greatest (therefore lowest bioavailability of Zn) during the harvest season of May-August (Fig 4.6), mainly due to greater consumption of legumes.

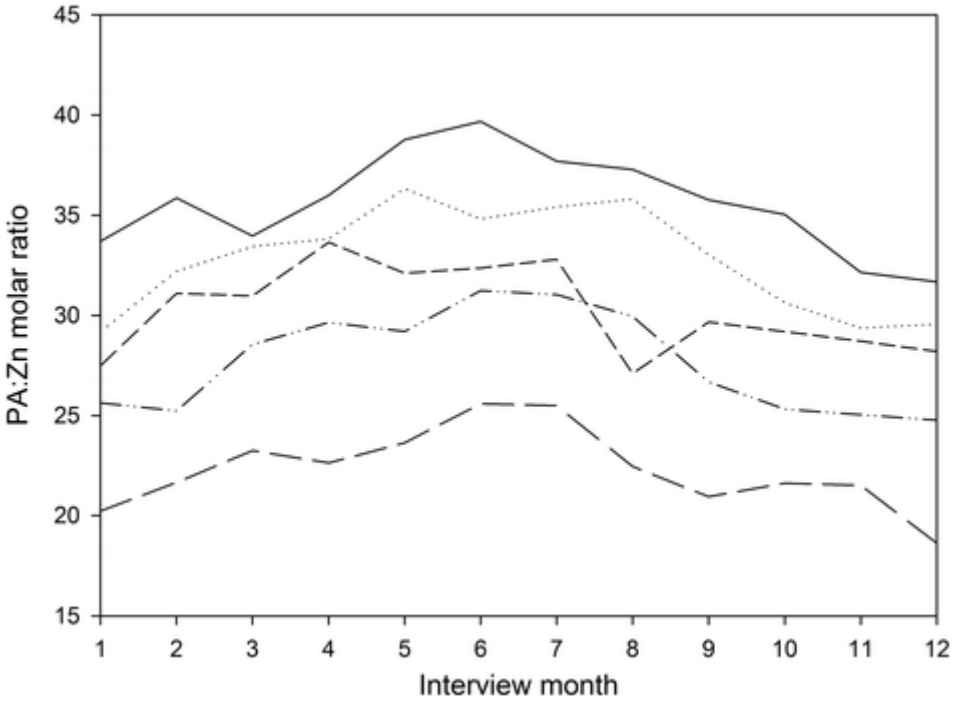


Fig 4.6 Seasonal phytic acid:zinc (PA:Zn) molar ratio in the diet. Median household dietary PA:Zn molar ratio by interview month. A diet with PA:Zn molar ratio >15 is likely to supply inadequate bioavailable Zn (Sandström 1989). Quintiles are 1 (poorest, continuous), 2 (dot), 3 (short-dash), 4 (dash-dot-dot) and 5 (wealthiest, long-dash).

4.6 Discussion

4.6.1 Comparison with previous estimates of dietary nutrient supplies

The present study estimated dietary mineral element supplies at household levels in Malawi by combining food supply data captured in the IHS3 (NSO 2014) with locally-generated food crop composition data (Joy *et al.* 2015a, Watts *et al.* 2015). This compares with previous efforts to quantify the prevalence of inadequate element intakes at wide scales in Malawi on the basis of national-level food supply data captured in Food Balance Sheets (FBSs) published by the FAO matched with published or local composition data (Chilimba *et al.* 2011, Broadley *et al.* 2012, Wessells *et al.* 2012a, Hurst *et al.* 2013, Joy *et al.* 2013, Joy *et al.* 2014, Joy *et al.*

2015a, Kumssa *et al.* 2015a, Kumssa *et al.* 2015b) or food consumption from household surveys matched with published composition data (Ecker *et al.* 2011, Verduzco-Gallo *et al.* 2014).

Where appropriate, the estimated prevalence of inadequate dietary element intakes are discussed in relation to the 2010 Global Burden of Disease study, coordinated by the Institute for Health Metrics and Evaluation (IHME), in which all-cause mortality and morbidity were assigned to 67 underlying risk factors (Lim *et al.* 2012, Murray *et al.* 2012, Institute for Health Metrics and Evaluation 2014). The Global Burden of Disease study used Disability-Adjusted Life-Years (DALYs) as a common currency to measure disease burden. A DALY is equivalent to a lost year of 'healthy life' and is the sum of years of life lost due to premature mortality and years of life lost due to a disability (Murray 1994).

4.6.2 Energy

Previously, Joy *et al.* (2014) estimated mean national dietary supply of energy in Malawi to be 2757 kcal $capita^{-1}$ d^{-1} based on FBS data while Verduzco-Gallo *et al.* (2014) reported mean consumption of 2305 kcal $capita^{-1}$ d^{-1} based on the IHS3. This compares to estimated mean and median consumption of 2381 and 2115 kcal $capita^{-1}$ d^{-1} in the present study. Verduzco-Gallo *et al.* (2014) reported mean consumption of 1232 kcal$capita^{-1}$ d^{-1} and a 37% prevalence of inadequate energy intakes for rural households. This compares to mean and median consumption of 1258 and 1208 kcal $capita^{-1}$ $d^{-1}$and an estimated 60% prevalence of inadequate energy intakes for rural households in the present study. The greater estimated prevalence of inadequate energy intakes in the present study is due to different assumptions regarding energy requirements: Verduzco-Gallo *et al.* (2014) defined 'minimum

calorie requirements' based on a light physical activity level (PAL) and low body mass index (FAO *et al.* 2004, Ecker *et al.* 2011). Thus, an adult male would be calorie deficient at intakes <2400 kcal d^{-1} compared to <3300 kcal d^{-1} in the present study where adults were assumed to lead a moderately active to active lifestyle with PAL of 1.9, consistent with expected activity given that 85% of the population are involved in agriculture, predominantly non-mechanised (FAO *et al.* 2004, NSO 2012b). Inadequate energy intakes are likely to be widespread among rural households, particularly during months of field preparation and weeding when PALs of 2.25 are estimated with requirements of 3850 kcal d^{-1} for men aged 18–30 (FAO *et al.* 2004).

Household spending data in the IHS3 support the finding of prevalent dietary energy insufficiency in poor households. Pauw *et al.* (2016) reported that, as the wealth of poorest rural households increased, the share of income spent on food also increased. This appears contrary to Engel's law which states that the share of household budget spent on food is inversely related to household real income (Engel 1857), a remarkably consistent observation across space and time (Kaus 2013). The apparent contradiction could be explained if the poorest households are calorie insufficient and additional income is used to purchase essential food.

4.6.3 Calcium

Previously, Broadley *et al.* (2012), Joy *et al.* (2014) and Kumssa *et al.* (2015a) estimated mean national dietary supplies of Ca to be 306, 592 and 259 mg *capita*$^{-1}$ d^{-1} based on FBS supply and published composition data. Subsequently, Joy *et al.* (2015a) estimated mean national dietary supplies of Ca to be 430 and 368 mg *capita*$^{-1}$ d^{-1} on calcareous and non-calcareous soils based on FBS supply and local

composition data. In the present study, estimated national mean and median supplies of Ca were 924 and 602 mg *capita*$^{-1}$ d^{-1}, with median supplies of 578 and 537 mg *capita*$^{-1}$ d^{-1} for rural households on calcareous and non-calcareous soils, respectively (Additional file 1: Table S16). The main reason for the greater estimated Ca supply in the present study is fish consumption. Mean national consumption of the food item 'Freshwater Fish' is reported in the 2009 FBS as 13 g *capita*$^{-1}$ d^{-1} on a fresh-weight (FW) basis (FAO 2014) which is equivalent to <3 g DW (Joy *et al.* 2015a). In contrast, estimated mean (± standard deviation, S.D.) consumption of fish in the present study is 19 ± 36 g *capita*$^{-1}$ d^{-1} DW.

Despite the greater estimated consumption of fish, estimated prevalence of inadequate Ca intakes remains high with 49% of all households consuming less than sum of member EARs. This is due to the high inter-household variation in Ca supplies related to spatial and socio-economic factors, and low contribution of other food groups, for example the common staple cereal, maize, to dietary Ca supply, especially dairy products.

In the IHME Global Burden of Disease study (Lim *et al.* 2013), the annual disease burden in Malawi due to diets low in Ca was estimated to be 720 DALYs (100 k population)$^{-1}$(Institute for Health Metrics and Evaluation 2014). However, DALYs attributable to low dietary Ca were based only on the increased risk of prostate and colorectal cancers and not other diseases associated with inadequate dietary Ca such as rickets and osteoporosis (WHO *et al.* 2004), and the study is likely to underestimate the burden.

4.6.4 Copper

Previously, Joy *et al.* (2014) estimated mean national dietary supply of Cu to be 3.0 mg*capita*$^{-1}$ d^{-1} based on FBS data and regional or international composition data, and subsequently, using local composition data, 2.6 and 2.0 mg *capita*$^{-1}$ d^{-1} on calcareous and non-calcareous soils, respectively (Joy *et al.* 2015a). In the present study, estimated median dietary Cu supply was 1.6 mg *capita*$^{-1}$ d^{-1} nationally, and 1.9 and 1.4 mg *capita*$^{-1}$ d^{-1} for rural households on calcareous and non-calcareous soils, respectively (Additional file 1: Table S16). Removal of the husk and endosperm during milling of maize grain reduces the concentration of Cu by ~80% (Additional file 1: Table S6). These losses were accounted for in the present study, resulting in lower estimated Cu supplies. Still, the estimated prevalence of inadequate Cu intakes is low which is consistent with previous findings (Joy *et al.* 2014).

4.6.5 Iron

Previously, Joy *et al.* (2014) estimated mean national dietary supply of Fe to be 29.1 mg*capita*$^{-1}$ d^{-1} based on FBS data and regional or international composition data, and subsequently, using local composition data, 23.2 and 18.4 mg *capita*$^{-1}$ d^{-1} on calcareous and non-calcareous soils, respectively (Joy *et al.* 2015a). Verduzco-Gallo *et al.* (2014) reported mean national dietary supplies of Fe to be 19.5 mg *capita*$^{-1}$ d^{-1} based on household survey data and regional or international composition data. In the present study, estimated national mean and median dietary Fe supplies were 20.4 and 16.6 mg*capita*$^{-1}$ d^{-1}.

The low estimated prevalence of inadequate Fe intakes is consistent with previous estimates based on FBS data (Joy *et al.* 2014, Joy *et al.* 2015a) and duplicate diet

composites (Siyame *et al.* 2013). However, household consumption and requirement data aggregated at household level will underestimate the prevalence of Fe deficiency among adolescent and pregnant women because of their greater Fe requirements (WHO *et al.* 2004). Verduzco-Gallo *et al.* (2014) report similar median Fe supplies of 19.5 mg $capita^{-1}$ d^{-1}, but a 49% prevalence of inadequate intakes on the basis of food supply data from IHS3 and this might be due to different assumptions of Fe requirement or bioavailability. Nationally, the majority of dietary Fe supply came from non-haem sources with only 14% from animal products including fish (Additional file 1: Table S26). Iron requirements were calculated assuming a low bioavailability (i.e. 10%) (WHO *et al.* 2004). However, a large proportion of Fe intake is likely to be attributable to inadvertent consumption of soil dust present on grains and leafy vegetables (Gibson *et al.* 2015, Joy *et al.* 2015a), the bioavailability of which has not been adequately established.

Disease burden due to Fe deficiency was quantified for Malawi and other nations in the IHME Global Burden of Disease study on the basis of anaemia prevalence (Lim *et al.* 2013). Thus, Fe deficiency was estimated to cause 1553 DALYs (100 k population)$^{-1}$(Institute for Health Metrics and Evaluation 2014). However, further research is required to determine whether Fe intakes are actually inadequate or whether other strategies to reduce Fe deficiency are required, for example focusing on diseases such as malaria or gut parasites that can reduce absorption and increase losses of Fe (Monsen 1988, Verhoef *et al.* 2001, Glinz *et al.* 2015).

4.6.6 Iodine

Estimated dietary supplies of I are limited in their relevance and accuracy without data on I intakes from salt. However, it is clear that supply of I from foods other

than salt is inadequate to meet the requirements of almost 100% of households, with mean and median supplies of 17.7 and 12.2 μg *capita*$^{-1}$ d^{-1} (Additional file 1: Table S16). The estimated proportion of households consuming adequately iodised salt in 2010 was 62% (Iodine Global Network 2014), suggesting that up to 38% of households are likely to be consuming inadequate I to meet requirements. Estimated supplies of I from foods other than salt is much lower than the previous estimate based on FBS data and regional or international composition data of 36.2 μg *capita*$^{-1}$ d^{-1} from food sources other than salt (Joy *et al.* 2014) due to the use of locally generated food composition data in the present study. Scenarios of salt iodisation and consumption are explored in the section 'Interventions to improve dietary micronutrient supplies'.

The national I status of Malawi was recently defined as 'adequate' on the basis of urinary I concentrations (UIC) in school-aged children (Iodine Global Network 2014). Also, the disease burden due to I deficiency has been quantified for Malawi and other nations in the IHME Global Burden of Disease study on the basis of goitre prevalence (De Benoist *et al.* 2004, Murray *et al.* 2012). Thus, a 28% prevalence of I deficiency in Malawi was estimated to cause 82 DALYs (100 k population)$^{-1}$ (Institute for Health Metrics and Evaluation 2014). However, this estimate is from a survey of goitre prevalence in school-aged children in seven districts of Malawi with endemic goitre conducted in 1996 (Mdebwe and Banda 1996, De Benoist *et al.* 2004); the study design bias and the use of goitre prevalence rather than UIC data is likely to over-estimate the burden of disease attributable to I deficiency, while the finding is likely to be out-dated considering that salt iodisation was only introduced as national policy in Malawi in 1995 (Kalimbira *et al.* 2005, Zimmermann 2015).

4.6.7 Magnesium

Previously, Broadley *et al.* (2012), Kumssa *et al.* (2015b), Joy *et al.* (2014) and Joy *et al.* (2012) estimated mean national dietary supply of Mg to be 530–789 mg *capita*$^{-1}$ d^{-1}, based on FBS data and regional or international composition data, and subsequently, using local composition data, 712 and 632 mg *capita*$^{-1}$ d^{-1} on calcareous and non-calcareous soils, respectively (Muthayya *et al.* 2013). Estimated supplies of Mg are lower in the present study, with national mean and median of 479 and 401 mg *capita*$^{-1}$ d^{-1}. Removal of the husk and endosperm during milling of maize grain reduces the concentration of Mg by ~80% (Additional file 1: Table S6). These losses were accounted for in the present study but not in previous studies which used whole grain composition data. Despite lower estimated Mg supplies, the estimated prevalence of inadequate Mg supplies remains low which is consistent with previous findings (Broadley *et al.* 2012, Joy *et al.* 2012, Joy *et al.* 2014), although deficiency may occur due to high dietary PA supplies (Kumssa *et al.* 2015b).

4.6.8 Selenium

Previously, Joy *et al.* (2014) estimated mean national dietary supply of Se to be 34 µg *capita*$^{-1}$ d^{-1} based on FBS data and regional or international composition data, and subsequently, using local composition data, 41 and 19 µg *capita*$^{-1}$ d^{-1} on calcareous and non-calcareous soils, respectively (Joy *et al.* 2015a). Similar estimates were found in the present study, with median Se supplies of 31 and 17 µg *capita* d^{-1} for rural households on calcareous and non-calcareous soils, respectively. Median supplies for households in expenditure quintile 5 were approximately 3–4 fold greater than those in expenditure quintile 1 on both soil types.

Using local composition data and 24 h dietary recall, Eick et al. [21] estimated mean dietary supply of Se to be 44 and 46 μg *capita*$^{-1}$ d^{-1} in Mangochi District among tuberculosis patients ($n = 40$) and controls ($n = 40$). In the present study, estimated median (Q1, Q3) Se supplies of rural households in Mangochi District were 39 (22, 71) μg *capita*$^{-1}$ d^{-1} on calcareous soils ($n = 80$) and 30 (19, 48) μg *capita*$^{-1}$ d^{-1} on non-calcareous soils ($n = 206$).

Hurst *et al.* (2013) estimated median dietary Se supplies of adult women in villages on calcareous Eutric Vertisols ($n = 55$) and non-calcareous ($n = 58$) soils to be 55 and 7 μg *capita*$^{-1}$ d^{-1}, respectively, based on mineral analyses of weighed duplicate diet composites. In the present study, median Se consumption per AME was 36.4 and 20.4 μg d^{-1} on calcareous and non-calcareous soils, respectively. The greater difference in dietary Se supplies between soil types found by Hurst *et al.* (2013) is likely to be due to the subset of calcareous and non-calcareous soils that were sampled. For example, the median Se concentration of maize grain from Eutric Vertisols was >0.3 mg kg^{-1}(FAO 2001, Hurst *et al.* 2013), approximately 10-fold greater than the median Se concentration of maize grain samples collected nationally from areas of calcareous soils used in the present study (median = 0.03 mg kg^{-1}, $n = 50$, Additional file 1: Table S5 and Additional file 1: Table S7). Eutric Vertisols, which cover ~0.5% of the land area of Malawi, are a subset of the calcareous soil grouping and might provide greater concentrations of phyto-available Se than other calcareous soils. The non-calcareous villages selected by Hurst *et al.* (2013) were not in a lakeshore EPA and low fish consumption may contribute to these extremely low Se supplies.

4.6.9 Zinc

Previously, Wessells *et al.* (2012a) estimated mean national dietary Zn supply to be 8.3 mg *capita*$^{-1}$ d^{-1} based on 2003–2007 FBS data and regional or international composition data while Joy *et al.* (2014) used 2009 FBS data to estimate 11.8 mg *capita*$^{-1}$ d^{-1}, and subsequently, using locally-generated composition data, 12.0 and 10.1 mg*capita*$^{-1}$ d^{-1} on calcareous and non-calcareous soils, respectively (Joy *et al.* 2015a). Kumssa *et al.* (2015a) estimated mean Zn supply for Malawi to be 14.1 mg capita^{-1} d^{-1} on the basis of 2011 FBS data and US food composition data. Verduzco-Gallo *et al.* (2014) reported mean national dietary supplies of Zn to be 10.8 mg *capita*$^{-1}$ d^{-1} based on household survey data and regional or international composition data. Similar estimates were found in the present study, with mean and median Zn supplies of 10.4 and 8.5 mg *capita*$^{-1}$ d^{-1}for all households and a median of 9.5 and 7.8 mg *capita*$^{-1}$ d^{-1} for rural households on calcareous and non-calcareous soils, respectively. However, Siyame *et al.* (2013) reported much lower dietary Zn supplies on the basis of duplicate diet composites, i.e. 6.4 and 4.8 mg *capita*$^{-1}$ d^{-1} for women living on calcareous and non-calcareous soils, respectively. The villages studied by Siyame *et al.* (2013) were not in lakeshore EPAs and low fish consumption might contribute to the very low Zn supplies in both the calcareous and non-calcareous areas.

The estimated prevalence of inadequate dietary Zn supplies in the current study is 57% nationally, and 49 and 62% for rural households on calcareous and non-calcareous soils, respectively. A high prevalence of inadequate Zn supplies has previously been estimated using FBS data, i.e. 41% nationally (Wessells *et al.* 2012a), 64% nationally (Joy *et al.* 2014), and 31 and 57% on calcareous and non-

calcareous soils (Joy *et al.* 2015a); and IHS3 data, i.e. 54% for rural households (Verduzco-Gallo *et al.* 2014).

Disease burden due to Zn deficiency was quantified for Malawi and other nations in the IHME Global Burden of Disease study on the basis of dietary Zn supplies (Lim *et al.* 2012, Wessells *et al.* 2012a). Thus, a 41% national prevalence of inadequate dietary Zn supplies was estimated to cause 791 DALYs (100 k population)$^{-1}$ (Institute for Health Metrics and Evaluation 2014). By extension, disease burden is likely to be >1000 DALYs (100 k population)$^{-1}$ for rural households on non-calcareous soils where the prevalence of inadequate dietary Zn supplies was 62% (Additional file 1: Table S20).

4.6.10 Phytate

Previously, Wessells *et al.* (2012a) estimated mean national dietary PA supply to be 2584 mg *capita*$^{-1}$ d^{-1} with a PA:Zn molar ratio of 31 based on 2003–2007 FBS data and regional or international composition data while Joy *et al.* (2014) used 2009 FBS data to estimate mean supply of 4510 mg *capita*$^{-1}$ d^{-1} and a PA:Zn of 38. Kumssa *et al.* (2015a) estimated mean PA supply of 3969 mg capita^{-1} d^{-1} and a PA:Zn molar ratio of 28 based on 2011 FBS data. The present study found mean and median PA supplies of 2795 and 2281 mg *capita*$^{-1}$ d^{-1} with a mean PA:Zn molar ratio of 29. These estimates are closer to the Wessells *et al.* (2012a) values owing to the adjustment in PA concentrations made due to milling.

The combination of a high prevalence of inadequate dietary Zn supplies and PA:Zn molar ratios >15 suggest that Zn deficiency is likely to be widespread in Malawi. This is consistent with anthropometric data, e.g. Gibson *et al.* (1998) reported 36 and 46% prevalence of low plasma and hair Zn concentrations, respectively, among

pregnant women in a rural area, and Siyame *et al.* (2013) reported >90% prevalence of Zn deficiency defined as plasma Zn <10.7 μmol L^{-1} among women living in rural areas on calcareous and non-calcareous soils. High dietary PA supplies might also increase the risks of Ca and Mg deficiencies due to inhibition of absorption (Bohn *et al.* 2004b, Fredlund *et al.* 2006). Although Thacher *et al.* (2009) reported that a phytate rich maize meal increased Ca absorption but strongly reduced Zn absorption in Nigerian children and absorption of Ca and Zn was not affected by the presence of rickets.

4.6.11 Inadequate supplies of multiple elements

Nationally, the prevalence of inadequate dietary supplies were greatest for Ca, Se and Zn. High dietary PA supplies are likely to increase risks of Ca and Zn deficiencies. Adequacy of dietary I supply is highly dependent on the concentration of I in salt and Fe deficiency is contingent on individuals' health, especially gut health and malaria. Thus, estimating the prevalence of I and Fe deficiencies based on food consumption data available in the IHS3 is problematic. Among rural households living on non-calcareous soils, concurrent dietary inadequacies of Ca, Se and Zn occurred in 81% of households in expenditure quintile 1 compared to 15% of households in expenditure quintile 5 (Table 4.4). Of the 5156 households nationally that had adequate energy supplies to meet requirements, 30, 56 and 27% still had inadequate supplies of Ca, Se and Zn, respectively, to meet sum of member EARs, while 16% had concurrent inadequate supplies of all three elements.

Table 4.4. Inadequate dietary supplies of multiple elements. Concurrent inadequate dietary supplies of calcium, selenium and zinc at household level by urban/rural location, expenditure quintile (1 = poorest, 5 = highest) and soil type (1 = calcareous; 2 = non-calcareous; 3 = undifferentiated).

Urban/ rural	Soil type	Expenditure quintile	n	Households with 'x' concurrent inadequate intakes			
				0	1	2	3
Urban	3	1	57	7	4	23	67
		2	135	9	14	30	47
		3	228	11	11	36	42
		4	442	21	15	32	32
		5	1311	45	16	21	17
Rural	1	1	493	6	8	24	62
		2	450	19	16	24	41
		3	385	30	17	24	29
		4	379	52	17	16	15
		5	340	68	14	10	9
	2	1	1000	2	3	14	81
		2	1297	6	9	23	62
		3	1424	11	12	27	49
		4	1467	19	19	28	34
		5	1335	44	23	18	15
	3	1	290	3	7	18	72
		2	291	10	12	27	51
		3	294	20	19	30	31
		4	294	29	22	30	19
		5	205	54	22	14	9

4.6.12 Interventions to improve dietary micronutrient supplies

Strategies to improve dietary micronutrient supplies include direct supplementation, food-based interventions (such as fortification of cereal flours at the processing stage or dietary diversification), and agricultural interventions (such as biofortification of crops through breeding or application of micronutrient-enriched fertilisers). Recently, it was demonstrated that agronomic biofortification of staple cereals may be a cost-effective strategy to reduce dietary Zn deficiencies in Malawi if Zn is delivered *via* foliar sprays, although such a strategy is likely to be less cost-effective than biofortification *via* crop breeding (Joy *et al.* 2015c). Here, two other scenarios are explored: the iodisation of salt and agronomic biofortification of maize grain with Se.

Over 99% of households in Malawi were not consuming adequate I through foods other than salt to meet requirements. However, the I status of Malawi has recently been defined as 'adequate' based on UIC of school-aged children (Iodine Global Network 2014); thus it is likely that salt iodisation has already been a considerable success. Yet challenges remain. The WHO recommend that adults consume <5 g d^{-1} of salt to limit risk of chronic disease due to excessive sodium intakes (Amine *et al.* 2002, World Health Organization 2007), yet the IHS3 data shows median salt supply per AME of 11.2 g d^{-1}. Even with universal coverage of salt iodised at the recommended 15 mg kg^{-1} and individual consumption of salt of 5 g d^{-1}, 93% of households in Malawi would have inadequate I supply to meet dietary requirements. The prevalence of inadequate dietary I supply would be 24 and <1% if salt consumption were 7.5 and 10 g *capita*$^{-1}$ d^{-1}, respectively. Alternatively, iodising salt at 30 mg kg^{-1} would supply adequate I to 99% of households if individual consumption of salt was 5 g d^{-1}.

Salt iodisation programmes need close monitoring. Iodisation of table salt is mandatory in Malawi yet only 62% of households in Malawi were consuming adequately iodised salt in 2010 (NSO 2011) suggesting problems with compliance or losses due to improper storage. A recent spot survey of adult women in Malawi living on calcareous ($n = 59$) and non-calcareous soils ($n = 59$) found that the median creatinine-corrected UIC was 203 μg L^{-1}. However, there was a 14% prevalence of I deficiency (UIC <100 μg L^{-1}) but 21% prevalence of excess (UIC >300 μg L^{-1}) (Watts *et al.* 2015). Thus, high household salt supplies or I concentration in salt might be contributing to excessive I intakes, risking hypo- and hyperthyroidism.

An agronomic biofortification programme for Se in Malawi could be effective without major changes in farm-level infrastructure through enrichment of existing fertilisers applied to maize (Chilimba *et al.* 2012a, b, Chilimba *et al.* 2014). Addition of 10 g Se ha^{-1} *via* a granular NPK fertiliser to maize grown on different soils in Malawi achieved a mean concentration of 0.276 mg Se kg^{-1} in the grain (Chilimba *et al.* 2012a) which is ~18-fold greater than the median Se concentration of maize grain samples from non-calcareous soils used in the present study. The approach has precedents, having largely eliminated dietary Se deficiency in Finland (Alfthan *et al.* 2015) and may be effective *via* a range of cereal crops (Broadley *et al.* 2009, White *et al.* 2009, Alfthan *et al.* 2015). Biofortification of staple crops has the potential to be highly equitable as staple foods are consumed on a daily basis by most low-income households and individuals with low status within the household. For example, 98% of all households in IHS3 and 94% of those in expenditure quintile 1 reported consuming maize. Furthermore, alternative options to increase dietary element supplies are limited in subsistence contexts. For example, Fiedler *et al.* (2013) showed that flour fortification during milling currently has limited reach in Zambia as few households purchase maize flour from large, centralised milling factories and those that do are generally wealthier with greater baseline Zn intakes.

Application of Se increases its concentration in all fractions of the grain including the endosperm (Lyons *et al.* 2003), although concentration is likely to be greater in the bran and embryo and previously it was demonstrated that refined maize flour has ~ half the concentration of Se than the whole grain (Additional file 1: Table S6). Thus, if all maize grown on non-calcareous soils received 10 g Se ha^{-1}, the median concentration of 'Maize *ufa* refined (fine flour)' would likely be ~0.138 mg Se kg^{-}

^{1} and maize products would supply ~37 μg Se *capita*$^{-1}$ d^{-1} compared to ~4 μg Se *capita*$^{-1}$ d^{-1} without biofortification. The prevalence of inadequate dietary Se supplies among rural households on non-calcareous soils would fall from 82 to 14%. However, the efficacy will depend on fertiliser use. Currently, maize production in Malawi requires ~140 kt of fertiliser nitrogen (N) to cover the ~2800 kha of production (Joy *et al.* 2015c). Usage is ~50 kt (i.e. 36% of requirement) of which ~35 kt is subsidised under the Farm Input Subsidy Programme (FISP) (International Fertilizer Development Center 2013). Thus, the efficacy of a biofortification scenario is likely to be ~36% of the universal application modelled above, i.e. reducing prevalence of inadequate dietary Se supplies from 82 to 57%. Nonetheless, Haug *et al.* (2007) emphasize that Se is a valuable resource which should not be wasted; e.g., with agronomic biofortification, usually only ~15% of soil-applied Se reaches cereal grain.

Agronomic biofortification with Se in areas where dietary deficiency occurs is likely to be highly cost-effective. If maize production in Malawi is assumed to be evenly distributed across different soil types then there are ~2000 kha of maize production on non-calcareous soils (Joy *et al.* 2015c). Application of 10 g ha^{-1} of Se would therefore cost US$ ~2 million year^{-1}, assuming exogenous Se costs US$ 100 kg^{-1} (other programmatic costs not included). Thus, for application of Se at 10 g ha^{-1}, a percentage point drop in national dietary Se deficiency prevalence would cost ~ US$ 27,000 yr^{-1}. If the Se status of individuals is assumed to be the same as their respective household status, then the cost per alleviated case of dietary Se deficiency would be ~ US$ 0.36 year^{-1}. The WHO TUL of intake for Se is 400 μg d^{-1} for adults and excessive Se intake may be defined as household supply per AME >400 μg d^{-1}. With universal application of 10 g ha^{-1} of Se on non-calcareous soils,

13 out of 12,117 households (~0.1%) would be expected to have excessive Se intake. The actual risk of excessive intakes is likely to be lower as all of these 13 households reported consuming >4000 kcal per AME d^{-1} which is implausibly high over a long period. Cost-effectiveness could be improved if Se-enriched fertiliser was distributed through the FISP scheme due to the greater prevalence of inadequate dietary Se supplies among poorer households. Furthermore, the modelled scenarios are likely to underestimate efficacy due to increases in the Se concentration of legumes intercropped with maize and of livestock products fed on maize stover or grain receiving Se-enriched fertiliser. Alfthan *et al.* (2015) reported an increase in the contribution of animal products to human dietary Se intakes in Finland following a national policy of agronomic biofortification with Se; milk products from 'conventional' production had ~2-fold greater Se concentration than from 'organic' production. There may also be benefits to livestock productivity and health (Broadley *et al.* 2006) which are not captured in the current study.

4.6.13 Use of household surveys to estimate the prevalence of inadequate dietary element supplies in Malawi

There are three main potential sources of dietary survey data: individual-level recall, household-level surveys and national-level FBSs. Individual-level 24 h diet recall is considered the 'gold standard' for dietary assessment by nutritionists, but the approach is often prohibitively expensive to conduct at large scales e.g. nationally (Fiedler 2013) and only small-scale surveys have been conducted in Malawi. Household surveys and FBSs are routinely conducted/compiled allowing longitudinal assessment of diets at national, e.g. Verduzco-Gallo *et al.* (2014), or international scales, e.g. Joy *et al.* (2013) and Kumssa *et al.* (2015a). The main

advantage of household surveys over FBSs is that they provide insights into the distribution of element supplies at sub-national levels. In the present study, there was a positive skew in the distribution of household supplies of all nutrients (Fig 4.7). Thus, 68% of households had per capita supplies of Ca less than the mean of all households, i.e. 924 mg *capita*$^{-1}$ d^{-1}. Estimates of dietary element supplies derived from FBSs have previously assumed a normal distribution of individual-level dietary supplies centred on mean per capita availability of an element in the national food supply; a 'cut-point' is set as the national mean EAR and prevalence of inadequate supplies is assumed to equal the proportion of the population with intakes below this level (Wessells *et al.* 2012a, Joy *et al.* 2014). If the positive skew in household level supplies also occurred at individual level, then the FBS approach is likely to underestimate the prevalence of inadequate dietary supplies.

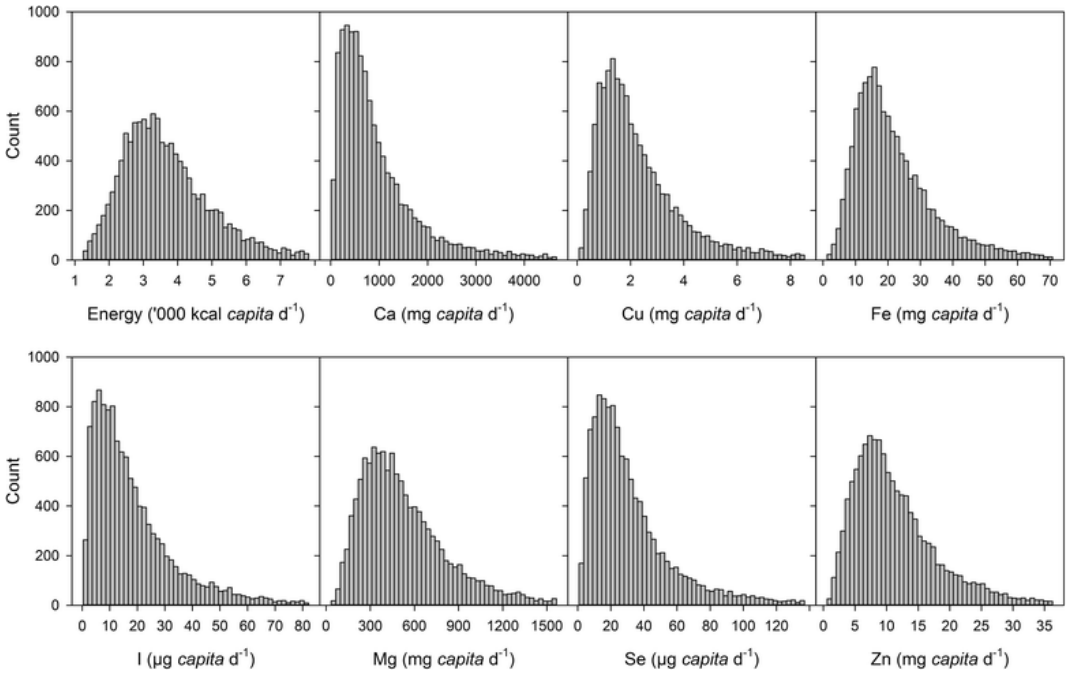


Fig 4.7 Distribution of household nutrient supplies. Dietary supplies of energy, calcium (Ca), copper (Cu), iron (Fe), iodine (I), magnesium (Mg), selenium (Se) and zinc (Zn). Iodine supply excludes salt.

There are three further advantages of deriving food consumption data from the IHS3 compared to the Malawi FBS. First, a greater variety of food items or break down of major food items are reported than the standard 94 edible items in the FBS, for example common indigenous vegetables, or refined and whole grain maize flour, and this allows more accurate matching of crop composition data. Second, FBSs generally rely on crop or livestock production data which may be restricted to commercialised major food crops; for example, sweet potato supply is not recorded in the most recent FBS (FAO 2014). Third, IHS3 gives insights into seasonality of supplies.

National-level estimates of inadequate dietary supplies derived from the analysis of FBSs might spur policy makers to intervene or support further research. However, inter-household and seasonal variation in food choice and dietary micronutrient intakes are likely to be required for effective policy making. For example, agronomic biofortification appears to be a promising strategy to address dietary Se deficiency in Malawi (Chilimba *et al.* 2012a, b, Hurst *et al.* 2013, Chilimba *et al.* 2014). A policy maker might question whether an intervention needs to reach wealthy households with their greater calorie intakes and more diverse diets. Despite greater consumption of nutrient-dense foods including fish, 55% of households in expenditure quintile 5 on non-calcareous soils had inadequate Se supplies to meet sum of member EARs. Thus, enriching subsidised fertiliser used by poorer households would not be sufficient in eliminating dietary Se deficiency. Valid concerns of potential toxicity might also be raised given the relatively narrow range between 'inadequate' and 'excessive' intakes of Se (IOM 2001); but in the present study, it was estimated that ~0.1% of the population would have excessive intakes if application of Se at 10 g ha^{-1} were universal on non-calcareous soils. And

this figure is likely to be lower if households with unrealistically high calorie intakes are removed. Similarly, Fiedler *et al.* (2013) used household survey data to address concerns over the effectiveness and safety of potential food fortification schemes in Zambia. However, close monitoring of fertiliser enrichment and distribution to appropriate areas would be required, similar to management considerations in the salt iodisation strategy.

4.7 Caveats

4.7.1 Dietary recall at household level

Dietary recall provides a proxy for food consumption and is subject to error. Recall error can occur when interviewees forget instances of food consumption ('recall loss') or include items which were consumed prior to the recall period ('telescoping errors'). These types of error are likely to be greater for longer recall periods. Furthermore, the ability of one household member to accurately report the consumption of all household members is questionable, and this might account for some of the association between decreasing dietary energy supplies with increasing household size (Fig 4.3). Under- or over-reporting of food consumption can also be intentional. For example, respondents in the UK and US national dietary surveys were estimated to under-report their individual calorie intakes by up to 41% (Rennie *et al.* 2007, Archer *et al.* 2013). This occurred in a society in which being overweight is generally perceived as undesirable, yet in the UK 71 and 58% of men and women, respectively, have a Body Mass Index >25 (Bates *et al.* 2014).

Social pressures in Malawi are likely to differ to those in the UK or US. For example, being overweight is generally perceived as healthy and socially desirable (Bentley *et al.* 2005) and it is interesting to note the high reported calorie

consumption of the wealthiest expenditure quintile, i.e. mean and median consumption per AME of 3837 and 3618 kcal d^{-1}compared to a requirement of 3300 kcal d^{-1} for adult males with a PAL of 1.9. Also, many wealthy individuals, particularly in urban areas, are unlikely to require such high calorie intakes due to lower PALs. For example, an adult male with a sedentary or light activity level would require 2500-3000 kcal d^{-1} (FAO *et al.* 2004).

In addition to recall errors, there are likely to be numerous reporting errors introduced by enumerators. The capping of food item consumption at maximum plausible levels is likely to have reduced, though not eliminated, the influence of such errors. Local units are a convenient way to report quantities consumed in an interview process but may encourage inaccurate reporting. For example, ~80% of households that recorded consumption of the food item 'Maize *ufa* refined (fine flour)' expressed the quantity consumed in units of small, medium or large pails. The accuracy of these units is subject to the interpretation of the interviewees and enumerators, as well as the conversion to metric units applied by the authors of the present study.

One quick way to assess the validity of food consumption data reported in the IHS3 is to look at the plausibility of dietary energy supplies. As reported in the Results, there were both implausibly low and high calorie intakes when households were aggregated at the EPA level. The energy requirement of an adult male with PAL of 1.9 and body mass of 70 kg is ~3300 kcal d^{-1} (FAO *et al.* 2004), yet 21% of households reported consumption per AME of <1650 kcal d^{-1}, i.e. half the requirement, and 8% reported >4950 kcal d^{-1}, i.e. double the requirement, and these levels are highly unlikely to be sustainable for periods of more than a few days. If

these households were excluded, the estimated national prevalence of inadequate dietary energy, Ca, Cu, Fe, I, Mg, Se and Zn supplies from foods other than salt would be 50, 44, 2, 10, 100, 1, 72 and 51%, respectively, compared to 57, 49, 6, 18, 99, 5, 74 and 57% prior to removal of households with 'implausible' dietary energy intakes. Thus, the results of the present study are reasonably robust against recall or reporting errors at a national scale and demonstrated some regional variation that could not be identified using national-level food supply data, e.g. greater Ca supplies in lakeshore EPAs. However, taking Ca as an example, removing households with implausible energy intakes would increase or decrease the prevalence of inadequate dietary supplies by >25% in 94 of 149 EPAs which suggests that further research is required to improve the accuracy of household survey food consumption data before intervention policies can be designed at the highly disaggregated level of the EPA.

Comparison between food supply in FBS and IHS3 reveals some consistencies and discrepancies. For example, mean national consumption of maize is reported as 365 g*capita*$^{-1}$ d^{-1} FW in the 2009 FBS (FAO 2014), equivalent to 327 g *capita*$^{-1}$ d^{-1} DW (Joy *et al.* 2015a); this compares to mean (± S.D.) consumption of maize products (i.e. item codes 101–105 and 820) of 320 (± 203) g *capita*$^{-1}$ d^{-1} DW in the household survey. However, mean consumption of freshwater fish differs by ~7-fold between the two food supply datasets (*cf.* discussion on Ca supplies). The original source of FBS fish supply data in Malawi is not publicly available which prevents further investigation of this discrepancy.

The present study estimated dietary element supplies and prevalence of inadequate supplies at the household level due to the availability of data in the IHS3. However,

intra-household variation in foods consumed is not captured. Energy requirement ratios of household members could be used to estimate distribution of foods within the household and hence the prevalence of inadequate supplies at the individual level. However, the reliability of this approach is questionable as it requires the assumption that all members within a household consume the same mixture of food items (Bouis *et al.* 2011).

Estimating dietary supplies at household level is likely to underestimate the prevalence of inadequate intakes at individual levels because poorer, rural households are more likely to have inadequate dietary element supplies and to have larger household size (Table 4.1 and 4.2; Additional file 1: Table S9 and Additional file 1: Table S10). If all individuals living in households with supplies less than sum of member EARs were assumed to have inadequate intakes, then the prevalence of dietary energy, Ca, Cu, Fe, I, Mg, Se and Zn deficiencies among individuals would be 61, 53, 7, 20, 99, 6, 77 and 63%, respectively, compared to 57, 49, 6, 18, 99, 5, 74 and 57% among households.

4.7.2 Food composition

As with food supply data, there is an accuracy-cost trade-off in the use of food composition data and *caveats* regarding composition data were discussed previously (Joy *et al.* 2015a). In the present study, households were assigned to 'calcareous', 'non-calcareous' and 'undifferentiated' soil types on the basis of their location. This is likely to improve the relevance of matched food composition data. A national-scale food crop survey can only capture some of the variation in crop composition due to soil factors or varietal differences. However, locally-generated data remain preferable to the use of regionally or internationally collated datasets,

particularly for elements such as Se where plant uptake is under strong geochemical control. However, it is inevitable that some households were misclassified due to GPS location being aggregated at EA level and displaced to ensure confidentiality or due to the resolution of soil maps.

4.7.3 Nutrient requirements

Nutrient requirements for age and sex categories are reported by a number of different public health bodies. In the present study, requirements were derived from FAO and WHO (FAO *et al.* 2004, WHO *et al.* 2004), and IOM (IOM 2000a, 2001) as described in the Methods. However, aggregated requirement values cannot fully capture inter-individual variation in requirements, e.g. due to different body sizes, activity levels, presence of infection, consumption of promoters or inhibitors of nutrient absorption etc. A preference for conservative (i.e. high) requirement values might over-estimate the prevalence of nutrient deficiencies.

4.7.4 Drinking water

Drinking water can contribute significantly to dietary element intakes but this was not quantified in the present study; consequently, the prevalence of inadequate dietary supplies may be overestimated. For example, an *ad hoc* survey of borehole waters ($n = 19$) revealed mean and median Ca concentration of 39.2 and 22.2 mg L^{-1} (range 3.2-209.3), I concentration of 15.4 and 12.6 μg L^{-1} (range 1.0-54.2) and Mg concentration of 31.4 and 24.8 mg L^{-1} (range 0.9-95.2; Additional file 1: Table S28). Mean concentrations of Cu, Fe, Se and Zn were 1.9, 0.3, 0.2 and 8.4 μg L^{-1} which is insignificant compared to dietary food intakes. A wider survey would be required to assess the contribution of drinking water to dietary element supplies, although this does illustrate the potential of some drinking waters to contribute significantly

to dietary Ca, Mg and I supplies as seen in other contexts, e.g. (Fordyce *et al.* 2000, Voutchkova *et al.* 2014).

4.8 Conclusions

Prevalence of dietary element supplies and deficiencies were quantified for Malawi by combining food consumption data captured in the most recent household survey (IHS3) with locally-generated food composition data, stratified by soil type. We estimate that 57% of households had inadequate dietary energy supplies for requirements of an active lifestyle, while >50% of households had inadequate dietary supplies of Ca, Se or Zn to meet requirements but <20% had inadequate dietary supplies of Cu, Fe and Mg. Among households with adequate energy supply, 30, 56 and 27% still had inadequate supplies of Ca, Se and Zn to meet requirements. Supply of I from foods other than salt is inadequate for >99% of households. Access to essential nutrients varied due to socioeconomic and environmental factors. For example, the median supply of Ca in rural households in the wealthiest and poorest expenditure quintiles was 1157 and 255 mg $capita^{-1}$ d^{-1}, respectively; the difference was largely driven by the consumption of fish with a median supply of Ca from fish of 737 and 246 mg $capita^{-1}$ d^{-1} in the wealthiest and poorest expenditure quintiles, respectively. The median supply of Se among rural households in areas of calcareous and non-calcareous soil was 30.7 and 17.3 μg $capita^{-1}$ d^{-1}, respectively; the difference was driven by food composition, with median Se concentration of 0.0138 and 0.0071 mg kg^{-1} in refined maize flour from calcareous and non-calcareous soils, respectively.

Nationally, cereals supplied >60% of dietary energy, >40% of Mg and Zn, >30% of Fe and >20% of Se, but <5% of Ca. Fish was an essential source of micronutrients

for many households, partly due to the preference for eating whole small fish (*usipa*) including bones. Overall, 77% of households recorded fish consumption during 7 days preceding their interview, and fish supplied 62, 47 and 26% of national dietary Ca, Se and Zn supplies. However, consumption of fish varies with greater access for wealthier households and those living in lakeshore EPAs.

Two strategies to increase dietary element supplies were modelled. We show that iodisation of salt at 15–30 mg kg^{-1} can ensure that the majority of households have adequate I supplies due to near universal consumption of salt, including in poorer, rural households. However, mean household salt supplies of 11.2 g per AME were greater than the WHO maximum recommended intake of 5 g $capita^{-1}$ d^{-1} and close monitoring of iodisation levels at production is required to avoid excessive I intakes. Agronomic biofortification with 10 g Se ha^{-1} of maize has the potential to reduce the prevalence of inadequate dietary Se supplies from 82 to 14% of households living on non-calcareous soils, and from 95 to 21% for the poorest subset of those households. However, if only those fertilisers currently in use were enriched, the prevalence of inadequate Se intakes among all households living on non-calcareous soils would fall from 82 to 57% The cost per alleviated case of dietary Se deficiency would be ~ US$ 0.36 $year^{-1}$, representing a highly cost-effective strategy.

Household surveys provide a valuable resource for assessing national diets, although the accuracy of food consumption data remains an issue: instances of implausibly high or low reported energy intakes suggest reporting errors, possibly due to purposeful under- and over-reporting. Also, there are unresolved discrepancies between national-level FBS and household survey datasets, for example the ~7-fold greater estimated consumption of fish based on IHS3. The

present study used food crop composition data generated for Malawi and demonstrated significant variation in dietary supplies of some elements depending on soil properties. However, locally-generated food crop composition data stratified by soil type are not available for many countries. Thus, work to improve the accuracy and spatial resolution of food crop composition data is required to extend the methodology to other countries, particularly for elements where plant uptake is under strong geochemical control.

CHAPTER 5. VARIATION IN THE MINERAL ELEMENT CONCENTRATION OF *MORINGA OLEIFERA* LAM. AND *M. STENOPETALA* (BAK. F.) CUF.: ROLE IN HUMAN NUTRITION

5.1 Authors contribution

All authors were involved in designing the study. **DBK** and EJMJ. collected samples from Kenya and Ethiopia, respectively. **DBK** processed the samples in the laboratory (i.e., milling, acid/base digestion, dilutions), and made the samples ready for ICP-MS analyses. SDY carried out ICP-MS analyses. **DBK** compiled the laboratory analyses data, carried out statistical analyses, and produced the initial draft of the manuscript. All authors contributed in drafting the manuscript.

5.2 Abstract

Background: *Moringa oleifera* (MO) and *M. stenopetala* (MS) (family Moringaceae; order Brassicales) are multipurpose tree/shrub species. They thrive under marginal environmental conditions and produce nutritious edible parts. The aim of this study was to determine the mineral composition of different parts of MO and MS growing in their natural environments and their potential role in alleviating human mineral micronutrient deficiencies (MND) in sub-Saharan Africa.

Methods: Edible parts of MO (n = 146) and MS (n = 50), co-occurring cereals/vegetables and soils (n = 95) underneath their canopy were sampled from localities in southern Ethiopia and Kenya. The concentrations of seven mineral elements, namely, calcium (Ca), copper (Cu), iodine (I), iron (Fe), magnesium

(Mg), selenium (Se), and zinc (Zn) in edible parts and soils were determined using inductively coupled plasma-mass spectrometry.

Results: In Ethiopian crops, MS leaves contained the highest median concentrations of all elements except Cu and Zn, which were greater in Enset (a.k.a., *false banana*). In Kenya, Mo flowers and MS leaves had the highest median Se concentration of 1.56 mg kg^{-1} and 3.96 mg kg^{-1}, respectively. The median concentration of Se in MS leaves was 7-fold, 10-fold, 23-fold, 117-fold and 147-fold more than that in brassica leaves, amaranth leaves, baobab fruits, sorghum grain and maize grain, respectively. The median Se concentration was 78-fold and 98-fold greater in MO seeds than in sorghum and maize grain, respectively. There was a strong relationship between soil total Se and potassium dihydrogen phosphate (KH_2PO_4)-extractable Se, and Se concentration in the leaves of MO and MS.

Conclusion: This study confirms previous studies that *Moringa* is a good source of several of the measured mineral nutrients, and it includes the first wide assessment of Se and I concentrations in edible parts of MO and MS grown in various localities. Increasing the consumption of MO and MS, especially the leaves as a fresh vegetable or in powdered form, could reduce the prevalence of MNDs, most notably Se deficiency.

5.3 Introduction

Human micronutrient deficiencies (MNDs) are widespread in sub-Saharan Africa (Joy *et al.* 2015b, Kumssa *et al.* 2015a, Gashu *et al.* 2016b). There is increasing interest in the potential role of underutilised crops to address MNDs and *Moringa* is one example (Olson *et al.* 2016). *Moringa* is the sole genus of the flowering plant family Moringaceae, order Brassicales; (APGIV 2016). It comprises 13 species of trees and shrubs (Table 5.1) , namely, *M. arborea, M. borziana, M. concanensis, M. drouhardii, M. hildebrandtii, M. longituba, M. oleifera, M. ovalifolia, M. peregrine, M. pygmaea, M. rivae, M. ruspoliana,* and *M. stenopetala* (TFLI 2014). Nine of the 13 species in the genus *Moringa* are native to lowlands of eastern Africa (i.e., south-eastern Ethiopia, Kenya and Somalia), of which, eight are considered endemic (NRC 2006, Roskov *et al.* 2014). The Horn of Africa is considered to be the centre of diversity of *Moringa* genus, but *Moringa oleifera* (MO) is the only species thought to originate outside Africa (Olson and Carlquist 2001, NRC 2006). *Moringa oleifera* and *M. stenopetala* (MS) are the two cultivated and most studied species (Shahzad *et al.* 2013, Kifleyohannes *et al.* 2014, Kushwaha *et al.* 2014, Ogunsina *et al.* 2014, Tesfaye *et al.* 2014, Toma *et al.* 2014, Zaffer *et al.* 2014, da Conceição *et al.* 2015, Forster *et al.* 2015, Matshediso *et al.* 2015, Olson *et al.* 2016).

Moringa oleifera (Fig 5.1a) is indigenous to the Himalayan foothills of south India (Paliwal and Sharma 2011). It has been naturalized to tropical and sub-tropical Asia; Middle East; Africa; and America (Morton 1991, Foidl *et al.* 2001, NRC 2006, Moyo *et al.* 2013, Popoola *et al.* 2013). This pantropical species is known by various names. In English, it is known as *drumstick* tree due to the shape of its pods,

never die tree due to its ability to thrive under marginal environmental conditions, and *mother's best friend* due to its nutritious edible parts that help revive malnourished children (Lim 2012). It is known as *Mlonge/ Mzunze/ Mjungu moto/ Mboga chungu/ Shingo* in Kenya (NRC 2006). *Moringa stenopetala* (Fig 5.1b) is native to southern Ethiopia and northern Kenya (Jahn 1991a, Abuye *et al.* 2003). In southern Ethiopia, it is locally known as *Haleko* in Walayita and Konso languages.

Moringa oleifera and MS are fast growing multipurpose woody plants which grow in diverse ecosystems (Morton 1991, Odee 1998, Foidl *et al.* 2001, NRC 2006, Undie *et al.* 2013), from very dry marginal lowland tropical climates to moist high altitude regions. They shed their leaves during long dry seasons. Their tuberous roots enable them to store water and withstand very long dry seasons. The MO tree can grow up to 5 – 15 m in height, with a diameter at breast height up to 25 cm (Morton 1991, Foidl *et al.* 2001, NRC 2006). A mature MS tree is usually larger in overall size and more drought tolerant than MO, with larger leaves, seeds and trunk. However, MS is slower-growing compared to MO. In experiments conducted in the Sudan, MS flowered after 2.5 years as compared to 11 months for MO (Price 2007).

5.3.1 Nutritional uses

Dietary diversification using underutilized crops/trees, such as *Moringa* spp. is one of the many alternative strategies to fight MNDs (Joy *et al.* 2014, Joy *et al.* 2015b, Kumssa *et al.* 2015a, Kumssa *et al.* 2015b, Mabhaudhi *et al.* 2016). However, data on nutritional contents of such under-utilised vegetables and understanding of environmental/genetic variation in trace elements concentration are limited. Ethnobotanical and biochemical studies carried out in various countries where *Moringa* grow show that these species are multipurpose. They are used for food,

medicine, fodder, fencing, firewood, gum and as a coagulant to treat dirty water (Morton 1991, Balemie and Kebebew 2006, Virchow 2008, Teklehaymanot and Giday 2010, Ocho *et al.* 2012, Degefu and Dawit 2013, Popoola *et al.* 2013). The foliage, immature pods, seeds, and roots are used both as food and medicine. Young shoots are also cooked and eaten (Lim 2012, Popoola *et al.* 2013). Leaves are either cooked or consumed raw as vegetables. *Moringa* leaves are used in a similar way as a cabbage and spinach thereby nicknamed 'cabbage tree' (Jahn 1991b). As a food or forage source, *Moringa* spp. can supply a wide range of essential macro and micro nutrients (Moyo *et al.* 2011, Lim 2012, Melesse *et al.* 2012, Olson *et al.* 2016). The mean concentration of Ca, Cu, Fe, Mg, and Zn in MO leaves collected from a garden in Jalisco State of Mexico were 16100, 9.6, 97.9, 2830 and 29.1 mg kg^{-1} dry weight (dw), respectively. Similarly, the concentrations of these elements in MS leaves were 12700, 9.1, 69.9, 3690 and 33.7 mg kg^{-1} dw, respectively (Olson *et al.* 2016). A mean Se concentration of 0.877 mg kg^{-1} dw was reported in MO leaves grown at six locations ranging from 0.455 mg kg^{-1} dw in Rwanda to 2.00 mg kg^{-1} dw in the Solomon Islands (Lyons *et al.* 2015). However, systematic analysis of Se has not been conducted at multiple sites within a country and concentrations of other elements such as iodine have not been reported.

5.3.2 Impact of environment on mineral element concentration in *Moringa* edible parts

The mineral element concentrations in different edible parts of *Moringa* spp. are affected by the environment in which they grow. For example, the effect of elevation and season on mineral micronutrient concentration of leaves and immature pods of MO and MS was studied in Ethiopia (Melesse *et al.* 2012).

Concentrations of Ca, Fe, and Zn in *Moringa* leaves grown in mid-altitude areas during the rainy season were 24800, 578 and 24.3 mg kg^{-1} dw for MO and 14900, 700 and 24.7 mg kg^{-1} dw for MS, respectively. In low altitude areas, the Ca, Fe, and Zn concentration in MO leaves during rainy season were 25700, 564 and 26 mg kg^{-1} dw, while in MS leaves, the concentrations were 24000, 581 and 28.1 mg kg^{-1} dw, respectively (Melesse *et al.* 2012). Other studies have compared MO samples collected from various sites without specifying environmental variables. For example, in their study on the mineral concentration of MO edible parts in two regions of Nigeria, it was reported that Ca, Mg, Fe and Cu concentration in the leaves, pods and seeds were higher in tissues collected from Sheda region than Kuje, Abuja (Anjorin *et al.* 2010). Similarly, a study conducted in the Punjab province of Pakistan indicated that the Ca, Mg, and Zn concentration in the leaves and pods of MO varied significantly by region (Aslam *et al.* 2005). For example, the Ca concentration in MO leaves in Bahawalnagar and Sadiqabad were 22900 and 19000 mg kg^{-1} dw respectively. A study conducted by Olson *et al.* (2016) indicated variation in leaf elemental concentration between 12 *Moringa* species grown in a common garden experiment.

5.3.3 Study aims

To our knowledge, no studies have explored the association between plant tissue element concentration of *Moringa* spp., and the site-specific physico-chemical properties of the soil. Previous studies assessing the variation in the elemental concentration in edible parts of *Moringa* spp. in various agro-ecological zones have typically been based on generic classifications, e.g., elevation (Melesse *et al.* 2012). Furthermore, there is some evidence that *Moringa* accumulates Se (Lyons *et al.* 2015) but this has not been widely confirmed in leaves or for other plant parts.

Iodine concentrations have not previously been reported in *Moringa* leaves. The objectives of this study were to:

- determine the multi-elemental concentration in the flowers, immature pods, leaves, and seed kernels of MO and MS grown in different agro-ecological zones in Ethiopia and Kenya;
- explore the association between MO and MS edible parts mineral element concentration and soil physico-chemical properties;
- assess the potential of consumption of MO and MS leaves in alleviating dietary micronutrient deficiencies in sub-Saharan Africa; and
- compare the mineral element concentrations in MS and MO edible parts with locally grown cereal and vegetable crops.

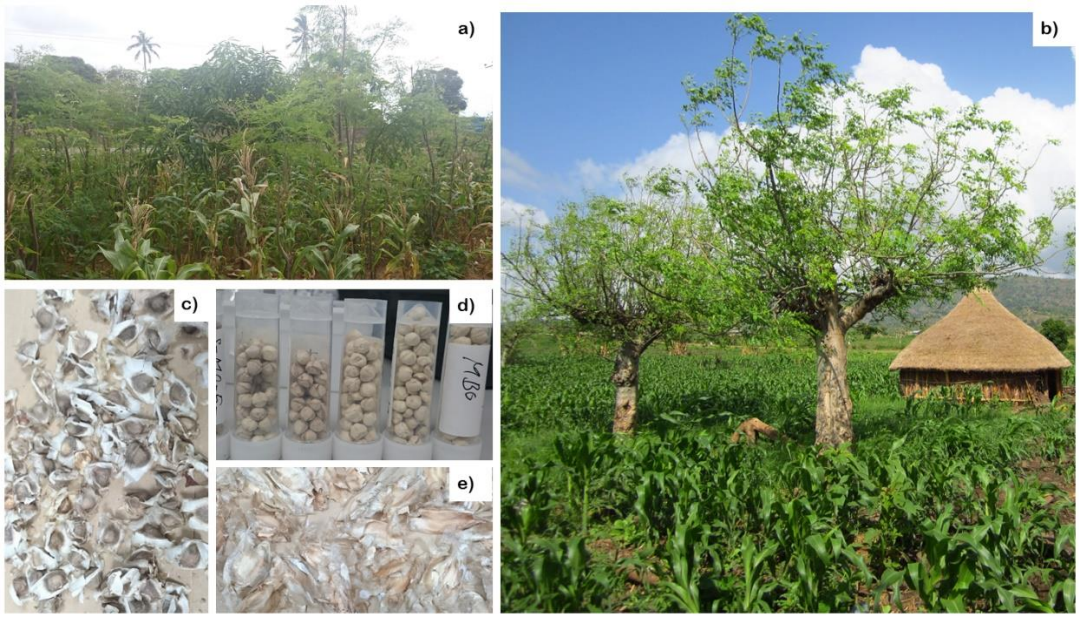


Fig 5.1. *M. oleifera* tree intercropped with maize at Malindi, Kenya (a); *M. stenopetala* trees intercropped with maize, southern Ethiopia (b); Husked *M. oleifera* seeds (c); kernel of *M. oleifera* (d) and husked *M. stenopetala* seeds (e).

5.4 Materials and methods

This study was conducted in southern Ethiopia and Kenya. Sample collections from localities in southern Ethiopia were carried out in December 2014 and April 2015, and in July 2015 from localities in Kenya (Fig 5.2). Edible parts of MO and MS

were sampled from plants that were cultivated by *Moringa* growing households after receiving their consent. The study was carried out on private/communal land with the owners' permission, and it did not involve endangered or protected species. The study received ethical approval from the University of Nottingham, School of Biosciences Research Ethics Committee (SB REC), approval number: SBREC140117A.

5.4.1 Study sites

Edible parts of MO, MS, other food crops, and soil samples, were collected from localities in southern Ethiopia and Kenya (Fig 5.2 and Appendix 5.1 - 5.2). Site selection was conducted by the guidance of local agricultural development agents who knew about the localities and households that cultivate *Moringa* trees. In addition, different sites with varying soil types were surveyed. The altitude of the locations ranged from 13 m a.s.l. in Malindi, Kenya to 1700 m a.s.l. in Hawassa, Ethiopia.

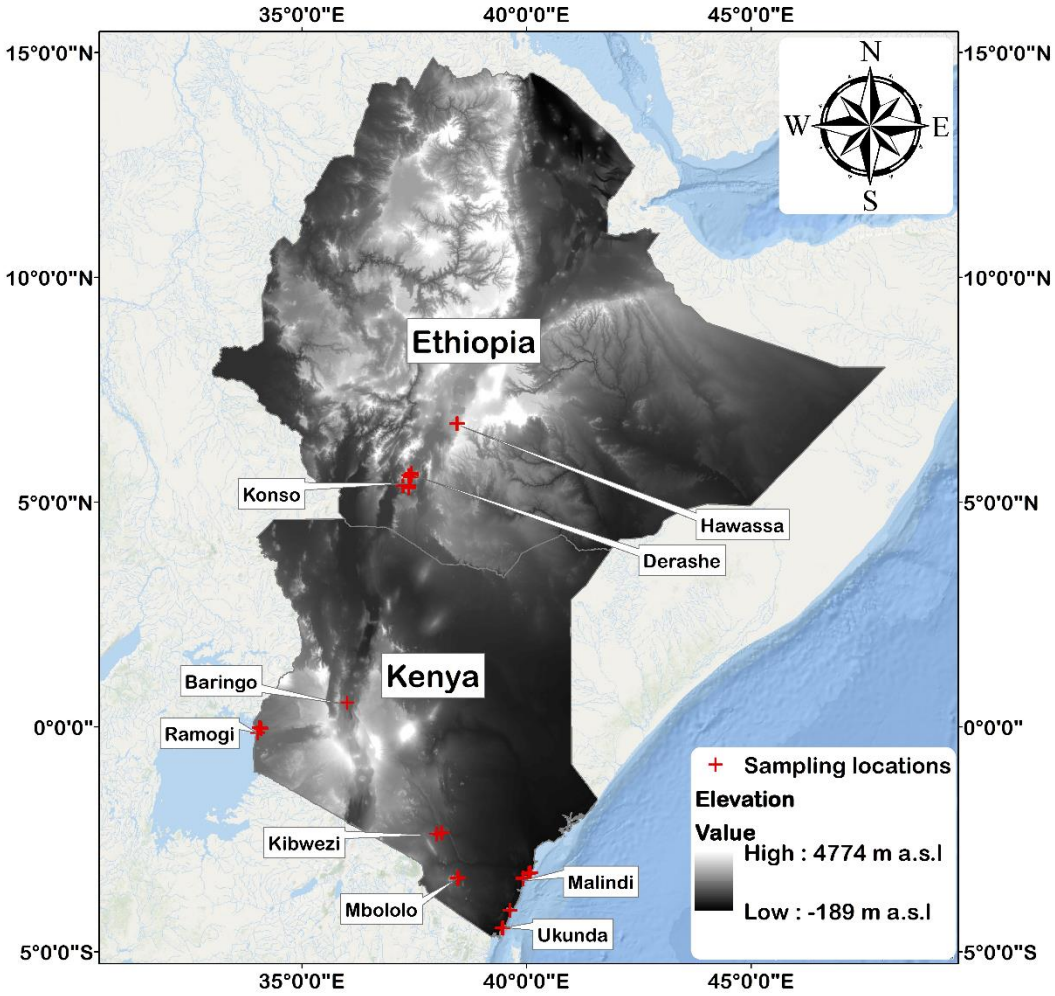


Fig 5.2. Sample collection localities in Ethiopia and Kenya, and grey scale altitudinal range for Ethiopia and Kenya. Country boundaries shape files were downloaded from the GADM Global Administrative Areas database (http://gadm.org/, Version 2, January 2012), and digital elevation data were downloaded from http://www.diva-gis.org/Data. Map was created using ESRI ArcMap™ 10.3.1 software.

Table 5.1. Species in the Moringaceae family order Brassicales, current and synonymous binomial names, and species distribution (Roskov *et al.* 2014).

Accepted binomial name	**Synonym**	**Distribution**
Moringa arborea		NE-Kenya
Moringa borziana	*Hyperanthera borziana*	S-Somalia, E-Kenya
Moringa concanensis	*Moringa concanensis*	SE-Pakistan (Baluchistan, Sind), India (widespread), W-Bangladesh
Moringa drouhardii		S-Madagascar
Moringa hildebrandtii		Madagascar (extinct in the wild, but frequently planted)
Moringa longituba	*Hyperanthera longituba*	NE-Kenya, SE-Ethiopia, Somalia
Moringa oleifera	*Anoma moringa*	Indigenous to N-India, Nepal, E-Pakistan; and Introduced in Costa Rica , Australia (Queensland), trop. Africa , Java , Malesia , Jamaica , Lesser Antilles (St. Martin , St. Barts , Antigua , Saba , St. Eustatius , St. Kitts , Montserrat , Guadeloupe , Martinique , St. Lucia , St. Vincent , Grenadines , Grenada , Barbados), Panama , Belize , Aruba , Bonaire , Curacao , Haiti , Dominican Republic , Bahamas , Cuba , Nicaragua , Mexico , Venezuela , Brazil (c), Seychelles , Somalia , New Caledonia , Fiji , Christmas Isl. (Austr.), Palau Isl.(Koror , Namoluk , Pohnpei), Society Isl.(Tahiti , Raiatea), Southern Marianas(Saipan , Rota , Guam), Niue , Mauritius , Réunion , Rodrigues , Madagascar , Yemen , Oman , Cape Verde Isl.(Santo Antao Isl. , Sal Isl. , Ilha de Maio , Ilha de Sao Tiago , Fogo Isl.), Ryukyu Isl. , Andamans , Nicobars , Myanmar [Burma] , Vietnam , Bhutan , Sikkim , Sri Lanka , Laos , Philippines , USA (Florida), U.S. Virgin Isl.
	Guilandia moringa	
	Hyperanthera arborea	
	Hyperanthera decandra	
	Hyperanthera moringa	
	Hyperanthera pterygosperma	
	Moringa domestica	
	Moringa edulis	
	Moringa erecta	
	Moringa moringa	
	Moringa nux-eben	
	Moringa octogona	
	Moringa parvifolia	
	Moringa polygona	

Accepted binomial name	**Synonym**	**Distribution**
	*Moringa pterygos*perma	
	Moringa robusta	
	Moringa sylvestris	
	Moringa zeylanica	
Moringa ovalifolia		South Africa (Transvaal), Namibia, SW-Angola
Moringa peregrina	*Gymnocladus arabica*	Egypt (Eastern Desert, SE-Egypt), Israel (E-Israel: Rift Valley, SC-Israel: Judean Desert, S-Negev Desert), Jordania (S-Jordania), Oman (Dhofar, Mascat & Oman), Saudi Arabia (C-Saudi Arabia, N-Saudi Arabia, NW-Saudi Arabia: Hejaz, SW-Saudi Arabia: Asir), Sinai peninsula (Southern Sinai), Yemen (Aden Desert, coastal Hadhramaut, NE-Yemen: Inner Hadhramaut, SW-Yemen, Tihama), United Arab Emirates, N-Sudan, N-Ethiopia, Eritrea, Somalia, India
	Hyperanthera aptera	
	Hyperanthera arborea	
	Hyperanthera monodynama	
	Hyperanthera peregrina	
	Hyperanthera semidecandra	
	Moringa aptera	
	Moringa arabica	
Moringa pygmaea		NE-Somalia
Moringa rivae subsp. *longisiliqua*		S-Ethiopia
Moringa rivae subsp. *rivae*	*Hyperanthera rivae*	S-Somalia, S-Ethiopia, Kenya
Moringa ruspoliana	*Hyperanthera ruspoliana*	Somalia, SE-Ethiopia, NE-Kenya
Moringa stenopetala	*Donaldsonia stenopetala*	
	Moringa streptocarpa	SW-Ethiopia, N-Kenya

5.4.2 Plant multi-elemental analyses

5.4.2.1 Sample collection and preparation

A total of 196 *Moringa* plant edible parts with ≥ 3 samples per site for each tissue (i.e., flowers, leaves, immature pods, seeds and roots) were collected from southern Ethiopia and Kenya (Appendix 5.1 and 5.2). The edible parts collected from MS were limited to leaves due to unavailability of other tissues during the sampling campaign. Cereal grains and vegetable crops were also collected from some of the farmers' fields that grew those crops in combination with *Moringa* trees in Kenya. Similarly, various cereal and pulse grains were acquired from households that took part in the survey from Ethiopia. Fresh *Moringa* leaves were washed in the field by using either tap or bottled water. Fresh edible plant samples collected from Ethiopia were air dried and those from Kenya were oven-dried at 40–50 °C at Kenyan Forestry Research Institute (KEFRI) headquarters in Nairobi and transferred to the University of Nottingham, UK, for further processing and chemical analyses. The dried edible parts, and grains were milled using an ultra-centrifugal mill to pass through a 1 mm screen (ZM 200, Retsch GmbH, Haan, Germany).

5.4.2.2 Nitric acid digestion of plant samples

Subsamples (c. 0.2 g) of the milled plant samples were weighed in triplicate for nitric acid (HNO_3) digestion and subsequent multi-elemental analysis. Samples were mixed with 6 mL of HNO_3 (PrimarPlus - Trace Analysis Grade (TAG), Fisher Scientific, Loughborough, UK) in microwave digestion tubes and digested at 140 °C for 20 min (Multiwave PRO, Anton Paar, St. Albans, UK). After cooling, the samples were diluted with 14 mL of Milli-Q water (MQW) (18.2 MΩ cm; Merck Millipore Milli-Q, Darmstadt, Germany) prior to multi-elemental analysis by

inductively coupled plasma-mass spectrometry (ICP-MS; iCAP-Q, Thermo-Scientific, Loughborough, UK) following a further 1-in-10 dilution with MQW.

5.4.2.3 TMAH-extractable plant iodine (I)

Iodine was extracted from 0.2 g milled plant material in 5 mL of 5% tetramethylammonium hydroxide (TMAH) solution (25% w/w aq. Soln., Electronic Grade, 99.9999% [metal basis] Alfa Aesar, Ward Hill, MA, USA), with microwave heating at 110 °C, for 20 min. The digested samples were diluted to 25 mL with MQW and centrifuged at 3000 rpm for 30 min (Heraeus Megafuge 40 Centrifuge, Thermo Scientific, Osterode am Harz, Germany) in a single use 50 mL centrifuge tubes (SUCT) (Fisherbrand, Fisher Scientific, Pittsburgh, USA). Supernatant solutions were then filtered using a 0.22 µm syringe filter (SF) (Millex PES, Merck Millipore Darmstadt, Germany) and transferred to sample tubes for ICP-MS analysis. Due to the high viscosity of the digestates from starchy seeds and grains which blocked the ICP-MS auto sampler needle, plant iodine analyses were conducted on *Moringa* leaves only.

5.4.3 Soil multi-elemental analysis

5.4.3.1 Sample collection and preparation

Thirty-three and 62 soil samples were collected from southern Ethiopia and Kenya, respectively (S2 Table). Each sample comprised soil pooled from five locations underneath the canopy of a *Moringa* tree spp. Bulked samples were air dried and sieved to pass through < 2 mm screen. A subsample of 30 g was taken to the University of Nottingham. From each sample, a 10 g subsample was Agate ball-milled (PM 400, Retsch, Haan, Germany) for multi-elemental analyses.

5.4.3.2 Multi-acid digestion of soils

Triplicate finely ground soil samples (c. 0.2 g) were digested for two days with 2.5 mL hydrofluoric acid (HF) (40% AR), 2 mL HNO_3 (70% TAG), 1 mL perchloric acid ($HClO_4$) (70% AR) and 2.5 mL MQW in PFA tubes on a Teflon-coated graphite block digester (Model A3, Analysco Ltd, Chipping Norton, UK). On the third day, the hot plate heating was turned off and 2.5 mL concentrated HNO_3 (70% TAG) and MQW were added and heated for 1 h at 50 °C. After cooling, the digestates were made up to 50 mL in plastic volumetric flasks. Multi-elemental analyses were undertaken by ICP-MS following a further 1-in-10 dilution.

5.4.3.3 Phosphate-extractable soil Se (Se-P)

Duplicate soil samples (< 2 mm; c. 2 g) were shaken in SUCT for 1 h on a rotary shaker with 20 mL of 0.016 M potassium dihydrogen phosphate (KH_2PO_4) (Stroud *et al.* 2012). The soil suspensions were centrifuged at 2200 rpm for 20 min and 10 mL of supernatant solution was filtered through a SF prior to Se-P analyses by ICP-MS.

5.4.3.4 TMAH-extractable soil iodine

Finely milled duplicate 2 g soil samples were mixed with 10 mL of 10 % TMAH in a SUCT. The soil suspensions were heated in an oven at 70 °C (Memmert GmbH + Co, D 06061, Model 500, Schwabach, Germany) for 3 h and then centrifuged at c. 3000 rpm for 20 min. The supernatant solution was diluted 1-in-10 with MQW prior to analysis for iodine by ICP-MS.

5.4.3.5 Soil pH

The < 2 mm sieved soil was mixed with deionized water at a ratio of 5 g:12.5 mL in SUCT and shaken for 30 min on a rotary shaker. The pH of the mixture was measured using combined pH meter and electrode (HI-209 pH/mV pH Meter, Hanna Instruments Ltd., Leighton Buzzard, UK). Prior to taking the pH readings, the electrode was calibrated using buffers at pH of 4.01 and 7.00. After each reading, the glass electrode was rinsed by deionized water before measuring the pH of the next sample.

5.4.4 Analytical quality control

For analytical quality control, blanks, duplicates, internal standards and certified reference materials were analysed in all instances of plant and soil analyses. The certified reference materials were tomato leaves (1573A), wheat flour (1567B), and Montana soil II (2711A) from the National Institute of Standards and Technology, Gaithersburg, MD, USA. Raw data of the plant and soil sample analytical results is presented as supplementary tables (Appendix 5.3 and 5.4).

5.4.5 Data analyses

Research data compilation and management were carried out using Microsoft© Excel© and Access© 2016 (Microsoft, Redmond, USA). Statistical analyses of the elemental concentration in edible plant parts and the soils were conducted using IBM® SPSS® Statistics version 22 (IBM Corp., New York, USA). The Shapiro-Wilk test for normality of the distribution of the data and Levene's test for homogeneity of variance were run to select between parametric and non-parametric analyses of variance (ANOVA) (S5 – S16 Tables). In addition, visual assessments of the data distributions were made. Due to the small sample size at each locality,

most of the plant and soil elemental concentration data did not meet the assumptions of parametric ANOVA; logarithmic transformation did not improve the non-normal distribution and heteroscedasticity of the elemental concentration data. Hence, Welch's robust test for equality of means was applied to test the variation in elemental concentration by locality. Spearman's rank correlation analysis was conducted using GenStat® version 17 (VSN International, Hemel Hempstead, UK) to assess the association between soil physico-chemical properties and *Moringa* leaves elemental concentration, and relationships between elemental concentrations in edible parts of various vegetables. Box plots of plant and soil elemental concentration and pH were drawn using Tableau® Desktop Professional Edition version 10.0.0 (Tableau Software Inc., Seattle, Washington, USA). Outliers were not included in the box plots. Plant edible parts with sample size < 3 per locality were excluded from statistical analyses. For instance, there was only one MO and MS sample at Baringo and Ramogi, respectively. These were not included in the data analyses.

5.5 Results

Plant edible parts and soil elemental concentration analytical results for calcium (Ca), copper (Cu), iodine (I), iron (Fe), magnesium (Mg), selenium (Se), and zinc (Zn); and soil pH are reported. The association between plant edible parts elemental concentration and soil properties, and variation in elemental concentration by location are also reported. Furthermore, comparisons are made among *Moringa* spp. edible parts, maize and sorghum grains, beans, amaranth leaves, baobab fruit, brassica leaves, and enset (*Ensete ventricosum* a.k.a., *false banana*), mineral element concentrations.

5.5.1 *Moringa* elemental concentration

The concentrations of mineral elements in *Moringa* leaves, immature pods, seeds and flowers and variations by localities are presented below, and summarised in Fig 5.3 - 5.7, Appendix 5.17 - 5.21 and Table 5.2.

5.5.2 Moringa oleifera leaf elemental concentration

The overall mean concentrations of Ca, Cu, I, Fe, Mg, Se and Zn in MO leaves were 18300, 6.92, 0.218, 202, 5390, 4.25 and 35.6 mg kg^{-1} dw, respectively (S17 Table). Mineral element concentration of the MO leaves varied significantly ($p < 0.05$) between localities, except for Ca (Fig 5.3 and Appendix 5.27). There was no systematic variation in the relative concentration at a given location for these elements, although Kibwezi had the highest values of the trace elements (Cu, Se, Zn).

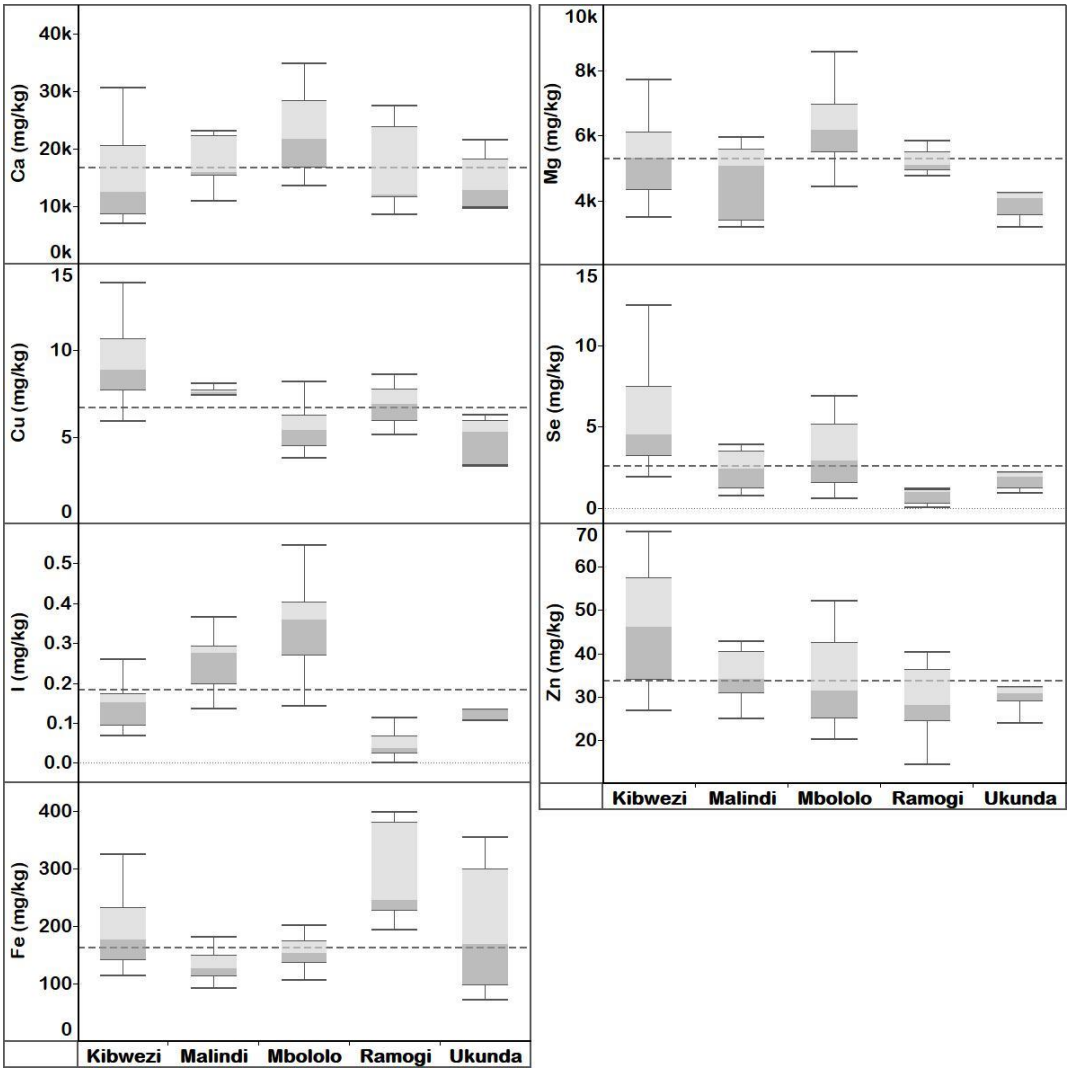


Fig 5.3. Quartiles of elemental concentration (mg kg^{-1} dw) in *M. oleifera* leaves collected from Kenya (Kibwezi, Malindi, Mbololo, Ramogi and Ukunda) localities. Median elemental concentration for each locality is where the light and dark grey shading boxes meet. The horizontal broken lines depict the overall median concentration for each element across localities.

5.5.3 *Moringa stenopetala* leaf elemental concentration

The overall mean concentrations of Ca, Cu, I, Fe, Mg, Se and Zn in MS leaves were 21100, 4.53, 0.07, 162, 6440, 1.66 and 22.2 mg kg^{-1} dw, respectively (Appendix 5.18). Mean Cu, I, Mg and Zn differed significantly between localities ($p < 0.05$), while Ca, Fe, and Se concentrations of MS leaves did not differ significantly between localities (Appendix 5.28). *Moringa stenopetala* leaves collected from Kenya (n = 5) had higher median concentrations of mineral elements than those

from Ethiopia (n = 36) except Cu and Zn (Fig 5.4 and Table 5.2). MS leaves from Hawassa, southern Ethiopia had significantly ($p < 0.05$) higher concentration of Zn and lower Mg than those from Baringo Island, Kenya. On the contrary, MS leaves collected from Baringo island contained significantly ($p < 0.05$) higher concentrations of Se and I than all samples from localities in Ethiopia. The concentration of Cu in MS leaves collected from Baringo island were significantly ($p < 0.05$) lower than samples from Derashe, southern Ethiopia.

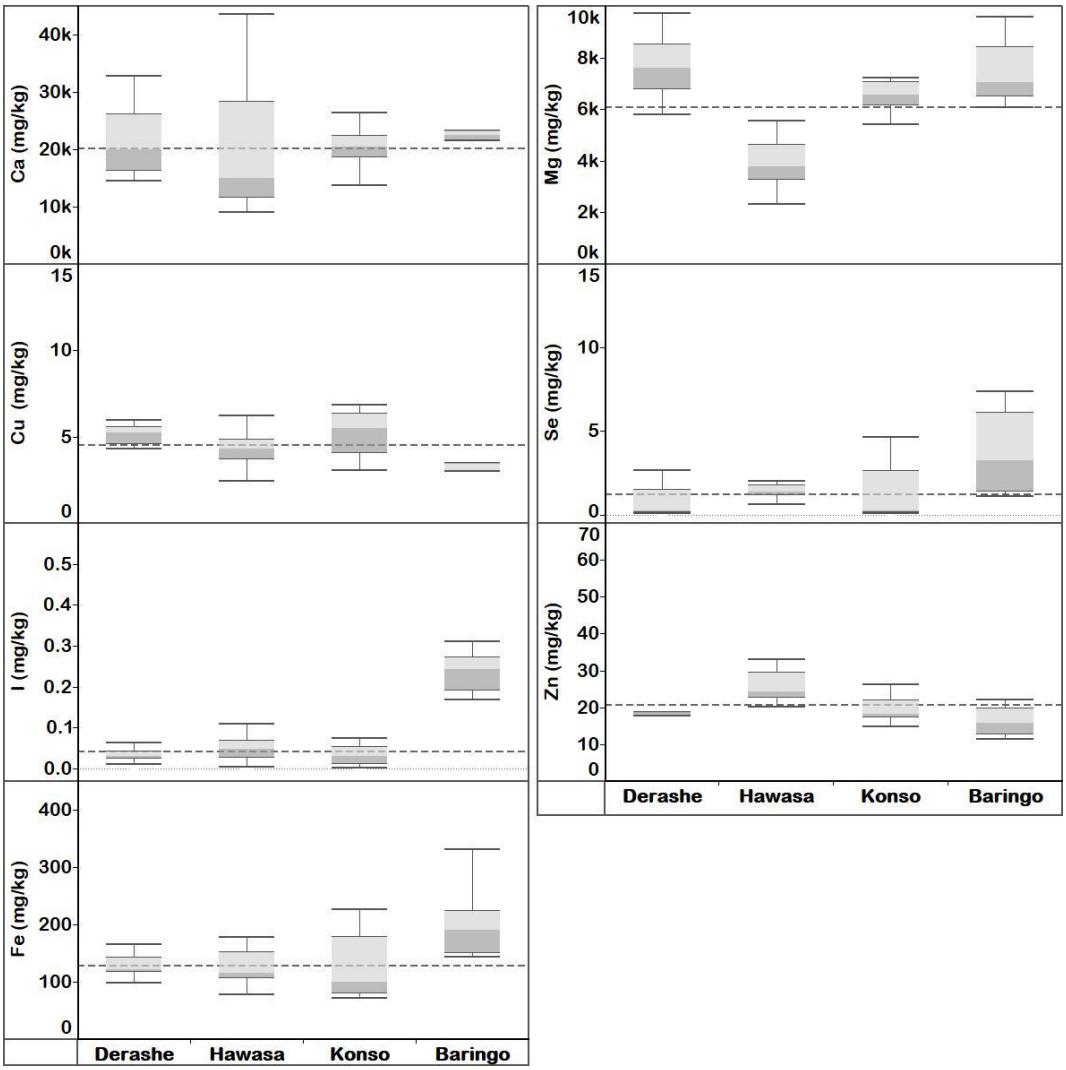


Fig 5.4. Quartiles of elemental concentration (mg kg^{-1} dw) in *M. stenopetala* leaves collected from Ethiopia localities (Derashe, Hawassa and Konso), and Kenya locality (Baringo). Median elemental concentration for each locality is where the light and dark grey shading boxes coincide. The horizontal broken lines depict the overall median concentration for each element across localities.

5.5.4 *Moringa oleifera* immature pods elemental concentration

The overall mean concentrations of Ca, Cu, Fe, Mg, Se and Zn in MO immature pods were 3600, 5.42, 65.4, 2860, 2.36 and 27.6 mg kg^{-1} dw, respectively (Appendix 5.19). The distribution of MO immature pods elemental concentration in comparison with the overall median value varied between the elements and locations (Fig 5.5). For example, the median elemental concentration in the immature pods collected from Kibwezi were generally higher than the overall median concentration in Kenya. The median Ca and Mg concentration in immature pods collected from Ramogi and Ukunda were below the overall median concentration in Kenya. There were significant differences ($p < 0.05$) in the Cu, Fe and Mg but not ($p \geq 0.05$) Ca, Se and Zn mean concentrations of MO immature pods collected from different localities (Appendix 5.30).

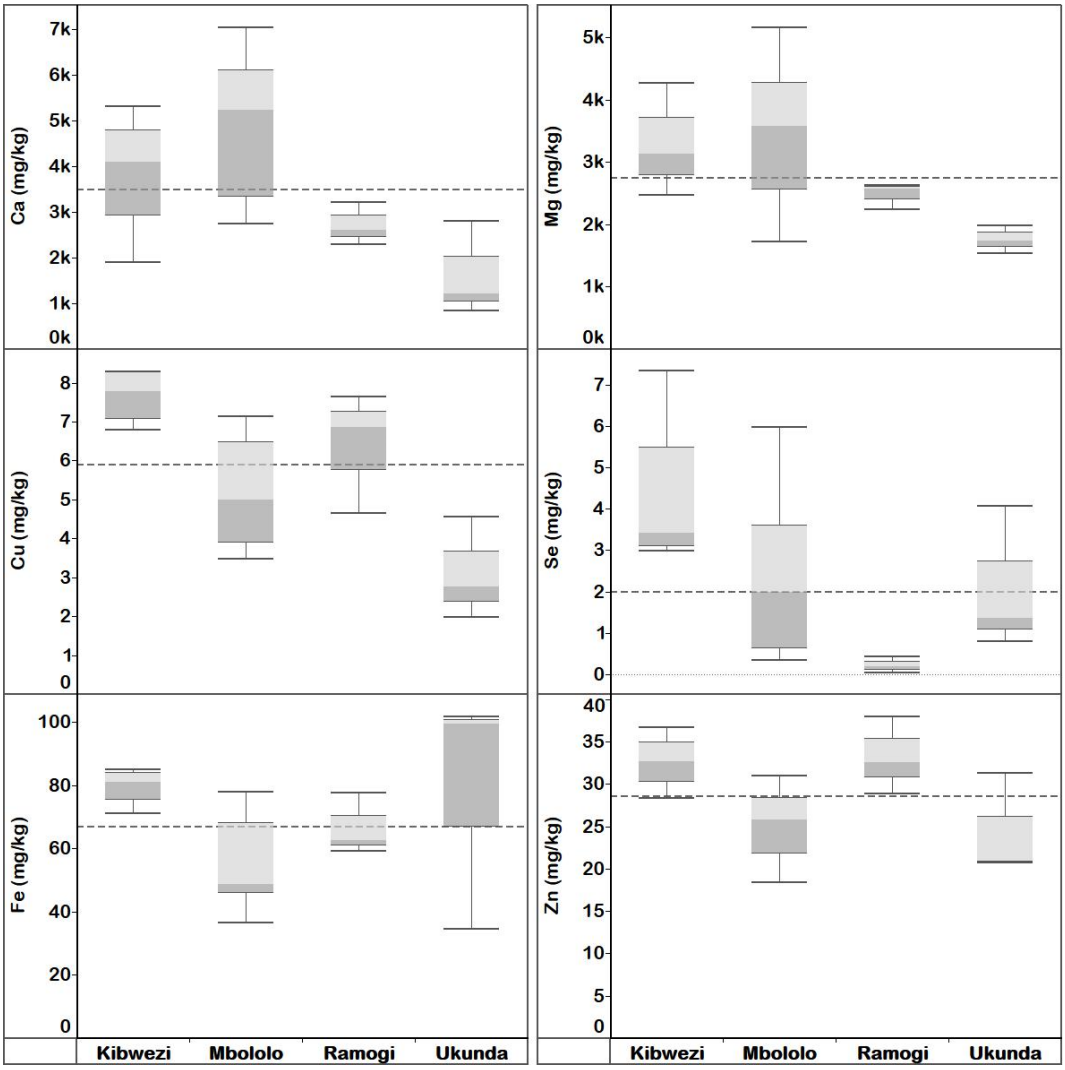


Fig 5.5. Quartiles of elemental concentration (mg kg^{-1} dw) in *M. oleifera* immature pods collected from Kenya localities. Median elemental concentration for each locality is where the light and dark grey shading boxes coincide. The horizontal broken lines depict the overall median concentration for each element across localities.

5.5.5 *Moringa oleifera* seeds elemental concentration

The overall mean concentrations of Ca, Cu, Fe, Mg, Se and Zn in MO seeds were 1310, 4.18, 49.2, 3080, 3.59 and 44.8 mg kg^{-1} dw, respectively (Appendix 5.20). Overall median elemental concentration in MO seeds varied between the elements and locations (Fig 5.6). There was no significant difference ($p \geq 0.05$) in the mean elemental concentration of MO seeds collected from different localities (Appendix 5.31).

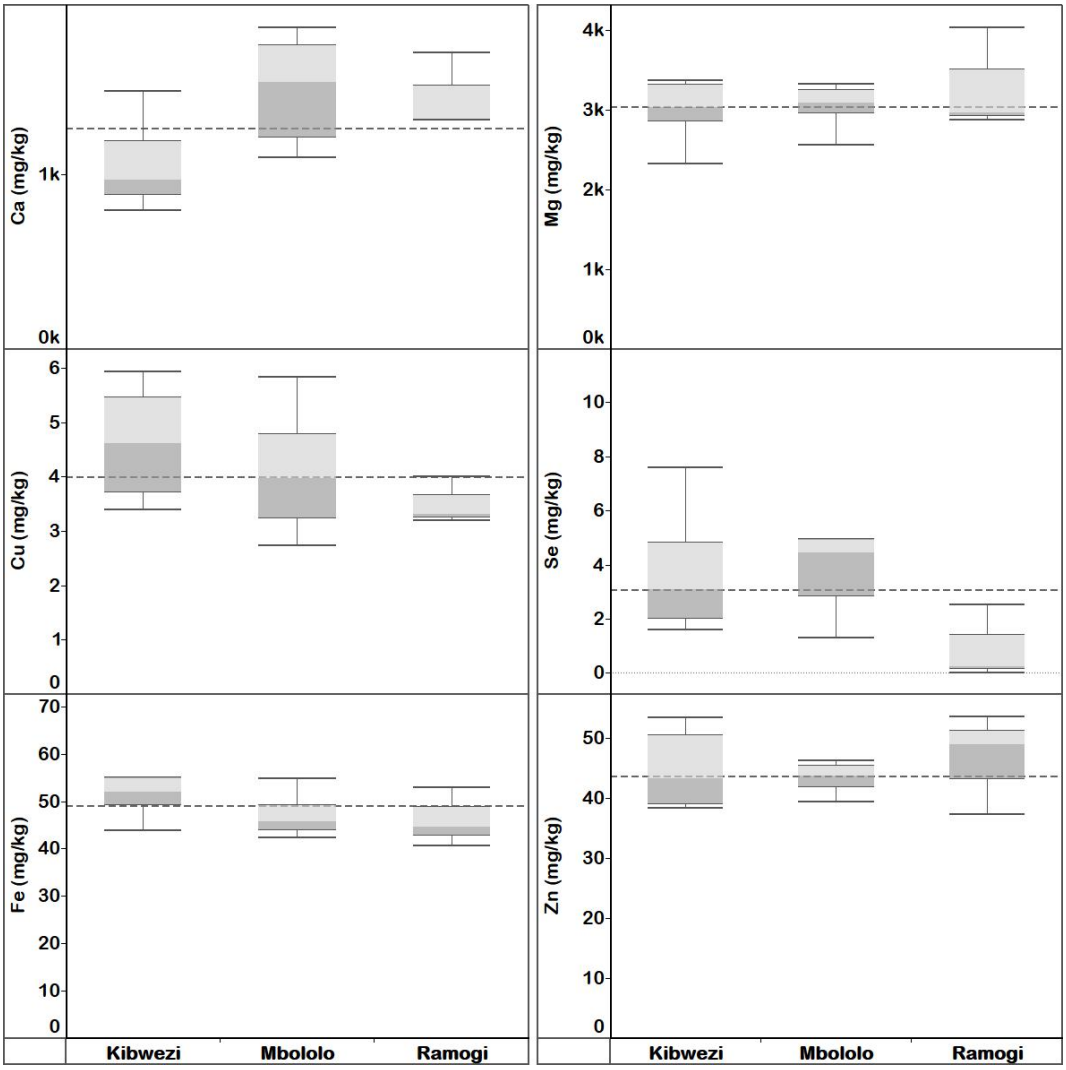


Fig 5.6. Quartiles of elemental concentrations in *M. oleifera* seeds (mg kg^{-1} dw) collected from Kenya localities. Median elemental concentration for each locality is where the light and dark grey shading boxes coincide. The horizontal broken lines depict the overall median concentration for each element across localities.

5.5.6 *Moringa oleifera* flowers elemental concentration

The overall mean concentrations of Ca, Cu, Fe, Mg, Se and Zn in MO flowers were 3650, 6.40, 253, 2830, 2.81 and 32.7 mg kg^{-1} dw, respectively (Appendix 5.21). The distribution of MO flowers elemental concentration in comparison with the overall median value varied between elements and locations (Fig 5.7). There were significant differences ($p < 0.05$) in the mean Ca, Cu, Fe, Mg and Se, but not Zn concentrations of MO flowers collected from different localities (Appendix 5.32).

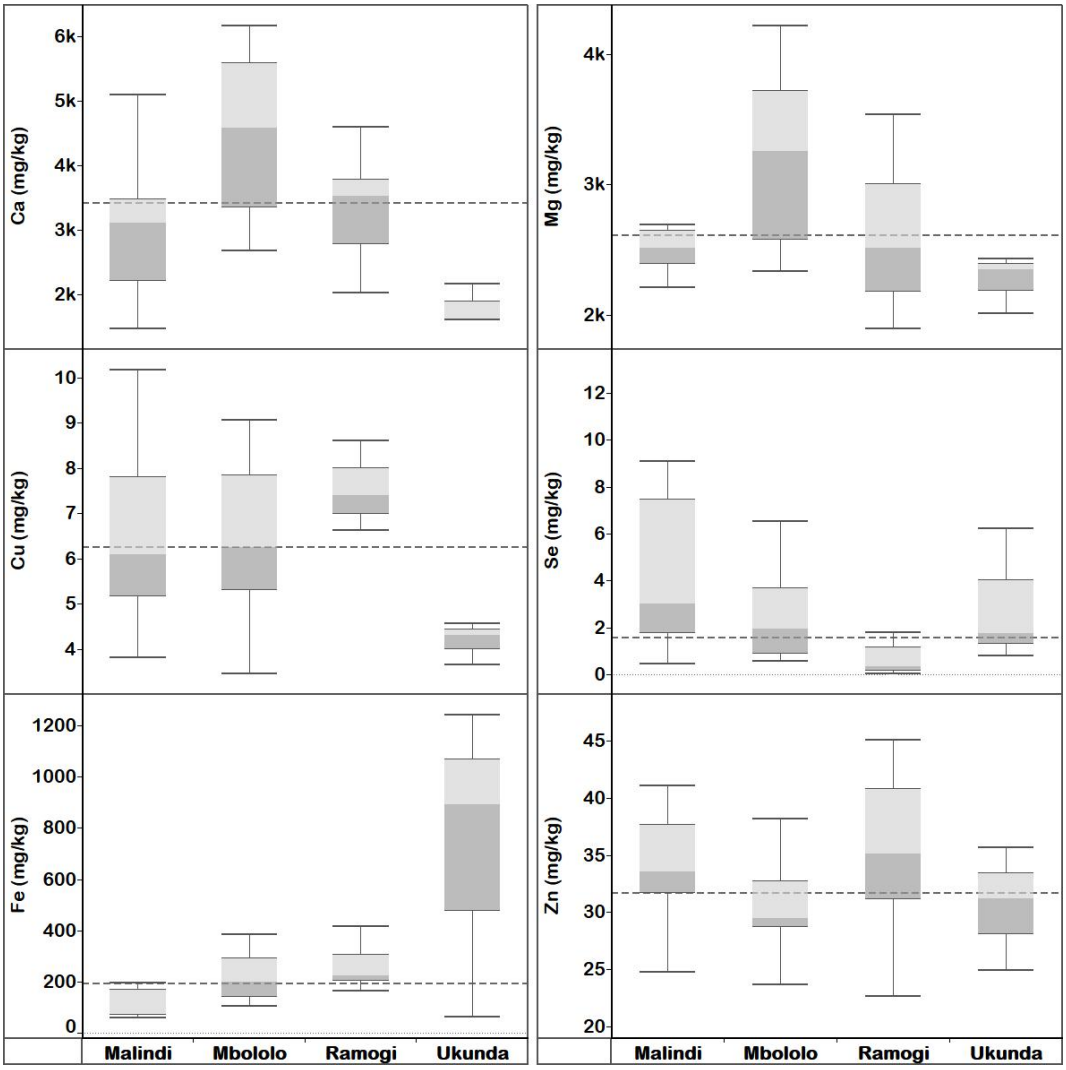


Fig 5.7. Quartiles of elemental concentrations (mg kg^{-1} dw) in *M. oleifera* flowers collected from Kenya localities. Median elemental concentration for each locality is where the light and dark grey shading boxes coincide. The horizontal broken lines depict the overall median concentration for each element across localities.

5.5.7 Comparison of elemental concentration between crops

For comparison, elemental concentration of *Moringa* spp. edible parts and other vegetables, fruits, and staple cereal crops are presented in Table 5.2. On a weight-for-weight basis, in Ethiopia, MS leaves contained the highest median concentrations of all elements except Cu and Zn. Median concentrations of Cu and Zn were highest in enset and beans, respectively (Table 5.2).

In Kenya, on weight-for-weight basis, *Moringa* edible parts had the highest median Se concentration ranging from 1.56 mg kg^{-1} in MO flowers to 3.96 mg kg^{-1} in MS leaves. The median concentration of Se in MS leaves was 7-fold, 10-fold, 23-fold, 117-fold and 147-fold more than that in brassica leaves, amaranth leaves, baobab fruits, sorghum grain and maize grain, respectively. The median Se concentration in MO seeds was 78-fold and 98-fold greater than sorghum and maize grain, respectively. Seeds of MO had the highest median Zn concentration while amaranth leaves contained comparable quantities of Zn with MO flowers and leaves. The median Zn concentration in MO seeds was 2-fold greater than in maize and sorghum grain (Table 5.2).

Table 5.2. Median elemental concentrations in cereals, vegetables, fruits and seeds grown in various parts of Ethiopia and Kenya, and the number of samples (n).

Crop	n	Median concentration (mg kg^{-1} dw)						
		Ca	Cu	I	Fe	Mg	Se	Zn
Ethiopia								
MS leaves	36	19400	4.71	0.093	117	6070	1.12	21.0
Maize grain	17	55.1	0.943		28.2	918	0.182	20.4
Enset	5	2,190	1.30		71.3	260	0.060	34.2
Sorghum grain	8	176	1.74		51.5	1350	0.097	16.1
Beans	4	1500	9.41		88.5	1760	0.150	24.9
Kenya								
Amaranth leaves	6	26700	6.88		339	12900	0.399	33.9
Baobab fruits	4	2660	7.63		9.40	1180	0.169	11.9
Brassica leaves	4	32000	9.25		104	7880	0.597	17.4
Maize grain	9	51.2	2.80		16.5	912	0.027	21.5
MO flowers	33	3420	6.25		4.73	2610	1.56	31.7
MO immature pods	25	3060	5.05		62.6	2610	1.99	27.8
MO leaves	56	16700	6.83	0.201	160	5400	2.73	33.3
MO seeds	32	1250	4.02		49.8	3100	2.64	46.0
MS leaves	5	22500	3.07	0.231	190	7210	3.96	15.7
Sorghum grain	6	103	6.24		33.7	1220	0.034	22.6

5.5.8 Soil pH and elemental concentrations

Ninety percent of the soil samples from Ethiopia and 97 % of those from Kenya had pH >7. The soil pH at the three localities in Ethiopia ranged from 6.12 in Hawassa to 8.67 in Derashe, with overall mean and median of 7.84 and 7.98, respectively. In Kenya, soil pH ranged from 6.63 in Mbololo to 8.65 in Malindi with overall mean and median of 7.88 and 7.85, respectively (Fig 5.8, Table 5.3 and Appendix 5.22). Welch's robust test of equality of means showed that the soil pH varied significantly between localities ($p < 0.05$) (Appendix 5.33). Similarly, Welch's robust tests of equality of mean soil elemental concentrations showed that there was significant difference ($p < 0.05$) between soils collected from various localities (Appendix 5.33). Descriptive statistics of the soil physico-chemical properties across all

localities in Ethiopia and Kenya are summarized in Table 5.3. Soil samples from Baringo, Kibwezi and Ramogi localities were the three with highest median phosphate-extractable Se concentration. Total Se concentration was highest in soils from Baringo, Hawassa and Ramogi. With respect to total soil iodine, Ramogi, Kibwezi and Mbololo soil samples had the highest concentrations. Total Zn concentration in soil samples from Hawassa were 2-fold, 4-fold and 3-fold more than the median Zn concentration from soils in Ethiopia, Kenya and overall median Zn concentrations.

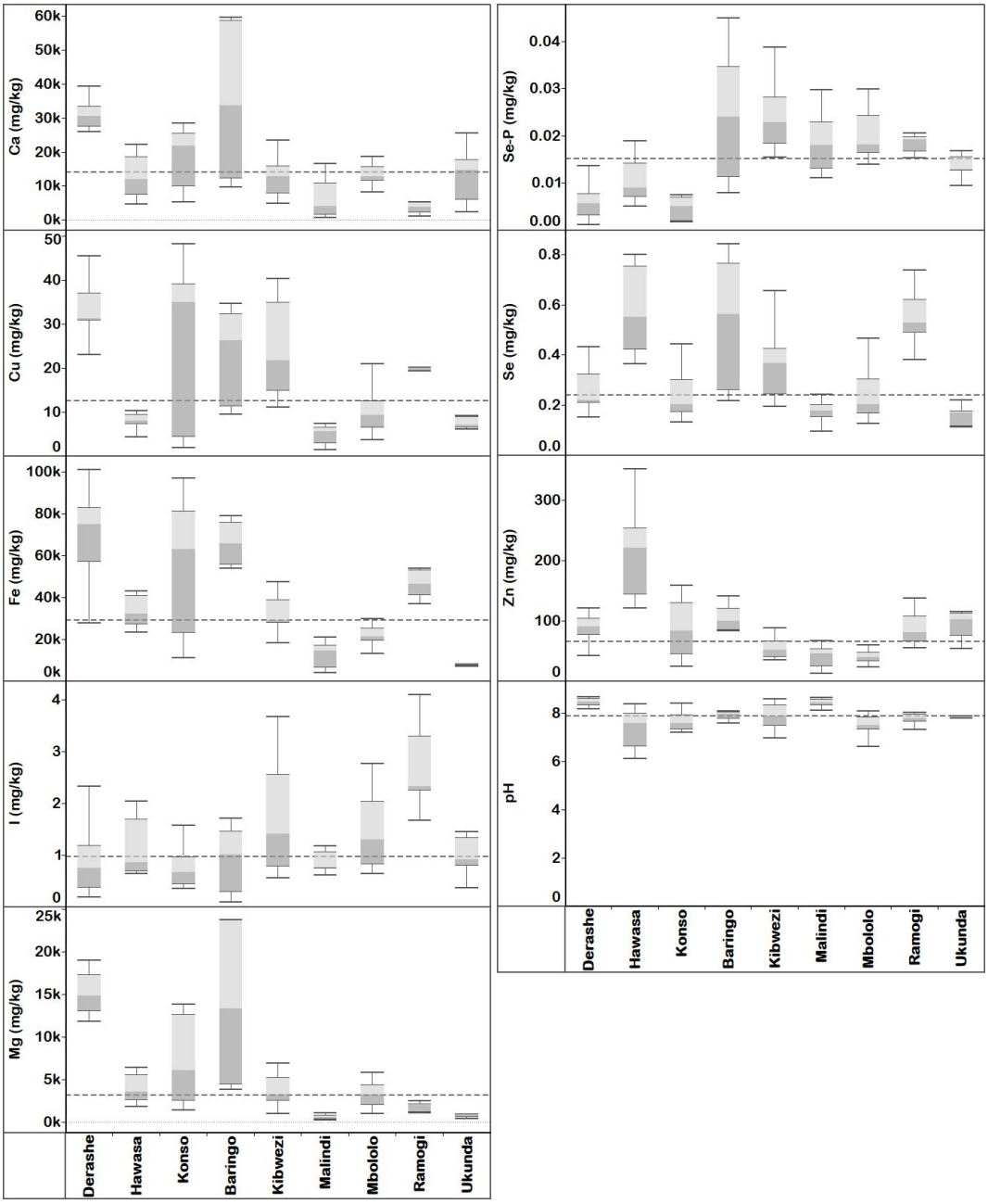


Fig 5.8. Quartiles of soil elemental concentration (mg kg^{-1} dw) and pH collected from southern Ethiopia (Derashe, Hawassa and Konso), and Kenya (Baringo, Kibwezi, Malindi, Mbololo, Ramogi and Ukunda) localities. Median elemental concentration for each locality is where the light and dark grey shading boxes coincide. The horizontal broken lines depict the overall median of each property across localities. Total soil elemental concentrations except for Se-P, which is KH_2PO_4-extractable fraction.

Table 5.3. Descriptive statistics of soil pH, and elemental concentrations by locality.

Locality	Statistic	Concentration (mg kg^{-1} dw)								
		Ca	Cu	I	Fe	Mg	Se	Se-P	Zn	pH
Baringo	n	5	5	5	5	5	5	5	5	5
	Mean	38400	23.7	0.923	66200	15000	0.550	0.027	97.7	7.88
	Median	52000	31.1	1.06	67000	19500	0.664	0.035	92.9	7.96
Derashe	n	12	12	12	12	12	12	12	12	12
	Mean	28400	33.4	0.851	69200	13700	0.255	0.006	88.0	8.40
	Median	30600	31.2	0.748	74900	14800	0.222	0.006	90.7	8.48
Hawassa	n	9	9	9	9	9	9	9	9	9
	Mean	15800	13.0	1.13	35400	4800	0.599	0.011	260	7.43
	Median	14100	8.62	0.941	32900	3800	0.622	0.010	229	7.66
Kibwezi	n	14	14	14	14	14	14	14	14	14
	Mean	14200	24.0	1.72	33000	4020	0.362	0.024	54.0	7.88
	Median	12800	21.7	1.41	29300	3260	0.366	0.023	51.5	7.89
Konso	n	12	12	12	12	12	12	12	12	12
	Mean	18700	26.2	0.771	56000	7120	0.239	0.004	85.725	7.595
	Median	21800	35.0	0.676	63100	6100	0.204	0.005	83.1	7.59
Malindi	n	11	11	11	11	11	11	11	11	11
	Mean	7290	4.72	0.955	12200	566	0.179	0.019	39.9	8.40
	Median	4000	5.63	0.768	14500	591	0.177	0.018	45.4	8.44
Mbololo	n	16	16	16	16	16	16	16	16	16
	Mean	13400	10.3	1.43	22300	3210	0.241	0.020	39.8	7.53
	Median	12900	9.38	1.30	21300	3260	0.203	0.018	39.7	7.50
Ramogi	n	8	8	8	8	8	8	8	8	8
	Mean	3550	35.5	3.24	68900	2210	0.550	0.018	87.8	7.81
	Median	3670	21.3	2.81	53600	2020	0.529	0.019	81.6	7.81
Ukunda	n	7	7	7	7	7	7	7	7	7
	Mean	12900	7.55	1.00	7690	715.3	0.155	0.014	92.3	7.94
	Median	14600	7.07	0.923	8040	839	0.169	0.013	102	7.83
Ethiopia	n	33	33	33	33	33	33	33	33	33
	Mean	21400	25.2	0.898	55200	8870	0.343	0.007	134	7.84
	Median	22300	30.7	0.773	51200	6700	0.281	0.006	101	7.98
Kenya	n	61	61	61	61	61	61	61	61	61
	Mean	13200	16.5	1.56	31000	3470	0.313	0.020	60.2	7.88
	Median	11800	11.3	1.20	23500	2300	0.239	0.018	53.5	7.85
Total	n	94	94	94	94	94	94	94	94	94
	Mean	16100	19.6	1.33	39500	5360	0.324	0.016	86.1	7.87
	Median	13700	13.0	0.972	29700	3130	0.240	0.015	66.0	7.88

5.5.9 Relationships between MO edible parts and other vegetables

The association between elemental concentrations of the edible parts of MO are presented in Appendix 5.34. Calcium concentration in MO flowers showed highly significant ($p < 0.01$) positive correlation with the Ca, Cu, Mg, and Se concentration in MO immature pods. Contrary to this, the Cu concentration in MO flowers had a significant ($p < 0.01$) negative correlation with the Ca in the seeds and leaves. The Se in the MO flower was highly significantly ($p < 0.01$) correlated with the Se in leaves and immature pods. The association between the elemental concentrations in MO edible parts and other vegetables cultivated on the same location are presented in Appendix 5.35 and 5.36. The Fe concentration in MO leaves showed significant ($p < 0.05$) positive correlation with the Fe, and negative correlation with the Se concentration in amaranth leaves (Appendix 5.35). Calcium concentration in MO leaves showed significant ($p < 0.01$) negative correlation with the Ca, Cu, and Mg concentration in brassica leaves (Appendix 5.36). Similarly, Ca concentration in amaranth leaves showed a significant ($p < 0.01$) negative correlation with the Ca, Cu, and Mg concentrations in brassica leaves (Appendix 5.37).

5.5.10 Relationships between *Moringa* leaves elemental concentration and soil properties

The concentration of Cu in MO leaves was significantly ($p < 0.05$) positively associated with total soil Cu and Fe concentrations. Similarly, MO leaf Fe concentration also showed a statistically significant ($p < 0.05$) positive correlation with soil Fe, Se and Zn. Selenium concentration in MO leaves showed a stronger significant ($p < 0.05$) positive correlation with phosphate extractable (Se-P) soil Se than the total soil Se (Appendix 5.23 and 5.24). The Ca and Zn concentration in

MO leaves showed no statistically significant ($p \geq 0.05$) correlation with any of the reported soil properties.

Moringa stenopetala leaf Fe concentration was significantly ($p < 0.05$) positively correlated with soil Mg, Se and Se-P. Similarly, MS leaves Mg concentration showed a statistically significant ($p < 0.05$) negative correlation with soil Zn concentration. The Se content of MS leaves indicated a strong and significant positive correlation with the soil Se-P (Appendix 5.25 and 5.26). However, the Ca, Cu and I concentration of MS leaves did not show significant ($p \geq 0.05$) correlation with any of the soil properties.

5.6 Discussion

5.6.1 Elemental concentration in *Moringa spp.* edible parts

This study is the first comprehensive analysis of Se concentrations in different edible parts of MO and MS grown in various localities. Four previous studies reported Se concentrations in MO leaves, from Niger (27.1 mg kg^{-1} dw) (Freiberger *et al.* 1998), Solomon Islands (2 mg kg^{-1} dw) (Lyons *et al.* 2015), South Africa (363 mg kg^{-1} dw, unusually high) (Moyo *et al.* 2011), and Mexico, Lombardia (0.096 mg kg^{-1} dw) and San Pedro (1.07 mg kg^{-1} dw) (Valdez-Solana *et al.* 2015). Our MO leaf Se concentration (mean = 4.25 and median = 2.73 mg kg^{-1} dw) is consistent with results from Solomon Islands and Mexico, but differ markedly from the South African data. The results of the analyses from Niger were based on two samples. Taking this into account, and the fact that a MO leaf sample (L-MO-29-MBO) from Mbololo, Kenya, had a mean Se concentration of 21.2 mg kg^{-1} dw from triplicate analyses, the findings from Niger were reasonably consistent with ours. Our attempt to verify the very high reported concentrations from the South Africa study by

contacting the authors was not successful. Iodine concentrations in MO and MS leaves or other parts have not been reported previously to our knowledge.

Table 5.4summarises 11 previous studies of MO leaf elemental concentrations alongside data from the present study. Allowing for differences in analytical method and likely inter-study variation in leaf maturity there are many broad similarities. For example, the mean Ca concentration in MO leaves in the present study is the fourth lowest following MO leaf samples collected from Kuje, Nigeria (Anjorin *et al.* 2010), Hawassa, Ethiopia (Debela *et al.* 2013) and Jalisco state, Mexico (Olson *et al.* 2016), while the mean Zn concentration is the second highest following MO leaves collected from Thailand (Limmatvapirat *et al.* 2015). Similarly, mean elemental concentrations in MS leaves collected from Ethiopia and Kenya in the current study indicated inconsistent variation when compared with previous studies. For instance, the mean concentration of Ca in MS leaves of 21 g kg^{-1} dw in the present study is comparable with that reported from Ethiopia (Melesse *et al.* 2012) (19.8 g kg^{-1} dw), and greater than the 12.7 g kg^{-1} dw reported from Mexico (Olson *et al.* 2016). However, the Fe concentration (162 mg kg^{-1} dw) in the present study is far lower than that reported from Ethiopia (666 mg kg^{-1} dw) (Melesse *et al.* 2012).

Table 5.4. Comparison of reported mineral element concentrations in MO leaf, sources, number of observation (n) and locations. The concentration values in the first row in bold are the result from current study.

Ca	Cu	I	Fe	Mg	Se	Zn	Source	n	Location
mean (mg kg^{-1} dw)									
18300	6.92	0.218	202	5370	4.25	35.6	Current study	56	Various localities, Kenya
16000	9.6	__	97.9	2800	__	29.1	(Olson *et al.* 2016)	23	Mexico, Jalisco State
25800	9.44	__	591	5520	__	24.7	(Melesse *et al.* 2012) †	6	Hawassa, Ethiopia
26200	9.58	__	561	5550	__	25.2	(Melesse *et al.* 2012)†	6	Arbaminch, Ethiopia
36500	8.25	__	490	5000	363	31	(Moyo *et al.* 2011)		Limpopo, South Africa
20000	7	__	___	3700	2.0	31	(Lyons *et al.* 2015)		Honaiara, Solomon Islands
3463	44	__	41	725	__	__	(Anjorin *et al.* 2010)	2	Kuje, Abuja, Nigeria
38270	43.6	__	78.8	806	__	__	(Anjorin *et al.* 2010)	2	Sheda, Abuja, Nigeria
22900	9.5	__	205	100	__	25.9	(Aslam *et al.* 2005)	2	Bahawalnager, Pakistan
19000	11.2	__	397	98.2	__	20.9	(Aslam *et al.* 2005)	2	Sadiqabad, Pakistan
26400	7.3	__	573	109	__	34.1	(Aslam *et al.* 2005)	2	Chenabnager, Pakistan
24000	8.85	__	226	4340	27.1	< 5	(Freiberger *et al.* 1998)	2	Zinger, Niger
20200	10.3	__	194	3230	0.096	10	(Valdez-Solana *et al.* 2015)	5	Lombardia, Mexico
26200	4.1	__	70.7	3400	1.07	16	(Valdez-Solana *et al.* 2015)	5	San Pedro, Mexico
12900	17.7	__	391	1800	__	28.2	(Debela *et al.* 2013)		Hawassa, Ethiopia
__	7.6	__	82.2	__	__	92.8	(Limmatvapirat *et al.* 2015)†	5	Thailand
18400		__	173	5630	__	24.8	(Leone *et al.* 2015)		Chad
27400	12.2	__	417	4900	__	30.9	(Leone *et al.* 2015)		Sahrawi camps, Algeria
21500	6.6	__	119	5340	__	21.8	(Leone *et al.* 2015)		Haiti

†Values were averaged

Mean elemental concentrations in MS leaves collected from Ethiopia and Kenya in the current study indicated inconsistent variation when compared with previous studies. For instance, the mean concentration of Ca in MS leaves of 21 g kg^{-1} dw in the present study is comparable with that reported from Ethiopia (Melesse *et al.* 2012) (19.8 g kg^{-1} dw), and greater than the 12.7 g kg^{-1} dw reported from Mexico (Olson *et al.* 2016). However, the Fe concentration (162 mg kg^{-1} dw) in the present

study is far lower than that reported from Ethiopia (666 mg kg^{-1} dw) (Melesse *et al.* 2012).

The mean concentration of Ca (3,600 mg kg^{-1} dw) in the immature pods of MO in the current study was higher than that reported from Ethiopia (2,740 mg kg^{-1} dw) (Melesse *et al.* 2012) and Pakistan (2,740 mg kg^{-1} dw) (Aslam *et al.* 2005). However, the Fe concentration (65.4 mg kg^{-1} dw) in the MO immature pods in this study was much lower than that reported from Ethiopia (510 mg kg^{-1} dw) (Melesse *et al.* 2012) and Pakistan (510 mg kg^{-1} dw) (Aslam *et al.* 2005). The Cu, Mg and Zn concentrations in the immature pods of MO reported from Ethiopia (Melesse *et al.* 2012) are comparable to our findings. However, the concentration of Cu in the immature pods of MO reported from Pakistan (26.6 mg kg^{-1} dw) (Aslam *et al.* 2005) was higher than the results in this study (5.42 mg kg^{-1} dw). Elemental concentration in MO seed kernel in the present study showed inconsistent variation as compared to previous studies in two regions of Nigeria (Anjorin *et al.* 2010). For example, the mean concentration of Cu (4.2 mg kg^{-1} dw) and Fe (49.2 mg kg^{-1} dw) in MO seed kernel in the present study is lower than the MO seed kernel samples collected from Sheda region of Nigeria with Cu and Fe concentration of 34.2 mg kg^{-1} dw and 118.5 mg kg^{-1} dw, respectively. Calcium concentration of 1310 mg kg^{-1} dw in the present study is higher than the concentration of Ca (1029 mg kg^{-1}) in MO seed kernel collected from Kuje region of Nigeria (Anjorin *et al.* 2010). The variation in elemental concentration in immature pods and seeds of MO this study and others can be attributed to the variation in environment in which MO grew, intra-specific variation in the MO, the variation in maturity levels of immature pods, and the difference in analytical method pursued. There are no studies we are aware of that report the elemental concentrations in the flowers of *Moringa* species.

5.6.2 Variation in *Moringa* spp. element concentration

Variation in the elemental concentrations of the different edible parts of MO and MS can be due to the impact of the environment/management, the effect of intra- and inter-specific genetic variation (Olson *et al.* 2016), and the interactions between the genetics and environment (Melesse *et al.* 2012). The seeds and/or planting materials sources of the *Moringa* trees from which the edible parts were sampled were not traceable and so this discussion is limited to environment/management factors. *S*amples were collected from trees that were grown and managed by households in various localities of Ethiopia and Kenya. Different households pursue various tree management regimes, such as, lone trees, hedgerow, woodlot, pollarding, lopping, watering, fertilizing, intercropping, etc. For example, some households place household wastes and manure that can supply nutrients to the trees and some may water their plants during dry season. Stages of growth, for instance, of the leaves at the time of surveying varies due to variation in management regime, and climate and soil type may all contribute further to variation of the elemental concentration in the edible parts.

The positive correlation between some of the *Moringa* edible parts elemental concentration and soil chemical properties indicate the significance of the soil environment in which the plants grow besides the inherent genetic ability of these species to absorb and translocate mineral elements to edible parts. In addition, it is not only the quantity of the mineral element available in the soil which impacts on the *Moringa* spp. edible parts elemental concentration but also the chemical form in which the element exists in the soil (Guo *et al.* 2014). The stronger positive correlation of *Moringa* leaves Se concentration with KH_2PO_4 extractable soil Se

than the total soil Se was an indication of the association between *Moringa* edible parts and phyto-available soil elemental concentration.

5.6.3 *Moringa* spp. role in human Se nutrition

Selenium deficiencies are widespread in sub-Saharan Africa (Joy *et al.* 2014, Joy *et al.* 2015b). For instance, based on 2009 food supply data from the Food and Agriculture Organization, national level Se deficiency risks in Ethiopia and Kenya were estimated to be 35.5 % and 58.3 %, respectively (Joy *et al.* 2014). Based on seven day dietary recall survey conducted in the year 2010–2011, Joy, Kumssa *et al.,* (Joy *et al.* 2015b) estimated that 81% of Malawian households had insufficient Se to meet dietary requirements. Similarly, in northwest Ethiopia, Gonder town, a cross-sectional study on school children (n=100) using blood serum concentration of mineral nutrients reported 62 % of the children were deficient in Se (Amare *et al.* 2012). Gashu *et al.* (Gashu *et al.* 2016a) reported Se deficiency risk in school children in the Amhara region of Ethiopia to be 58% (n=349).

Moringa spp. edible parts contain high concentrations of Se and the leaves have similar levels of the six other reported mineral elements to other leafy vegetables grown in the same localities. Table 5.5 summarizes the Recommended Daily Allowances (RDA) for an adult male of Ca, Cu, I, Fe, Mg, Se and Zn, concentrations of these mineral elements in MO and MS leaves collected from various localities of Ethiopia and Kenya, and percentage of RDA fulfilled by consuming 100 g of fresh *Moringa* leaves per day. The RDA is a daily nutrient intake level that fulfils the nutrient requirements of ~ 98 % of the healthy individuals in an age- and sex-specific population (IOM 2003). *Moringa oleifera* grown without Se fertilizer can provide 100 % of the RDA of a healthy adult man which is comparable with Se

obtained from a similar quantity of carrots biofortified with 1 kg ha^{-1} of Se fertilizer (Smolen *et al.* 2016), and maize biofortified with 5 g of Se ha^{-1} at the level of the Malawian population maize consumption (Chilimba *et al.* 2012a). A daily consumption of 100 g fresh leaves of MS grown in Ethiopia can fulfil 41 % of the Se RDA, while MS grown in Kenya can provide 144 % of the Se RDA for a healthy adult man. Consumption of fresh leaves or leaf powders of MO and MS, for example, can help at least to reduce the many MNDs and alleviate Se deficiency if interventions target vulnerable populations living in localities where these *Moringa* species grow vigorously. Besides, *Moringa* leaf powders can be stored for use during the dry season and transported and traded with areas where *Moringa* is not cultivated to fight against MNDs. In areas where rain fed agriculture is practiced, other vegetables, for example, *Brassica* can be used to diversify sources of dietary mineral elements and *Moringa* leaf powders can be stored and used when they are needed most during the dry season.

Table 5.5. Recommended Daily Allowance (RDA) (IOM 2003) for 19 - 70 yrs. old adult males (mg $capita^{-1}$ d^{-1}), median elemental concentration in 100 g fresh *Moringa* leaves (mg) from Kenya and Ethiopia and percentage of RDA fulfilled by consuming 100 g fresh *Moringa* leaves.

	Ca	**Cu**	**I**	**Fe**	**Mg**	**Se**	**Zn**
RNI	1000	0.9	0.15	18	320	0.055	8
MO Kenya	334	0.137	0.004	3.18	108	0.055	0.665
% of RNI fulfilled	33	15	3	18	34	100	8
MS Ethiopia	387	0.094	0.002	2.34	121	0.022	0.419
% of RNI fulfilled	39	10	1	13	38	41	5
MS Kenya	450	0.061	0.005	3.80	144	0.079	0.314
% of RNI fulfilled	45	7	3	21	45	144	4

5.6.4 Contamination of *Moringa* leaves by soil dust

Edible plant parts externally exposed to the atmosphere, such as, leaves, immature pods and flowers are liable to extraneous soil dust contamination. This affects the Fe concentration in contaminated tissues (Joy *et al.* 2015a). To reduce extraneous Fe contamination, *Moringa* leaf samples were washed using bottled or tap water in the field during collection. Correlations between the Fe, aluminium (Al), titanium (Ti) and vanadium (V) concentrations in leaves were determined as an indicator of soil dust contamination. The high positive correlation (Table 5.6) between these leaf elemental concentrations indicates that washing did not eliminate soil dust contamination and there is an extraneous Fe. Even if precautionary measure was taken to simulate the usual processing in dietary usage of *Moringa* leaves, there was indication that the leaves had been contaminated with soil dust. The extent of such sources of Fe was assessed for *Moringa* leaves, as an example, where paired plant and soil samples were collected using Equation 5.1. and results presented in Appendix 5.38.

Equation 5.1 Estimating the quantity of extraneous Fe due to soil dust contamination in *Moringa* leaves.

$$P_{Fe} = \frac{V_{plant} * Fe_{soil}}{V_{soil} * Fe_{plant}}$$

Where, P_{Fe} is the proportion of Fe from extraneous source; $V_{plant}\ and\ Fe_{plant}$ are elemental concentrations in the plant; and $V_{soil}\ and\ Fe_{soil}$ are concentrations in the soil.

Table 5.6. Correlation among leaf Fe, Al, Ti and V concentration. D.f. = degrees of freedom, r = correlation coefficient, R^2 = coefficient of determination.

Elements		D.f.	r	R^2	p-value
Leaf Fe	Leaf Al	103	0.904	0.818	< 0.0001
	Leaf Ti	103	0.619	0.384	< 0.0001
	Leaf V	103	0.899	0.809	< 0.0001

5.7 Conclusion

In addition to the high selenium concentration, *Moringa* spp. leaves are rich in proteins and β-carotene (Leone *et al.* 2015, Olson *et al.* 2016), possess anti-oxidant properties (Kushwaha *et al.* 2014), contain low concentrations of anti-nutrients (Gidamis *et al.* 2003, Devi *et al.* 2007, Ogbe and Affiku 2011, Nouman *et al.* 2013), may be used in treating ailments (Kifleyohannes *et al.* 2014), the seeds are used as water coagulant (Ahmed *et al.* 2010), and they grow under marginal environmental conditions providing much needed ecological services (for example, shade, wind break, etc.). *Moringa oleifera* is naturalized while MS is indigenous to Kenya and Ethiopia. Where these species grow, the population have indigenous knowledge of their multiple uses including the high nutritive values (Jiru *et al.* 2006). Nonetheless, the utilization of these species as food is limited to specific localities and communities (NRC 2006), they are neglected in terms of research and development, and the trees can be classed as underutilized crops (Padulosi *et al.* 2008, Mayes *et al.* 2012). Agricultural and health extension work to popularise the production and consumption of MO and MS may be a useful strategy to complement efforts to alleviate dietary MNDs through dietary diversification, and use of *Moringa* leaf powders to fortify meals in the dry season when other leafy green vegetables are not available. In addition, variations in mineral micronutrient concentrations suggest that breeding efforts to increase the nutritional value of

Moringa foliage may be successful. However, research is also required to determine the bioavailability of nutrients from *Moringa* edible parts. The Moringaceae belongs to the same order (Brassicales) as Brassicaceae (APGIV 2016) which are known to be Se accumulators (White *et al.* 2004, White 2016). Hence further studies on the Se concentrations in edible portions of the other *Moringa* species are important to understand and exploit the potential of the family in the fight against human Se undernutrition. Besides, research is needed to elucidate the mechanism employed by *Moringa* to free up soil Se.

Appendix 5.1. Number of *Moringa* edible part samples collected from Ethiopia and Kenya by locality and species. MO, *M. oleifera*; MS, *M. stenopetala*.

Species	Country	Locality	Flower	Immature pod	Leaf	Root	Seed
MO	Kenya	Baringo	1				
	Kenya	Kibwezi	1	4	14		9
	Kenya	Malindi	7	2	11		2
	Kenya	Mbololo	16	13	16		16
	Kenya	Ramogi	7	5	8		7
	Kenya	Ukunda	3	3	7		1
MS	Kenya	Baringo	3	1	5	1	2
	Ethiopia	Derashe	1	1	12		
	Ethiopia	Hawassa			14		
	Ethiopia	Konso			16		1
	Kenya	Ramogi			1		

Appendix 5.2. Number of soil samples (n) collected from the different localities in Ethiopia and Kenya.

Country	Locality	N
Ethiopia	Derashe	12
	Hawassa	9
	Konso	12
Kenya	Baringo	6
	Kibwezi	14
	Malindi	11
	Mbololo	16
	Ramogi	8
	Ukunda	7

Appendix 5.3. Descriptive statistics on elemental concentration (mg kg^{-1}) of plant Certified Reference Materials (CRM).

	1567B						1573A					
Element	**Ca**	**Cu**	**Fe**	**Mg**	**Se**	**Zn**	**Ca**	**Cu**	**Fe**	**Mg**	**Se**	**Zn**
N	8	8	8	8	8	8	7	7	7	7	7	7
Mean	189.700	1.700	12.910	333.400	1.130	10.340	51066.000	4.350	333.400	11162.000	0.080	30.460
Median	189.400	1.870	12.810	327.500	1.100	10.050	53182.000	4.470	348.300	11600.000	0.080	31.600
Minimum	169.200	0.000	11.920	302.800	1.060	9.270	38332.000	3.330	246.700	8443.000	0.060	23.630
Maximum	205.100	2.260	14.840	362.800	1.230	11.530	55428.000	4.940	373.100	12177.000	0.090	33.640
Lower quartile	182.700	1.690	12.150	317.800	1.070	9.670	50338.000	4.240	320.000	11018.000	0.070	29.310
Upper quartile	199.700	2.100	13.290	355.600	1.200	11.250	54173.000	4.580	360.900	11836.000	0.090	32.740
Standard deviation	11.990	0.720	0.950	22.220	0.070	0.890	5916.000	0.500	42.690	1266.000	0.010	3.400
Standard error of mean	4.240	0.250	0.340	7.860	0.020	0.310	2236.000	0.190	16.130	478.500	0.000	1.280
Coefficient of variation	6.320	42.290	7.360	6.670	6.170	8.560	11.590	11.540	12.800	11.340	13.070	11.150

Appendix 5.4. Descriptive statistics on elemental concentration (mg kg^{-1}) of soil Certified Reference Materials (CRM) (2711A).

Element	Ca	Cu	I	Fe	Mg	Se	Se-P	Zn
N	3	3	3	3	3	3	3	3
Mean	19,501.000	100.700	1.060	25,678.000	7,928.000	1.800	0.140	334.900
Median	19,934.000	99.700	1.060	26,340.000	8,182.000	1.840	0.140	337.200
Minimum	17,684.000	93.870	1.050	23,376.000	7,096.000	1.620	0.130	308.900
Maximum	20,885.000	108.400	1.060	27,319.000	8,506.000	1.920	0.140	358.700
Lower quartile	18,246.000	95.330	1.050	24,117.000	7,368.000	1.680	0.130	316.000
Upper quartile	20,648.000	106.200	1.060	27,075.000	8,425.000	1.900	0.140	353.300
Standard deviation	1,644.000	7.310	0.010	2,053.000	738.100	0.160	0.000	24.960
Standard error of mean	949.200	4.220	0.010	1,186.000	426.200	0.090	0.000	14.410
Coefficient of variation	8.430	7.260	0.890	8.000	9.310	8.670	1.940	7.450

Appendix 5.5. Shapiro-Wilk test of normality of the distribution of soil elemental concentration by locality.

Element	Locality	Shapiro-Wilk Statistic	d.f.	*P*
Ca	Derashe	0.83	12	0.021
	Hawassa	0.886	9	0.18
	Konso	0.842	12	0.03
	Baringo	0.783	6	0.041
	Kibwezi	0.859	14	0.029
	Malindi	0.788	11	0.007
	Mbololo	0.982	16	0.977
	Ramogi	0.966	8	0.862
	Ukunda	0.941	7	0.644
Cu	Derashe	0.899	12	0.156
	Hawassa	0.502	9	0
	Konso	0.805	12	0.011
	Baringo	0.864	6	0.203
	Kibwezi	0.903	14	0.125
	Malindi	0.903	11	0.199
	Mbololo	0.946	16	0.423
	Ramogi	0.749	8	0.008
	Ukunda	0.855	7	0.137
I	Derashe	0.892	12	0.126
	Hawassa	0.823	9	0.037
	Konso	0.909	12	0.207
	Baringo	0.938	6	0.646
	Kibwezi	0.889	14	0.078
	Malindi	0.768	11	0.004
	Mbololo	0.926	16	0.207
	Ramogi	0.956	8	0.768
	Ukunda	0.941	7	0.649
Fe	Derashe	0.921	12	0.297
	Hawassa	0.864	9	0.105
	Konso	0.895	12	0.137
	Baringo	0.933	6	0.603
	Kibwezi	0.908	14	0.146
	Malindi	0.861	11	0.058
	Mbololo	0.944	16	0.407
	Ramogi	0.762	8	0.011
	Ukunda	0.884	7	0.244
Mg	Derashe	0.817	12	0.015
	Hawassa	0.852	9	0.078
	Konso	0.869	12	0.063
	Baringo	0.812	6	0.075
	Kibwezi	0.847	14	0.02

Element	Locality	Shapiro-Wilk Statistic	d.f.	*P*
	Malindi	0.952	11	0.673
	Mbololo	0.966	16	0.768
	Ramogi	0.871	8	0.155
	Ukunda	0.719	7	0.006
Se	**Derashe**	0.886	12	0.104
	Hawassa	0.862	9	0.102
	Konso	0.891	12	0.123
	Baringo	0.911	6	0.444
	Kibwezi	0.945	14	0.487
	Malindi	0.973	11	0.914
	Mbololo	0.846	16	0.012
	Ramogi	0.978	8	0.953
	Ukunda	0.874	7	0.2
Se-P	**Derashe**	0.948	12	0.602
	Hawassa	0.922	9	0.411
	Konso	0.839	12	0.027
	Baringo	0.869	6	0.222
	Kibwezi	0.938	14	0.391
	Malindi	0.874	11	0.089
	Mbololo	0.875	16	0.033
	Ramogi	0.724	8	0.004
	Ukunda	0.948	7	0.714
Zn	**Derashe**	0.951	12	0.648
	Hawassa	0.801	9	0.021
	Konso	0.938	12	0.472
	Baringo	0.911	6	0.446
	Kibwezi	0.92	14	0.219
	Malindi	0.901	11	0.191
	Mbololo	0.971	16	0.849
	Ramogi	0.942	8	0.633
	Ukunda	0.877	7	0.212
pH	**Derashe**	0.809	12	0.012
	Hawassa	0.868	9	0.118
	Konso	0.922	12	0.299
	Baringo	0.88	6	0.269
	Kibwezi	0.955	14	0.645
	Malindi	0.905	11	0.215
	Mbololo	0.959	16	0.642
	Ramogi	0.954	8	0.747
	Ukunda	0.668	7	0.002

Appendix 5.6. Levene's test of homogeneity of variances of soil elemental concentration based on mean and median. D.f. 1 is the degree of freedom of the numerator, and d.f. 2 is the degree of freedom of the denominator.

Element		Levene Statistic	d.f. 1	d.f. 2	*P*
Ca	**Based on Mean**	11.233	8	86	0
	Based on Median	7.967	8	86	0
	Based on Median and with adjusted d.f.	7.967	8	60	0
	Based on trimmed mean	10.324	8	86	0
Cu	**Based on Mean**	8.078	8	86	0
	Based on Median	2.696	8	86	0.011
	Based on Median and with adjusted d.f.	2.696	8	24	0.029
	Based on trimmed mean	7.008	8	86	0
I	**Based on Mean**	6.125	8	86	0
	Based on Median	4.875	8	86	0
	Based on Median and with adjusted d.f.	4.875	8	29	0.001
	Based on trimmed mean	5.955	8	86	0
Fe	**Based on Mean**	10.434	8	86	0
	Based on Median	4.888	8	86	0
	Based on Median and with adjusted d.f.	4.888	8	21	0.002
	Based on trimmed mean	9.136	8	86	0
Mg	**Based on Mean**	14.734	8	86	0
	Based on Median	11.067	8	86	0
	Based on Median and with adjusted d.f.	11.067	8	38	0
	Based on trimmed mean	13.41	8	86	0
Se	**Based on Mean**	7.215	8	86	0
	Based on Median	5.379	8	86	0
	Based on Median and with adjusted d.f.	5.379	8	69	0
	Based on trimmed mean	6.984	8	86	0
Se-P	**Based on Mean**	11.445	8	86	0
	Based on Median	8.481	8	86	0
	Based on Median and with adjusted d.f.	8.481	8	66	0
	Based on trimmed mean	10.997	8	86	0
Zn	**Based on Mean**	6.206	8	86	0
	Based on Median	4.393	8	86	0
	Based on Median and with adjusted d.f.	4.393	8	10	0.015
	Based on trimmed mean	5.216	8	86	0
pH	**Based on Mean**	3.463	8	86	0.002
	Based on Median	2.658	8	86	0.012
	Based on Median and with adjusted d.f.	2.658	8	46	0.017
	Based on trimmed mean	3.389	8	86	0.002

Appendix 5.7. Test of normality of the distribution of MO leaves elemental concentration by locality in Kenya.

Element	Locality	Shapiro-Wilk Statistic	d.f.	*P*
Ca	**Kibwezi**	0.906	14	0.136
	Malindi	0.895	11	0.162
	Mbololo	0.918	16	0.156
	Ramogi	0.815	8	0.041
	Ukunda	0.74	7	0.01
Cu	**Kibwezi**	0.972	14	0.906
	Malindi	0.866	11	0.068
	Mbololo	0.94	16	0.344
	Ramogi	0.961	8	0.817
	Ukunda	0.886	7	0.256
I	**Kibwezi**	0.702	14	0
	Malindi	0.486	11	0
	Mbololo	0.919	16	0.16
	Ramogi	0.708	8	0.003
	Ukunda	0.762	7	0.017
Fe	**Kibwezi**	0.916	14	0.192
	Malindi	0.929	11	0.397
	Mbololo	0.79	16	0.002
	Ramogi	0.643	8	0
	Ukunda	0.881	7	0.229
Mg	**Kibwezi**	0.968	14	0.852
	Malindi	0.81	11	0.013
	Mbololo	0.884	16	0.045
	Ramogi	0.944	8	0.65
	Ukunda	0.867	7	0.175
Zn	**Kibwezi**	0.904	14	0.129
	Malindi	0.948	11	0.615
	Mbololo	0.951	16	0.502
	Ramogi	0.929	8	0.509
	Ukunda	0.957	7	0.791
Se	**Kibwezi**	0.87	14	0.043
	Malindi	0.656	11	0
	Mbololo	0.777	16	0.001
	Ramogi	0.808	8	0.035
	Ukunda	0.735	7	0.009

Appendix 5.8. Levene's test of homogeneity of variances of MO leaves elemental concentration by localities in Kenya based on mean and median. D.f. 1 is the degree of freedom of the numerator, and d.f. 2 is the degree of freedom of the denominator.

Element		Levene Statistic	d.f. 1	d.f. 2	*P*
Ca	**Based on Mean**	1.402	4	51	0.247
	Based on Median	0.585	4	51	0.675
	Based on Median and with adjusted df	0.585	4	22	0.677
	Based on trimmed mean	1.146	4	51	0.346
Cu	**Based on Mean**	1.18	4	51	0.331
	Based on Median	1.06	4	51	0.386
	Based on Median and with adjusted df	1.06	4	37	0.39
	Based on trimmed mean	1.164	4	51	0.338
I	**Based on Mean**	8.953	4	51	0
	Based on Median	1.835	4	51	0.136
	Based on Median and with adjusted df	1.835	4	14	0.177
	Based on trimmed mean	5.764	4	51	0.001
Fe	**Based on Mean**	4.08	4	51	0.006
	Based on Median	2.673	4	51	0.042
	Based on Median and with adjusted df	2.673	4	10	0.093
	Based on trimmed mean	3.209	4	51	0.02
Mg	**Based on Mean**	0.045	4	51	0.996
	Based on Median	0.089	4	51	0.985
	Based on Median and with adjusted df	0.089	4	40	0.985
	Based on trimmed mean	0.057	4	51	0.994
Zn	**Based on Mean**	4.831	4	51	0.002
	Based on Median	4.157	4	51	0.005
	Based on Median and with adjusted df	4.157	4	35	0.007
	Based on trimmed mean	4.729	4	51	0.003
Se	**Based on Mean**	2.582	4	51	0.048
	Based on Median	1.527	4	51	0.208
	Based on Median and with adjusted df	1.527	4	33	0.217
	Based on trimmed mean	2.19	4	51	0.083

Appendix 5.9. Test of normality of the distribution of MS leaves elemental concentration by locality.

Element	Locality	Shapiro-Wilk statistic	d.f.	*P*
Ca	**Derashe**	0.941	8	0.622
	Hawassa	0.846	14	0.019
	Konso	0.969	14	0.87
	Baringo	0.901	5	0.417
Cu	**Derashe**	0.935	8	0.558
	Hawassa	0.964	14	0.785
	Konso	0.927	14	0.274
	Baringo	0.958	5	0.795
I	**Derashe**	0.804	8	0.032
	Hawassa	0.896	14	0.1
	Konso	0.721	14	0.001
	Baringo	0.846	5	0.182
Fe	**Derashe**	0.941	8	0.619
	Hawassa	0.9	14	0.111
	Konso	0.447	14	0
	Baringo	0.873	5	0.277
Mg	**Derashe**	0.967	8	0.871
	Hawassa	0.963	14	0.776
	Konso	0.913	14	0.174
	Baringo	0.798	5	0.078
Se	**Derashe**	0.691	8	0.002
	Hawassa	0.962	14	0.758
	Konso	0.728	14	0.001
	Baringo	0.946	5	0.709
Zn	**Derashe**	0.65	8	0.001
	Hawassa	0.903	14	0.126
	Konso	0.975	14	0.94
	Baringo	0.98	5	0.933

Appendix 5.10. Levene's test of homogeneity of variances of MS leaves elemental concentration by localities based on mean and median. D.f. 1 is the degree of freedom of the numerator, and d.f. 2 is the degree of freedom of the denominator.

Element		Levene Statistic	d.f. 1	d.f. 2	*P*
Ca	**Based on Mean**	5.154	3	37	0.004
	Based on Median	2.404	3	37	0.083
	Based on Median and with adjusted df	2.404	3	22	0.095
	Based on trimmed mean	4.466	3	37	0.009
Cu	**Based on Mean**	2.828	3	37	0.052
	Based on Median	2.846	3	37	0.051
	Based on Median and with adjusted df	2.846	3	32	0.053
	Based on trimmed mean	2.815	3	37	0.052
I	**Based on Mean**	3.927	3	37	0.016
	Based on Median	1.838	3	37	0.157
	Based on Median and with adjusted df	1.838	3	19	0.175
	Based on trimmed mean	3.202	3	37	0.034
Fe	**Based on Mean**	1.99	3	37	0.132
	Based on Median	0.788	3	37	0.508
	Based on Median and with adjusted df	0.788	3	14	0.521
	Based on trimmed mean	1.097	3	37	0.363
Mg	**Based on Mean**	5.675	3	37	0.003
	Based on Median	2.065	3	37	0.122
	Based on Median and with adjusted df	2.065	3	7	0.197
	Based on trimmed mean	5.223	3	37	0.004
Se	**Based on Mean**	5.771	3	37	0.002
	Based on Median	1.818	3	37	0.161
	Based on Median and with adjusted df	1.818	3	23	0.172
	Based on trimmed mean	5.01	3	37	0.005
Zn	**Based on Mean**	4.696	3	37	0.007
	Based on Median	0.846	3	37	0.477
	Based on Median and with adjusted df	0.846	3	11	0.497
	Based on trimmed mean	3.358	3	37	0.029

Appendix 5.11. Test of normality of the distribution of MO immature pods elemental concentration by locality.

Element	Locality	Shapiro-Wilk statistic	d.f.	*P*
Ca	**Kibwezi**	0.938	4	0.64
	Mbololo	0.933	8	0.548
	Ramogi	0.967	3	0.649
	Ukunda	0.887	3	0.345
Cu	**Kibwezi**	0.88	4	0.34
	Mbololo	0.909	8	0.349
	Ramogi	0.93	3	0.487
	Ukunda	0.951	3	0.573
I	**Kibwezi**	0.886	4	0.366
	Mbololo	0.828	8	0.057
	Ramogi	0.866	3	0.283
	Ukunda	0.784	3	0.077
Fe	**Kibwezi**	0.916	4	0.514
	Mbololo	0.9	8	0.288
	Ramogi	0.891	3	0.359
	Ukunda	0.777	3	0.062
Mg	**Kibwezi**	0.927	4	0.577
	Mbololo	0.956	8	0.773
	Ramogi	0.868	3	0.289
	Ukunda	0.998	3	0.914
Se	**Kibwezi**	0.743	4	0.033
	Mbololo	0.901	8	0.292
	Ramogi	0.985	3	0.765
	Ukunda	0.877	3	0.316
Zn	**Kibwezi**	0.982	4	0.913
	Mbololo	0.947	8	0.677
	Ramogi	0.988	3	0.789
	Ukunda	0.77	3	0.044

Appendix 5.12. Levene's test of homogeneity of variances of MO immature pods elemental concentration by localities.

Element	Levene statistic	d.f. 1	d.f. 2	*P*
Ca	1.531	4	15	0.244
Cu	1.704	4	15	0.201
I	4.787	4	15	0.011
Fe	7.541	4	15	0.002
Mg	4.463	4	15	0.014
Se	1.318	4	15	0.308
Zn	0.448	4	15	0.772

Appendix 5.13. Test of normality of the distribution of MO seeds elemental concentration by locality.

Element	Locality	Shapiro-Wilk statistic	d.f.	*P*
Ca	**Kibwezi**	0.909	6	0.429
	Mbololo	0.885	7	0.251
	Ramogi	0.752	3	0.005
Cu	**Kibwezi**	0.953	6	0.765
	Mbololo	0.955	7	0.774
	Ramogi	0.849	3	0.239
I	**Kibwezi**	0.713	6	0.008
	Mbololo	0.827	7	0.075
	Ramogi	0.825	3	0.176
Fe	**Kibwezi**	0.914	6	0.461
	Mbololo	0.917	7	0.444
	Ramogi	0.958	3	0.606
Mg	**Kibwezi**	0.915	6	0.473
	Mbololo	0.912	7	0.41
	Ramogi	0.808	3	0.133
Se	**Kibwezi**	0.897	6	0.358
	Mbololo	0.842	7	0.104
	Ramogi	0.821	3	0.166
Zn	**Kibwezi**	0.903	6	0.389
	Mbololo	0.922	7	0.489
	Ramogi	0.942	3	0.536

Appendix 5.14. Levene's test of homogeneity of variances of MO seeds elemental concentration by localities.

Element	Levene statistic	d.f. 1	d.f. 2	*P*
Ca	0.878	2	13	0.439
Cu	1.208	2	13	0.33
I	0.846	2	13	0.451
Fe	0.45	2	13	0.647
Mg	2.343	2	13	0.135
Se	0.322	2	13	0.731
Zn	1.51	2	13	0.257

Appendix 5.15. Test of normality of the distribution of MO flowers elemental concentration by locality.

Element	Locality	Shapiro-Wilk statistic	d.f.	*P*
Ca	**Malindi**	0.956	7	0.787
	Mbololo	0.884	16	0.045
	Ramogi	0.941	7	0.646
	Ukunda	0.755	3	0.011
Cu	**Malindi**	0.963	7	0.843
	Mbololo	0.965	16	0.747
	Ramogi	0.936	7	0.601
	Ukunda	0.941	3	0.532
I	**Malindi**	0.478	7	0
	Mbololo	0.357	16	0
	Ramogi	0.857	7	0.143
	Ukunda	0.905	3	0.4
Fe	**Malindi**	0.788	7	0.031
	Mbololo	0.916	16	0.146
	Ramogi	0.921	7	0.477
	Ukunda	0.947	3	0.558
Mg	**Malindi**	0.942	7	0.655
	Mbololo	0.915	16	0.142
	Ramogi	0.959	7	0.809
	Ukunda	0.887	3	0.344
Se	**Malindi**	0.886	7	0.256
	Mbololo	0.742	16	0.001
	Ramogi	0.861	7	0.153
	Ukunda	0.879	3	0.321
Zn	**Malindi**	0.974	7	0.924
	Mbololo	0.903	16	0.091
	Ramogi	0.947	7	0.705
	Ukunda	0.991	3	0.822

Appendix 5.16. Levene's test of homogeneity of variances of MO flowers elemental concentration by localities.

Element	**Levene statistic**	**d.f. 1**	**d.f. 2**	***P***
Ca	2.282	3	29	0.1
Cu	2.127	3	29	0.118
I	1.687	3	29	0.192
Fe	20.373	3	29	0
Mg	6.803	3	29	0.001
Se	2.506	3	29	0.079
Zn	0.283	3	29	0.837

Appendix 5.17. Descriptive statistics of MO leaves elemental concentration (mg kg^{-1}) by locality.

Locality		Element						
		Ca	Cu	I	Fe	Mg	Se	Zn
Kibwezi	N	14	14	14	14	14	14	14
	Mean	14,960.160	9.179	0.152	193.383	5,400.566	5.742	45.791
	Median	12,562.529	8.873	0.152	176.566	5,316.767	4.529	46.204
	Std. Deviation	7,279.706	2.215	0.072	67.845	1,226.437	3.610	13.667
	Std. Error of Mean	1,945.583	0.592	0.019	18.132	327.779	0.965	3.653
	Minimum	7,037.418	5.902	0.069	114.234	3,509.752	1.937	26.908
	Maximum	30,580.651	13.900	0.308	325.598	7,716.198	12.502	68.153
Mbololo	N	16	16	16	16	16	16	16
	Mean	22,873.437	5.479	0.338	167.725	6,552.916	5.819	33.451
	Median	21,665.554	5.418	0.358	153.490	6,356.211	3.968	31.424
	Std. Deviation	7,280.129	1.221	0.100	56.486	1,513.341	5.941	10.356
	Std. Error of Mean	1,820.032	0.305	0.025	14.122	378.335	1.485	2.589
	Minimum	13,638.531	3.777	0.143	106.918	4,432.313	0.569	18.099
	Maximum	34,850.861	8.226	0.545	312.735	10,823.719	21.210	52.116
Ramogi	N	8	8	8	8	8	8	8
	Mean	16,316.006	6.912	0.067	402.855	4,915.632	0.839	29.592
	Median	11,908.853	7.125	0.037	310.538	5,090.188	0.620	26.224
	Std. Deviation	7,401.103	1.176	0.067	321.066	1,492.768	0.927	12.740
	Std. Error of Mean	2,616.685	0.416	0.024	113.514	527.773	0.328	4.504
	Minimum	8,551.457	5.161	0.001	193.676	2,524.808	0.031	14.399
	Maximum	27,546.755	8.590	0.204	1,171.058	7,485.904	2.851	54.391

Locality		Element						
		Ca	**Cu**	**I**	**Fe**	**Mg**	**Se**	**Zn**
Malindi	**N**	11	11	11	11	11	11	11
	Mean	17,199.049	7.268	0.247	129.044	4,470.598	3.215	34.392
	Median	15,946.089	7.559	0.254	125.327	4,750.177	2.576	33.722
	Std. Deviation	4,661.604	1.932	0.064	45.294	1,210.021	3.272	5.344
	Std. Error of Mean	1,405.526	0.583	0.019	13.657	364.835	0.987	1.611
	Minimum	10,924.482	3.005	0.135	74.934	3,094.520	0.774	25.053
	Maximum	23,153.497	10.424	0.366	227.622	5,958.689	12.537	42.851
Ukunda	**N**	7	7	7	7	7	7	7
	Mean	18,734.135	5.180	0.203	182.456	4,496.256	3.163	28.940
	Median	13,579.370	5.758	0.132	120.809	4,183.929	2.160	29.829
	Std. Deviation	13,045.504	1.391	0.160	109.738	1,292.254	3.093	6.622
	Std. Error of Mean	4,930.737	0.526	0.060	41.477	488.426	1.169	2.503
	Minimum	9,620.751	3.299	0.056	71.183	3,193.185	0.944	17.486
	Maximum	46,591.232	6.786	0.476	354.907	7,020.716	9.720	38.228
Total	**N**	56	56	56	56	56	56	56
	Mean	18,326.317	6.923	0.218	201.973	5,364.822	4.245	35.606
	Median	16,680.849	6.830	0.201	158.824	5,395.087	2.725	33.272
	Std. Deviation	8,138.245	2.223	0.130	155.263	1,551.893	4.376	11.958
	Std. Error of Mean	1,087.519	0.297	0.017	20.748	207.380	0.585	1.598
	Minimum	7,037.418	3.005	0.001	71.183	2,524.808	0.031	14.399
	Maximum	46,591.232	13.900	0.545	1,171.058	10,823.719	21.210	68.153

Appendix 5.18. Descriptive statistics for MS leaves elemental concentration (mg kg^{-1}) by locality.

Locality		Element						
		Ca	**Cu**	**I**	**Fe**	**Mg**	**Se**	**Zn**
Derashe	**N**	8	8	9	8	8	8	8
	Mean	22,089.115	5.156	0.029	125.665	7,991.397	1.371	24.644
	Median	21,554.019	5.342	0.029	120.825	7,969.240	0.260	18.599
	Std. Deviation	6,586.317	0.612	0.019	23.300	1,551.189	2.136	14.139
	Std. Error of Mean	2,328.615	0.216	0.006	8.238	548.428	0.755	4.999
	Minimum	14,522.685	4.282	0.001	98.536	5,806.893	0.080	15.424
	Maximum	32,888.314	5.984	0.063	164.959	10,197.398	6.175	56.923
Hawassa	**N**	14	14	14	14	14	14	14
	Mean	20,047.473	4.448	0.051	132.050	4,042.961	1.392	26.497
	Median	15,000.700	4.314	0.049	116.357	3,970.442	1.339	24.578
	Std. Deviation	11,523.479	1.180	0.033	43.608	1,030.168	0.412	5.318
	Std. Error of Mean	3,079.779	0.315	0.009	11.655	275.324	0.110	1.421
	Minimum	9,037.683	2.445	0.004	77.680	2,330.675	0.633	20.081
	Maximum	43,581.690	6.839	0.109	244.233	6,037.686	2.036	39.516
Konso	**N**	14	14	10	14	14	14	14
	Mean	20,594.913	4.759	0.050	196.409	6,953.243	1.329	18.759
	Median	19,943.419	4.721	0.031	109.520	6,699.051	0.240	18.115
	Std. Deviation	3,956.855	1.625	0.064	283.696	1,241.152	1.766	3.987
	Std. Error of Mean	1,057.514	0.434	0.020	75.821	331.712	0.472	1.066
	Minimum	13,727.290	1.290	0.001	72.034	5,415.358	0.036	10.532
	Maximum	28,905.411	6.854	0.221	1,165.833	9,147.332	4.643	26.173

Locality		Element						
		Ca	Cu	I	Fe	Mg	Se	Zn
Baringo	N	5	5	5	5	5	5	5
	Mean	23,544.784	3.154	0.237	207.359	9,198.380	3.783	16.135
	Median	22,494.501	3.067	0.243	189.925	7,206.049	3.962	15.696
	Std. Deviation	10,822.890	0.959	0.058	76.183	4,145.333	2.541	3.967
	Std. Error of Mean	4,840.144	0.429	0.026	34.070	1,853.849	1.136	1.774
	Minimum	10,058.043	1.759	0.169	143.434	6,069.678	1.098	11.497
	Maximum	40,319.734	4.418	0.312	330.570	16,234.149	7.396	22.001
Total	N	41	41	38	41	41	41	41
	Mean	21,059.274	4.534	0.070	161.965	6,435.852	1.658	22.229
	Median	20,045.246	4.464	0.038	122.286	6,178.372	1.208	20.383
	Std. Deviation	8,304.721	1.343	0.079	169.411	2,553.561	1.777	8.204
	Std. Error of Mean	1,296.980	0.210	0.013	26.458	398.799	0.277	1.281
	Minimum	9,037.683	1.290	0.001	72.034	2,330.675	0.036	10.532
	Maximum	43,581.690	6.854	0.312	1,165.833	16,234.149	7.396	56.923

Appendix 5.19. Descriptive statistics for MO immature pods elemental concentration (mg kg^{-1}) by locality.

Locality		Element					
		Ca	**Cu**	**Fe**	**Mg**	**Se**	**Zn**
Kibwezi	**N**	4	4	4	4	4	4
	Mean	3,856.707	7.668	79.559	3,252.703	4.299	32.598
	Median	4,102.595	7.793	81.074	3,137.504	3.422	32.690
	Std. Deviation	1,428.533	0.725	6.069	745.385	2.058	3.451
	Std. Error of Mean	714.266	0.362	3.034	372.693	1.029	1.725
	Minimum	1,901.944	6.792	71.075	2,470.055	2.991	28.306
	Maximum	5,319.696	8.293	85.015	4,265.749	7.360	36.708
Malindi	**N**	2	2	2	2	2	2
	Mean	2,168.956	3.943	57.668	1,829.595	2.216	23.703
	Median	2,168.956	3.943	57.668	1,829.595	2.216	23.703
	Std. Deviation	634.132	0.630	2.427	75.156	2.202	4.991
	Std. Error of Mean	448.399	0.445	1.717	53.144	1.557	3.529
	Minimum	1,720.558	3.497	55.952	1,776.451	0.659	20.174
	Maximum	2,617.355	4.388	59.385	1,882.738	3.773	27.232
Mbololo	**N**	8	8	8	8	8	8
	Mean	4,893.752	5.170	55.007	3,470.189	2.337	25.164
	Median	5,238.603	5.002	48.886	3,586.754	1.985	25.832
	Std. Deviation	1,599.666	1.405	14.666	1,172.171	2.015	4.458
	Std. Error of Mean	565.567	0.497	5.185	414.425	0.712	1.576
	Minimum	2,746.143	3.477	36.496	1,730.162	0.337	18.369
	Maximum	7,050.451	7.132	77.817	5,169.376	5.978	31.031

Locality		Element					
		Ca	**Cu**	**Fe**	**Mg**	**Se**	**Zn**
Ramogi	**N**	3	3	3	3	3	3
	Mean	2,707.043	6.378	66.491	2,485.935	0.211	33.159
	Median	2,607.266	6.852	62.760	2,575.122	0.183	32.575
	Std. Deviation	472.942	1.549	9.805	212.458	0.200	4.587
	Std. Error of Mean	273.053	0.895	5.661	122.663	0.115	2.649
	Minimum	2,291.950	4.646	59.100	2,243.421	0.027	28.891
	Maximum	3,221.912	7.634	77.614	2,639.264	0.424	38.010
Ukunda	**N**	3	3	3	3	3	3
	Mean	1,626.182	3.094	78.576	1,759.059	2.079	24.308
	Median	1,223.586	2.754	99.364	1,747.287	1.369	20.965
	Std. Deviation	1,037.495	1.326	38.163	226.071	1.753	6.033
	Std. Error of Mean	598.998	0.766	22.033	130.522	1.012	3.483
	Minimum	850.323	1.971	34.532	1,539.103	0.792	20.686
	Maximum	2,804.636	4.558	101.831	1,990.785	4.076	31.273
Total	**N**	20	20	20	20	20	20
	Mean	3,595.721	5.417	65.442	2,858.325	2.360	27.575
	Median	3,055.087	5.048	62.626	2,607.193	1.985	27.769
	Std. Deviation	1,760.177	1.894	19.245	1,057.747	2.069	5.702
	Std. Error of Mean	393.588	0.424	4.303	236.519	0.463	1.275
	Minimum	850.323	1.971	34.532	1,539.103	0.027	18.369
	Maximum	7,050.451	8.293	101.831	5,169.376	7.360	38.010

Appendix 5.20. Descriptive statistics for MO seeds elemental concentration (mg kg^{-1}) by locality.

Locality		Element					
		Ca	**Cu**	**Fe**	**Mg**	**Se**	**Zn**
Kibwezi	**N**	6	6	6	6	6	6
	Mean	1,048.234	4.625	53.301	2,992.807	3.700	44.576
	Median	970.460	4.624	51.958	3,034.416	3.087	43.199
	Std. Deviation	250.337	0.974	8.130	380.763	2.220	6.113
	Std. Error of Mean	102.200	0.398	3.319	155.446	0.906	2.496
	Minimum	797.616	3.403	43.892	2,332.004	1.602	38.296
	Maximum	1,477.118	5.934	67.880	3,375.255	7.586	53.427
Mbololo	**N**	7	7	7	7	7	7
	Mean	1,483.042	4.083	46.972	3,057.265	4.626	44.201
	Median	1,532.522	3.974	45.785	3,094.985	4.440	43.657
	Std. Deviation	308.508	1.134	4.415	264.794	3.196	4.172
	Std. Error of Mean	116.605	0.429	1.669	100.083	1.208	1.577
	Minimum	1,100.833	2.742	42.400	2,561.397	1.301	39.388
	Maximum	1,843.624	5.833	54.850	3,332.850	11.162	52.182
Ramogi	**N**	3	3	3	3	3	3
	Mean	1,444.051	3.502	46.062	3,296.738	0.938	46.531
	Median	1,317.493	3.306	44.598	2,972.231	0.257	48.859
	Std. Deviation	220.268	0.437	6.189	641.072	1.394	8.384
	Std. Error of Mean	127.172	0.252	3.573	370.123	0.805	4.841
	Minimum	1,316.265	3.197	40.737	2,882.802	0.015	37.228
	Maximum	1,698.394	4.003	52.852	4,035.181	2.542	53.505

Locality		Element					
		Ca	**Cu**	**Fe**	**Mg**	**Se**	**Zn**
Total	**N**	16	16	16	16	16	16
	Mean	1,312.678	4.177	49.175	3,077.994	3.587	44.779
	Median	1,265.461	3.988	48.982	3,033.608	3.069	43.585
	Std. Deviation	332.262	1.016	6.779	379.266	2.811	5.438
	Std. Error of Mean	83.065	0.254	1.695	94.816	0.703	1.360
	Minimum	797.616	2.742	40.737	2,332.004	0.015	37.228
	Maximum	1,843.624	5.934	67.880	4,035.181	11.162	53.505

Appendix 5.21. Descriptive statistics for MO flowers elemental concentration (mg kg^{-1}) by locality.

Locality		Element					
		Ca	**Cu**	**Fe**	**Mg**	**Se**	**Zn**
Malindi	**N**	7	7	7	7	7	7
	Mean	2,999.801	6.573	115.719	2,499.195	4.415	34.002
	Median	3,106.231	6.102	75.749	2,514.860	3.017	33.544
	Std. Deviation	1,203.615	2.156	60.699	177.194	3.532	5.391
	Std. Error of Mean	454.924	0.815	22.942	66.973	1.335	2.037
	Minimum	1,467.317	3.811	60.936	2,214.470	0.448	24.828
	Maximum	5,089.660	10.166	195.986	2,693.418	9.086	41.082
Mbololo	**N**	16	16	16	16	16	16
	Mean	4,436.461	6.333	219.815	3,177.500	3.027	31.311
	Median	4,579.819	6.252	198.502	3,256.363	1.938	29.478
	Std. Deviation	1,275.506	1.681	92.249	648.829	3.231	6.801
	Std. Error of Mean	318.876	0.420	23.062	162.207	0.808	1.700

Locality		Element					
		Ca	**Cu**	**Fe**	**Mg**	**Se**	**Zn**
	Minimum	2,674.313	3.464	105.882	2,338.926	0.570	20.798
	Maximum	6,160.185	9.066	384.560	4,219.717	12.997	47.248
Ramogi	**N**	7	7	7	7	7	7
	Mean	3,316.487	7.323	259.851	2,615.373	0.668	35.219
	Median	3,519.246	7.390	223.272	2,512.281	0.336	35.153
	Std. Deviation	904.368	1.100	88.747	602.025	0.702	8.069
	Std. Error of Mean	341.819	0.416	33.543	227.544	0.265	3.050
	Minimum	2,017.286	5.296	164.522	1,895.676	0.030	22.652
	Maximum	4,590.331	8.603	416.965	3,540.519	1.805	45.042
Ukunda	**N**	3	3	3	3	3	3
	Mean	1,788.378	4.177	731.886	2,264.732	2.930	30.605
	Median	1,607.169	4.306	891.968	2,350.993	1.768	31.181
	Std. Deviation	317.121	0.461	604.931	221.914	2.890	5.362
	Std. Error of Mean	183.090	0.266	349.257	128.122	1.669	3.096
	Minimum	1,603.414	3.666	63.014	2,012.640	0.801	24.978
	Maximum	2,154.551	4.560	1,240.676	2,430.564	6.220	35.656
Total	**N**	33	33	33	33	33	33
	Mean	3,653.410	6.398	252.778	2,831.399	2.812	32.647
	Median	3,415.872	6.253	194.733	2,613.325	1.561	31.676
	Std. Deviation	1,394.577	1.759	234.971	631.349	3.073	6.654
	Std. Error of Mean	242.765	0.306	40.903	109.904	0.535	1.158
	Minimum	1,467.317	3.464	60.936	1,895.676	0.030	20.798
	Maximum	6,160.185	10.166	1,240.676	4,219.717	12.997	47.248

Appendix 5.22. Descriptive statistics for soil elemental concentration (mg kg^{-1}) and pH by locality.

Locality		Element								
		Ca	**Cu**	**I**	**Fe**	**Mg**	**Se**	**Se-P**	**Zn**	**pH**
Derashe	**N**	12	12	12	12	12	12	12	12	12
	Mean	28,403.214	33.383	0.851	69,188.339	13,662.486	0.255	0.006	87.945	8.402
	Median	30,610.044	31.206	0.748	74,910.278	14,821.577	0.222	0.006	90.673	8.480
	Std. Deviation	9,945.190	5.987	0.607	22,443.902	5,213.568	0.083	0.004	23.943	0.281
	Std. Error of Mean	2,870.929	1.728	0.175	6,478.996	1,505.027	0.024	0.001	6.912	0.081
	Minimum	3,748.715	22.956	0.196	27,817.561	1,399.504	0.152	0.001	41.849	7.640
	Maximum	39,399.055	45.387	2.332	101,233.506	19,021.016	0.433	0.014	121.585	8.670
Hawassa	**N**	9	9	9	9	9	9	9	9	9
	Mean	15,752.996	12.951	1.131	35,393.976	4,796.370	0.599	0.011	259.637	7.426
	Median	14,126.779	8.624	0.941	32,902.160	3,798.195	0.622	0.010	228.647	7.660
	Std. Deviation	10,644.605	14.884	0.550	11,592.536	3,092.009	0.181	0.005	155.522	0.821
	Std. Error of Mean	3,548.202	4.961	0.183	3,864.179	1,030.670	0.060	0.002	51.841	0.274
	Minimum	4,682.805	4.373	0.646	23,331.785	1,780.680	0.363	0.005	121.331	6.120
	Maximum	39,050.078	52.372	2.049	61,432.348	11,775.353	0.799	0.019	627.064	8.380
Konso	**N**	12	12	12	12	12	12	12	12	12
	Mean	18,702.525	26.223	0.771	55,996.273	7,119.375	0.239	0.004	85.725	7.595
	Median	21,813.748	34.991	0.676	63,083.976	6,065.041	0.204	0.005	83.056	7.590
	Std. Deviation	8,615.910	17.638	0.379	31,041.357	4,842.516	0.097	0.002	44.051	0.582
	Std. Error of Mean	2,487.199	5.092	0.109	8,960.868	1,397.914	0.028	0.001	12.716	0.168
	Minimum	5,335.662	1.880	0.351	11,151.671	1,370.094	0.133	0.002	25.041	6.180

Locality		Element								
		Ca	Cu	I	Fe	Mg	Se	Se-P	Zn	pH
	Maximum	28,536.562	48.195	1.577	96,930.589	13,842.646	0.442	0.007	159.458	8.420
Baringo	**N**	6	6	6	6	6	6	6	6	6
	Mean	34,628.300	23.363	0.932	65,997.154	13,728.112	0.535	0.024	105.030	7.903
	Median	33,793.565	26.262	1.020	65,946.575	13,371.579	0.562	0.024	100.074	7.975
	Std. Deviation	24,475.117	11.009	0.635	10,184.250	9,603.637	0.263	0.015	22.608	0.182
	Std. Error of Mean	9,991.925	4.494	0.259	4,157.703	3,920.668	0.107	0.006	9.230	0.074
	Minimum	9,594.741	9.478	0.091	53,803.144	3,780.249	0.218	0.008	83.502	7.600
	Maximum	59,681.313	34.610	1.709	79,060.505	23,780.005	0.843	0.045	141.534	8.080
Kibwezi	**N**	14	14	14	14	14	14	14	14	14
	Mean	14,159.658	23.967	1.718	33,018.946	4,020.536	0.362	0.024	53.998	7.879
	Median	12,800.030	21.693	1.406	29,296.002	3,255.077	0.366	0.023	51.488	7.890
	Std. Deviation	8,428.409	10.437	1.064	8,774.911	2,738.148	0.128	0.007	16.704	0.499
	Std. Error of Mean	2,252.587	2.789	0.284	2,345.193	731.801	0.034	0.002	4.464	0.133
	Minimum	4,803.902	11.103	0.557	18,399.321	1,003.312	0.194	0.016	35.085	6.970
	Maximum	36,880.760	40.265	3.672	47,632.026	11,591.483	0.655	0.039	88.822	8.590
Malindi	**N**	11	11	11	11	11	11	11	11	11
	Mean	7,288.130	4.715	0.955	12,225.555	565.782	0.179	0.019	39.900	8.408
	Median	4,001.650	5.634	0.768	14,461.795	590.613	0.177	0.018	45.418	8.440
	Std. Deviation	8,384.839	2.121	0.371	6,324.562	272.922	0.044	0.007	19.006	0.217
	Std. Error of Mean	2,528.124	0.639	0.112	1,906.927	82.289	0.013	0.002	5.731	0.066
	Minimum	651.092	1.529	0.615	4,085.513	214.004	0.096	0.011	12.632	7.960
	Maximum	26,319.427	7.371	1.650	21,098.543	1,054.796	0.243	0.030	66.631	8.650
Mbololo	**N**	16	16	16	16	16	16	16	16	16

Locality		Element								
		Ca	Cu	I	Fe	Mg	Se	Se-P	Zn	pH
	Mean	13,393.396	10.247	1.434	22,315.490	3,211.001	0.241	0.020	39.843	7.531
	Median	12,898.732	9.375	1.298	21,298.290	3,256.232	0.203	0.018	39.681	7.495
	Std. Deviation	2,669.684	4.675	0.654	6,182.955	1,413.737	0.111	0.005	10.455	0.383
	Std. Error of Mean	667.421	1.169	0.164	1,545.739	353.434	0.028	0.001	2.614	0.096
	Minimum	8,158.021	3.766	0.648	13,104.357	979.712	0.127	0.014	23.871	6.630
	Maximum	18,612.468	21.015	2.773	37,236.338	5,787.440	0.467	0.030	60.408	8.100
Ramogi	**N**	8	8	8	8	8	8	8	8	8
	Mean	3,546.217	35.476	3.236	68,858.642	2,210.946	0.550	0.018	87.830	7.814
	Median	3,671.381	21.340	2.813	53,558.972	2,016.352	0.529	0.019	81.582	7.805
	Std. Deviation	1,440.642	25.722	2.019	40,116.229	1,237.976	0.109	0.004	28.454	0.306
	Std. Error of Mean	509.344	9.094	0.714	14,183.229	437.691	0.039	0.001	10.060	0.108
	Minimum	1,204.121	17.324	0.584	37,039.730	1,072.656	0.382	0.008	55.431	7.330
	Maximum	5,354.220	90.307	7.076	149,900.869	4,696.946	0.739	0.021	137.908	8.380
Ukunda	**N**	7	7	7	7	7	7	7	7	7
	Mean	12,849.236	7.551	1.001	7,687.053	715.256	0.155	0.014	92.343	7.937
	Median	14,636.954	7.073	0.923	8,035.247	839.060	0.169	0.013	102.307	7.830
	Std. Deviation	8,436.241	1.344	0.391	1,128.622	226.664	0.041	0.003	23.584	0.383
	Std. Error of Mean	3,188.599	0.508	0.148	426.579	85.671	0.015	0.001	8.914	0.145
	Minimum	2,407.880	6.112	0.373	5,504.539	370.677	0.113	0.009	54.502	7.600
	Maximum	25,606.797	9.219	1.455	9,118.297	887.839	0.220	0.017	115.146	8.780
Ethiopia	**N**	33	33	33	33	33	33	33	33	33
	Mean	21,425.631	25.207	0.898	55,174.580	8,865.141	0.343	0.007	133.963	7.842
	Median	22,288.739	30.729	0.773	51,189.400	6,704.319	0.281	0.006	100.715	7.980

Locality		Element								
		Ca	Cu	I	Fe	Mg	Se	Se-P	Zn	pH
	Std. Deviation	10,859.675	15.567	0.523	26,868.612	5,850.130	0.198	0.005	114.104	0.708
	Std. Error of Mean	1,890.427	2.710	0.091	4,677.225	1,018.377	0.034	0.001	19.863	0.123
	Minimum	3,748.715	1.880	0.196	11,151.671	1,370.094	0.133	0.001	25.041	6.120
	Maximum	39,399.055	52.372	2.332	101,233.506	19,021.016	0.799	0.019	627.064	8.670
Kenya	**N**	62	62	62	62	62	62	62	62	62
	Mean	13,206.180	16.584	1.548	31,523.478	3,531.454	0.316	0.020	61.477	7.883
	Median	12,006.562	11.336	1.188	24,280.453	2,335.435	0.240	0.018	53.540	7.855
	Std. Deviation	12,193.497	14.983	1.185	26,086.050	4,782.849	0.185	0.008	30.686	0.456
	Std. Error of Mean	1,548.576	1.903	0.150	3,312.932	607.422	0.024	0.001	3.897	0.058
	Minimum	651.092	1.529	0.091	4,085.513	214.004	0.096	0.008	12.632	6.630
	Maximum	59,681.313	90.307	7.076	149,900.869	23,780.005	0.843	0.045	141.534	8.780
Total	**N**	95	95	95	95	95	95	95	95	95
	Mean	16,061.358	19.579	1.322	39,739.124	5,384.209	0.325	0.015	86.656	7.869
	Median	13,778.776	13.034	0.974	29,851.116	3,134.211	0.240	0.015	66.631	7.880
	Std. Deviation	12,333.323	15.660	1.049	28,557.161	5,745.736	0.189	0.009	79.039	0.553
	Std. Error of Mean	1,265.372	1.607	0.108	2,929.903	589.500	0.019	0.001	8.109	0.057
	Minimum	651.092	1.529	0.091	4,085.513	214.004	0.096	0.001	12.632	6.120
	Maximum	59,681.313	90.307	7.076	149,900.869	23,780.005	0.843	0.045	627.064	8.780

Appendix 5.23. Spearman's rank correlation (N = 56, d.f. = 54) between the elemental concentration of MO leaves and soil properties.

MO Leaves	**Ca**	1																
	Cu	-0.383	1.000															
	Fe	0.101	-0.024	1.000														
	I	0.011	-0.124	-0.125	1.000													
	Mg	0.581	-0.158	0.121	-0.164	1.000												
	Se	0.183	-0.055	-0.253	-0.427	0.397	1.000											
	Zn	-0.422	0.463	-0.091	-0.195	0.001	0.227	1.000										
Soil	**Ca**	0.213	-0.052	-0.213	-0.080	0.078	0.235	0.103	1.000									
	Cu	-0.183	0.277	0.476	-0.286	0.049	-0.068	0.133	0.139	1.000								
	Fe	-0.077	0.281	0.498	-0.298	0.152	-0.087	0.114	0.047	0.910	1.000							
	I	-0.034	0.136	0.245	-0.137	0.046	-0.160	0.068	0.030	0.600	0.614	1.000						
	Mg	0.094	0.118	0.197	-0.324	0.284	0.232	0.148	0.539	0.702	0.721	0.442	1.000					
	Se	-0.127	0.216	0.464	-0.337	0.079	0.026	0.091	-0.189	0.790	0.814	0.579	0.499	1.000				
	Se-P	0.139	0.107	-0.022	-0.256	0.324	0.437	0.169	-0.026	0.345	0.367	0.301	0.427	0.594	1.000			
	Zn	-0.134	-0.001	0.306	-0.027	-0.171	-0.306	-0.061	0.083	0.457	0.328	0.273	0.045	0.330	-0.061	1.000		
	pH	-0.074	0.177	-0.308	0.039	-0.271	-0.005	-0.006	0.023	-0.255	-0.238	-0.234	-0.337	-0.065	-0.016	0.132	1.000	
		Ca	**Cu**	**Fe**	**I**	**Mg**	**Se**	**Zn**	**Ca**	**Cu**	**Fe**	**I**	**Mg**	**Se**	**Se-P**	**Zn**	**pH**	
		MO Leaves							**Soil**									

Appendix 5.24. The *t* probabilities for the Spearman's rank correlation between the elemental concentration of MO leaves and soil properties. Significant correlations are in bold.

MO Leaves	**Ca**																
	Cu	**0.004**															
	Fe	0.457	0.862														
	I	0.935	0.364	0.358													
	Mg	**0.000**	0.244	0.373	0.228												
	Se	0.178	0.688	0.060	**0.001**	**0.002**											
	Zn	**0.001**	**0.000**	0.506	0.149	0.993	0.092										
Soil	**Ca**	0.116	0.702	0.114	0.557	0.568	0.081	0.452									
	Cu	0.178	**0.039**	**0.000**	**0.033**	0.722	0.616	0.327	0.307								
	Fe	0.572	**0.036**	**0.000**	**0.026**	0.264	0.526	0.402	0.732	**0.000**							
	I	0.802	0.318	0.069	0.316	0.735	0.240	0.618	0.824	**0.000**	**0.000**						
	Mg	0.490	0.387	0.146	0.015	0.034	0.085	0.278	0.000	**0.000**	**0.000**	**0.001**					
	Se	0.351	0.110	0.000	0.011	0.562	0.847	0.503	0.162	**0.000**	**0.000**	**0.000**	**0.000**				
	Se-P	0.306	0.434	0.872	0.057	**0.015**	**0.001**	0.212	0.850	**0.009**	**0.005**	**0.024**	**0.001**	0.000			
	Zn	0.324	0.993	**0.022**	0.846	0.206	**0.022**	0.654	0.541	**0.000**	**0.014**	**0.042**	0.742	**0.013**	0.657		
	pH	0.589	0.191	0.021	0.774	0.044	0.973	0.964	0.869	0.057	0.078	0.083	**0.011**	0.633	0.906	0.330	
		Ca	**Cu**	**Fe**	**I**	**Mg**	**Se**	**Zn**	**Ca**	**Cu**	**Fe**	**I**	**Mg**	**Se**	**Se-P**	**Zn**	**pH**
		MO Leaves							**Soil**								

Appendix 5.25. Spearman's rank correlation (N = 32, d.f. = 30) between the elemental concentration of MS leaves and soil properties.

MS Leaves	**Ca**	**1**															
	Cu	0.240	1.000														
	Fe	0.153	-0.080	1.000													
	I	-0.117	-0.351	0.367	1.000												
	Mg	0.788	0.057	0.069	-0.073	1.000											
	Se	0.048	-0.221	0.340	0.235	0.025	1.000										
	Zn	-0.259	0.289	-0.128	-0.164	-0.458	0.219	1.000									
Soil	**Ca**	0.195	0.023	0.342	-0.123	0.267	-0.009	-0.051	1.000								
	Cu	0.142	0.280	0.212	-0.132	0.112	-0.304	-0.120	0.717	1.000							
	Fe	0.347	0.151	0.288	0.058	0.224	-0.100	-0.142	0.677	0.730	1.000						
	I	0.127	0.176	0.079	0.014	-0.165	0.135	0.243	-0.113	-0.001	0.187	1.000					
	Mg	0.348	0.133	0.410	-0.005	0.327	-0.062	-0.154	0.856	0.679	0.840	0.067	1.000				
	Se	-0.173	-0.129	0.441	0.240	-0.484	0.422	0.245	0.005	0.003	0.126	0.620	0.085	1.000			
	Se-P	-0.194	-0.239	0.444	0.283	-0.218	0.609	-0.009	-0.135	-0.214	-0.156	0.486	-0.103	0.712	1.000		
	Zn	-0.306	0.025	0.251	0.176	-0.687	0.147	0.442	0.046	0.093	0.200	0.443	0.087	0.803	0.274	1.000	
	pH	0.214	0.270	0.083	-0.333	0.297	-0.019	0.231	0.637	0.494	0.410	-0.039	0.500	-0.139	-0.204	-0.135	1.000
		Ca	**Cu**	**Fe**	**I**	**Mg**	**Se**	**Zn**	**Ca**	**Cu**	**Fe**	**I**	**Mg**	**Se**	**Se-P**	**Zn**	**pH**
		MS Leaves							**Soil**								

Appendix 5.26. The *t* probabilities for the Spearman's rank correlation between the elemental concentration of MS leaves and soil properties. Significant correlations are in bold.

MS Leaves	**Ca**																
	Cu	0.186															
	Fe	0.402	0.665														
	I	0.523	0.049	**0.039**													
	Mg	**0.000**	0.757	0.709	0.690												
	Se	0.793	0.223	0.057	0.196	0.894											
	Zn	0.152	0.108	0.484	0.370	0.008	0.229										
Soil	**Ca**	0.284	0.899	0.055	0.503	0.139	0.962	0.782									
	Cu	0.439	0.120	0.244	0.472	0.542	0.090	0.512	**0.000**								
	Fe	0.052	0.409	0.110	0.754	0.219	0.584	0.439	**0.000**	**0.000**							
	I	0.488	0.336	0.668	0.938	0.368	0.462	0.181	0.538	0.995	0.307						
	Mg	0.051	0.469	**0.020**	0.979	0.068	0.738	0.400	**0.000**	**0.000**	**0.000**	0.717					
	Se	0.343	0.482	**0.012**	0.185	0.005	0.016	0.177	0.976	0.986	0.490	**0.000**	0.644				
	Se-P	0.288	0.188	**0.011**	0.117	0.230	**0.000**	0.962	0.460	0.239	0.393	**0.005**	0.576	**0.000**			
	Zn	0.088	0.892	0.166	0.336	**0.000**	0.423	**0.011**	0.802	0.612	0.273	**0.011**	0.635	**0.000**	0.129		
	pH	0.239	0.134	0.653	0.063	0.099	0.920	0.203	**0.000**	**0.004**	**0.020**	0.832	**0.004**	0.448	0.264	0.462	
		Ca	**Cu**	**Fe**	**I**	**Mg**	**Se**	**Zn**	**Ca**	**Cu**	**Fe**	**I**	**Mg**	**Se**	**Se-P**	**Zn**	**pH**
		MS leaves							**Soil**								

Appendix 5.27. Welch's robust test of equality of mean elemental concentrations in MO leaves across localities in Kenya, d.f. 1 (degrees of freedom of the numerator), d.f. 2 (degrees of freedom of the denominator), and the *p* value.

Element	**Welch statistic**	**d.f. 1**	**d.f. 2**	***p***
Ca	2.362	4	21	0.087
Cu	9.203	4	22	0.000
I	3.682	4	17	0.024
Fe	3.107	4	20	0.039
Mg	4.438	4	21	0.009
Zn	3.561	4	22	0.022
Se	8.246	4	22	0.000

Appendix 5.28. Welch's robust test of equality of mean elemental concentrations in MS leaves across localities. Refer to Appendix 5.27 for abbreviations.

Element	**Welch statistic**	**d.f. 1**	**d.f. 2**	***p***
Ca	0.216	3	12	0.883
Cu	5.538	3	15	0.009
I	18.296	3	13	0.000
Fe	1.883	3	14	0.180
Mg	21.481	3	12	0.000
Se	1.315	3	11	0.319
Zn	8.266	3	14	0.002

Appendix 5.29. Welch's robust test of equality of mean elemental concentrations in MO and MS leaves. Refer to Appendix 5.27 for abbreviations.

Element	**Welch statistic**	**d.f. 1**	**d.f. 2**	***p***
Ca	2.607	1	85	0.110
Cu	43.118	1	92	0.000
I	26.356	1	86	0.000
Fe	1.416	1	82	0.238
Mg	5.677	1	61	0.020
Se	15.975	1	77	0.000
Zn	42.651	1	95	0.000

Appendix 5.30. Welch's robust test of equality of mean elemental concentrations in MO immature pods across localities. Refer to Appendix 5.27 for abbreviations.

Element	**Welch statistic**	**d.f. 1**	**d.f. 2**	***p***
Ca	3.91	4	5	0.082
Cu	10.37	4	5	0.013
Fe	7.91	4	6	0.016
Mg	9.81	4	6	0.008
Se	4.77	4	4	0.077
Zn	2.82	4	4	0.160

Appendix 5.31. Welch's robust test of equality of mean elemental concentrations in MO seeds across localities. Refer to Appendix 5.27 for abbreviations.

Element	**Welch statistic**	**d.f. 1**	**d.f. 2**	***P***
Ca	4.441	2	6	0.061
Cu	2.767	2	8	0.119
I	1.448	2	9	0.288
Fe	1.426	2	5	0.321
Mg	0.256	2	5	0.785
Se	3.933	2	8	0.066
Zn	0.093	2	5	0.913

Appendix 5.32. Welch's robust test of equality of mean elemental concentrations in MO flowers across localities. Refer to Appendix 5.27 for abbreviations.

Element	**Welch statistic**	**d.f. 1**	**d.f. 2**	***p***
Ca	17.47	3	13	0.000
Cu	14.88	3	12	0.000
Fe	5.3	3	7	0.031
Mg	5.96	3	9	0.016
Se	4.41	3	7	0.047
Zn	0.63	3	8	0.615

Appendix 5.33. Welch's robust tests of equality of mean soil elemental concentrations across localities. Refer to Appendix 5.27 for abbreviations.

Element	**Welch's statistic**	**d.f.1**	**d.f.2**	***p***
Ca	24.956	8	29	0.000
Cu	33.272	8	30	0.000
Fe	51.82	8	29	0.000
Mg	21.974	8	29	0.000
Se	18.016	8	29	0.000
Zn	16.211	8	28	0.000
pH	10.983	8	30	0.000
I	3.126	8	29	0.011
Se_p	28.387	8	29	0.000

Appendix 5.34. Correlation between elemental concentrations of MO edible parts (flower, immature pod, leaf and seed). The figures below the yellow diagonal are correlation coefficients and those above the diagonal are p values. ** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed). N = 18

		Flower						Seed						Leaf						Immature pods					
		Ca	Cu	Fe	Mg	Se	Zn	Ca	Cu	Fe	Mg	Se	Zn	Ca	Cu	Fe	Mg	Se	Zn	Ca	Cu	Fe	Mg	Se	Zn
Flower	Ca																								
	Cu	0.096																							
	Fe	0.259	0.608**																						
	Mg	0.818**	0.261	0.36																					
	Se	0.424	-0.284	-0.238	0.42																				
	Zn	0.079	0.647**	0.284	0.284	-0.063																			
Seed	Ca	-0.164	-0.525*	-0.315	-0.015	0.098	-0.447																		
	Cu	0.317	0.278	0.22	0.585*	0.189	0.127	-0.342																	
	Fe	-0.141	0.732**	0.364	0.007	-0.323	0.391	-0.42	0.292																
	Mg	0.069	0.401	-0.022	0.104	-0.063	0.480*	-0.389	0.102	0.075															
	Se	0.465	-0.292	-0.203	0.560*	0.785**	-0.032	0.259	0.22	-0.397	-0.053														
	Zn	0.018	0.785**	0.482*	0.331	-0.065	0.385	-0.154	0.346	0.719**	0.075	-0.055													
Leaf	Ca	0.35	-0.484*	-0.571*	0.288	0.226	-0.249	0.459	0.034	-0.428	0.015	0.434	-0.416												
	Cu	0.102	0.713**	0.505*	0.16	-0.309	0.251	-0.288	0.135	0.393	0.214	-0.317	0.467	-0.42											
	Fe	-0.03	-0.073	0.164	-0.315	-0.428	-0.102	0.129	-0.697**	-0.354	0.23	-0.278	-0.34	-0.005	0.11										
	Mg	0.408	-0.304	-0.15	0.562*	0.375	-0.084	0.515*	0.094	-0.482*	-0.042	0.579*	-0.079	0.583*	-0.16	0.02									
	Se	0.408	-0.296	-0.17	0.422	0.926**	-0.036	0.034	0.337	-0.187	-0.232	0.730**	-0.069	0.201	-0.412	-0.579*	0.29								
	Zn	0.028	0.472*	0.583*	0.212	0.096	0.608**	-0.234	0.003	0.344	-0.168	0.129	0.406	-0.484*	0.321	-0.119	-0.096	0.176							
Immature pods	Ca	0.746**	0.131	0.143	0.542*	0.348	-0.032	-0.247	0.255	0.106	0.135	0.278	0.015	0.356	-0.038	-0.046	0.28	0.366	-0.119						
	Cu	-0.051	0.639**	0.412	-0.061	-0.307	0.129	-0.106	-0.189	0.42	0.187	-0.408	0.428	-0.416	0.777**	0.278	-0.253	-0.424	0.187	0.104					
	Fe	-0.003	0.255	0.43	-0.137	-0.224	-0.09	0.053	-0.404	0.226	0.156	-0.414	0.104	-0.315	0.379	0.531*	-0.108	-0.333	0.053	0.234	.628**				
	Mg	0.593**	0.179	0.148	0.523*	0.104	-0.139	-0.034	0.236	0.156	0.162	0.131	0.115	0.37	0.205	0.02	0.422	0.053	-0.232	0.856**	0.3	0.401			
	Se	0.527*	-0.247	-0.195	0.523*	0.938**	-0.036	0.112	0.267	-0.191	-0.137	0.769**	0.015	0.243	-0.358	-0.498*	0.366	0.930**	0.059	0.434	-0.342	-0.22	0.185		
	Zn	-0.24	0.507*	0.187	-0.251	-0.527*	0.218	-0.16	-0.218	0.558*	0.335	-0.678**	0.253	-0.354	0.519*	0.228	-0.414	-0.583*	-0.051	0.02	.767**	.655**	0.255	-0.463	

Appendix 5.35. Correlation between the elemental composition of MO and amaranth leaves. * Correlation is significant at the 0.05 level (2-tailed). N = 6.

	Ca_MO	Cu_MO	Fe_MO	Mg_MO	Se_MO	Zn_MO	Ca_AM	Cu_AM	Fe-AM	Mg_AM	Se_AM	Zn_AM
Ca_MO												
Cu_MO	-0.257											
Fe_MO	-0.314	0.6										
Mg_MO	0.029	-0.086	0.543									
Se_MO	0.486	0.143	-0.371	-0.2								
Zn_MO	0.086	-0.029	-0.086	-0.657	0.029							
Ca_AM	-0.657	-0.086	-0.143	-0.371	-0.714	0.029						
Cu_AM	0.086	-0.029	0.371	-0.086	-0.657	0.543	0.257					
Fe-AM	-0.657	0.429	0.829*	0.6	-0.6	-0.429	0.257	0.143				
Mg_AM	-0.371	0.771	0.371	0.143	0.029	-0.6	0.143	-0.371	0.543			
Se_AM	-0.086	-0.771	-0.829*	-0.371	0.086	0.143	0.314	-0.314	-0.543	-0.543		
Zn_AM	0.029	-0.029	0.714	0.543	-0.371	0.257	-0.314	0.6	0.429	-0.371	-0.429	

Appendix 5.36. Correlation between the elemental composition of MO and brassica (BO) leaves. ** Correlation is significant at the 0.05 level (2-tailed). N = 4.

	Ca_MO	Cu_MO	Fe_MO	Mg_MO	Se_MO	Zn_MO	Ca_BO	Cu_BO	Fe_BO	MG_BO	Se_BO	Zn_BO
Ca_MO												
Cu_MO	-0.4											
Fe_MO	0.6	-0.4										
Mg_MO	0.8	-0.8	0.8									
Se_MO	0.2	0.8	-0.2	-0.4								
Zn_MO	0.2	0.8	-0.2	-0.4	1.000**							
Ca_BO	-1.000**	0.4	-0.6	-0.8	-0.2	-0.2						
Cu_BO	-1.000**	0.4	-0.6	-0.8	-0.2	-0.2	1.000**					
Fe_BO	-0.8	0	-0.8	-0.6	-0.4	-0.4	0.8	0.8				
MG_BO	-1.000**	0.4	-0.6	-0.8	-0.2	-0.2	1.000**	1.000**	0.8			
Se_BO	0.4	0.6	0.4	0	0.8	0.8	-0.4	-0.4	-0.8	-0.4		
Zn_BO	1.000**	-0.4	0.6	0.8	0.2	0.2	-1.000**	-1.000**	-0.8	-1.000**	0.4	

Appendix 5.37. Correlation between the elemental composition of amaranth (AM) and brassica (BO) leaves. ** Correlation is significant at the 0.05 level (2-tailed). N = 3.

	Ca_AM	Cu_AM	Fe-AM	Mg_AM	Se_AM	Zn_AM	Ca_BO	Cu_BO	Fe_BO	MG_BO	Se_BO	Zn_BO
Ca_AM	1											
Cu_AM	-0.5	1										
Fe-AM	0.5	0.5	1									
Mg_AM	0.5	-1.000**	-0.5	1								
Se_AM	1.000**	-0.5	0.5	0.5	1							
Zn_AM	-0.5	1.000**	0.5	-1.000**	-0.5	1						
Ca_BO	1.000**	-0.5	0.5	0.5	1.000**	-0.5	1					
Cu_BO	1.000**	-0.5	0.5	0.5	1.000**	-0.5	1.000**	1				
Fe_BO	1.000**	-0.5	0.5	0.5	1.000**	-0.5	1.000**	1.000**	1			
MG_BO	1.000**	-0.5	0.5	0.5	1.000**	-0.5	1.000**	1.000**	1.000**	1		
Se_BO	-1.000**	0.5	-0.5	-0.5	-1.000**	0.5	-1.000**	-1.000**	-1.000**	-1.000**	1	
Zn_BO	-1.000**	0.5	-0.5	-0.5	-1.000**	0.5	-1.000**	-1.000**	-1.000**	-1.000**	1.000**	1

Appendix 5.38. Proportion of *Moringa* leaf Fe (P_{Fe}) from soil dust contamination.

Household_ID	Locality	Country	P_{Fe}
1	Kibwezi	Kenya	0.809305853
2	Kibwezi	Kenya	0.859005451
3	Kibwezi	Kenya	0.880694033
5	Kibwezi	Kenya	0.686836882
6	Kibwezi	Kenya	0.790896981
7	Kibwezi	Kenya	0.914348163
8	Kibwezi	Kenya	0.728524428
9	Kibwezi	Kenya	0.978012654
10	Kibwezi	Kenya	0.770764476
11	Kibwezi	Kenya	0.613141009
12	Kibwezi	Kenya	0.736813005
13	Kibwezi	Kenya	0.895118086
14	Kibwezi	Kenya	0.840541219
15	Mbololo	Kenya	0.407015466
16	Mbololo	Kenya	0.728357998
17	Mbololo	Kenya	0.683977188
18	Mbololo	Kenya	0.91696714
19	Mbololo	Kenya	0.996428126
20	Mbololo	Kenya	0.778215466
22	Mbololo	Kenya	0.983934603
23	Mbololo	Kenya	0.961204111
24	Mbololo	Kenya	0.978656011
25	Mbololo	Kenya	0.624392268
26	Mbololo	Kenya	0.637432072
27	Mbololo	Kenya	0.85143411
28	Mbololo	Kenya	0.943004811
29	Mbololo	Kenya	0.760552745
30	Mbololo	Kenya	0.701161466
33	Baringo	Kenya	0.365518828
34	Baringo	Kenya	0.560120274
35	Baringo	Kenya	0.543983319
36	Baringo	Kenya	0.72056563
37	Ramogi	Kenya	0.629400275
37	Ramogi	Kenya	0.625361807
38	Ramogi	Kenya	0.706371613
39	Ramogi	Kenya	0.615997707
40	Ramogi	Kenya	0.633150905
41	Ramogi	Kenya	0.779096764
42	Ramogi	Kenya	0.756923493
43	Ramogi	Kenya	0.651634992
44	Ramogi	Kenya	0.461083773
45	Malindi	Kenya	0.287802589

46	Malindi	Kenya	0.181402798
47	Malindi	Kenya	0.338070557
48	Malindi	Kenya	0.161195396
49	Malindi	Kenya	0.499887482
50	Malindi	Kenya	0.198489403
51	Malindi	Kenya	0.122729593
52	Malindi	Kenya	0.083928768
53	Malindi	Kenya	0.07489876
54	Malindi	Kenya	0.133678784
55	Malindi	Kenya	0.104314445
56	Ukunda	Kenya	0.263802257
57	Ukunda	Kenya	0.130284408
58	Ukunda	Kenya	0.615410268
59	Ukunda	Kenya	0.288910726
60	Ukunda	Kenya	0.188914971
61	Ukunda	Kenya	0.665464213
62	Ukunda	Kenya	0.640253663
ETH001	Derashe	Ethiopia	0.514756847
ETH002	Derashe	Ethiopia	0.423974733
ETH003	Derashe	Ethiopia	0.491634939
ETH004	Derashe	Ethiopia	0.550493328
ETH005	Derashe	Ethiopia	0.317038756
ETH006	Derashe	Ethiopia	0.416570011
ETH007	Derashe	Ethiopia	0.444265184
ETH008	Derashe	Ethiopia	0.289068157
ETH009	Derashe	Ethiopia	0.580631549
ETH010	Derashe	Ethiopia	0.515820976
ETH011	Derashe	Ethiopia	0.38483893
ETH012	Derashe	Ethiopia	0.374644317
ETH013	Konso	Ethiopia	0.181160699
ETH014	Konso	Ethiopia	0.303575394
ETH015	Konso	Ethiopia	0.289842849
ETH016	Konso	Ethiopia	0.263198556
ETH017	Konso	Ethiopia	0.567542744
ETH018	Konso	Ethiopia	0.553921233
ETH019	Konso	Ethiopia	0.59314284
ETH020	Konso	Ethiopia	0.608341499
ETH021	Konso	Ethiopia	0.431007573
ETH022	Konso	Ethiopia	0.868115319
ETH023	Konso	Ethiopia	0.386893576
ETH024	Konso	Ethiopia	0.664501033
Eth-Haw-1	Hawasa	Ethiopia	0.703761092
Eth-Haw-3	Hawasa	Ethiopia	0.553031446
Eth-Haw-5	Hawasa	Ethiopia	0.653323945

Eth-Haw-6	Hawasa	Ethiopia	0.834365082
Eth-Haw-7	Hawasa	Ethiopia	0.392734789
Eth-Haw-9	Hawasa	Ethiopia	0.650769482

CHAPTER 6. CHALLENGES AND OPPORTUNITIES FOR MORINGA GROWERS IN SOUTHERN ETHIOPIA AND KENYA

6.1 Abstract

Moringa oleifera (MO) and *M. stenopetala* (MS) are two commonly cultivated species of the Moringaceae family. Some households in southern Ethiopia (S. ETH) and Kenya (KEN) plant MS and MO, respectively. The edible parts of these species are rich in amino acids, vitamins and minerals, especially selenium. Despite their nutritional value, *Moringa* is sometimes considered as a "famine food". The aim of this study was to determine the extent of dietary utilization of these plants by *Moringa* Growing Households (MGHs). *Moringa* growing households were surveyed in 2015. Twenty-four and 56 heads of MGHs from S. ETH and KEN, respectively, were interviewed using semi-structured questionnaires. Subsistence agriculture was the main source of livelihood for all MGHs in S. ETH and 71% of those in KEN. All MGHs in S. ETH cultivated MS while those in KEN cultivated MO. Of the MGH heads in S. ETH, 71% had grown MS as long as they remember; the median cultivation period of MO in KEN was 15 years. All MGHs in S. ETH and 79% in KEN used *Moringa* leaves as a source of food. Forms of consumption of leaves were boiled fresh leaves, and leaf powder used in tea or mixed with other dishes. Other uses of *Moringa* include as medicine, fodder, shade, agroforestry, and as a source of income. Although MO and MS have multiple uses, MGHs face several challenges, including a lack of reliable information on nutritional and medicinal values, inadequate access to markets for their products, and pest and disease stresses to their plants. Research and development to address these

challenges and to promote the use of these species in the fight against *hidden hunger* are necessary.

6.2 Introduction

Consumption of diverse diets, with balanced supplies of macro and micro-nutrients is required for normal human growth and physiological development. However, availability of optimally diverse diets may be constrained by wealth and/or education (including loss of traditional knowledge of indigenous crops). Human diets have been inadvertently simplified in food systems during the Green-Revolution era (Welch *et al.* 1999, Miller *et al.* 2013), where agricultural production focused on provision of sufficient energy. In populations depending on cereal-based diets with low nutrient density, dietary simplification and shortage of access to animal source food exacerbates deficiency of vitamins and minerals, also known as *hidden hunger* (Frison *et al.* 2011, Pingali 2012, Sharma *et al.* 2017). *Moringa oleifera* (MO) *and M. stenopetala* (MS) are underutilized tropical tree species that can play an important role in dietary diversification and contribute to alleviation of *hidden hunger* in less developed tropical and subtropical countries (Lyons *et al.* 2015, Stevens *et al.* 2015, Olson *et al.* 2016, Kumssa *et al.* 2017). In particular, *Moringa* can be a rich source of some micronutrients that are commonly deficient in cereal-based diets, e.g. selenium (Kumssa *et al.* 2017).

Moringa oleifera and MS are the two widely cultivated species of the Moringaceae family, which comprises 13 species. Previous ethnobotanical and biochemical studies in countries where *Moringa* is grown show that these species are multipurpose (Jahn 1991b, Anwar *et al.* 2007, Padayachee and Baijnath 2012, Gopalakrishnan *et al.* 2016). Various tissues are used as food, herbal medicine,

fodder, hedges, firewood, gum and for water purification (Morton 1991, Balemie *et al.* 2006, Virchow 2008, Monera and Maponga 2010, Teklehaymanot *et al.* 2010, Mengistu *et al.* 2012, Ocho *et al.* 2012, Degefu *et al.* 2013, Popoola *et al.* 2013). The foliage, immature pods, seeds, roots and young shoots are used as food and herbal medicine (Lim 2012, Popoola *et al.* 2013). *Moringa stenopetala* leaves are used in a similar way as cabbage and spinach and the tree is nicknamed the 'cabbage tree' (Tenaye *et al.* 2009). Fresh MO and MS leaves are either boiled or consumed raw as vegetables, and leaf powders are mixed with other staple foods to increase the mineral, amino acid and vitamin density in the diets (Anwar *et al.* 2007, Tenaye *et al.* 2009, Teklehaymanot *et al.* 2010, Moyo *et al.* 2011, Lim 2012, Melesse *et al.* 2012, Popoola *et al.* 2013, Stevens *et al.* 2015, Olson *et al.* 2016, Kumssa *et al.* 2017).

Despite their nutritious edible parts, *Moringa* spp. are sometimes classified as "famine food", consumed by humans at times of food scarcity (Sena *et al.* 1998, Lockett *et al.* 2000, Tenaye *et al.* 2009). Similarly, preliminary information indicates that the human dietary usage of the edible parts of these species is limited. For example, in S. ETH, *Moringa* tends to be cultivated by communities living in marginal environments, with small land holdings due to high population density (Kumssa *et al.* 2017). In these areas, there is often a reliance on rain-fed agriculture as a source of livelihood and there are frequent food crop failures due to drought (Jahn 1991b, Jiru *et al.* 2006). The use of indigenous and locally available vegetables such as *Moringa* as a human food is often linked with low social class status in many communities in Africa and Asia (Jahn 1991b, Virchow 2008, Ebert 2014, Cernansky 2015).

Few studies have assessed the ethnobotany of MO and MS, and these have focused primarily on their medicinal uses (Mutheeswaran *et al.* 2011, Ocho *et al.* 2012, Semenya *et al.* 2012, Popoola *et al.* 2013, Sivasankari *et al.* 2014, Silambarasan and Ayyanar 2015, Stevens *et al.* 2015, Kamau *et al.* 2016, Lunyera *et al.* 2016). There is a lack of information on the ethnobotany of MO and MS with emphasis on its use as human food source in S. ETH and KEN. The aim of this study was to assess the current extent of dietary utilization of *Moringa* edible parts by MGHs in S. ETH and KEN. This will help to identify where challenges and opportunities exist to widen the use of *Moringa* and reduce human mineral micronutrient deficiencies.

6.3 Materials and Methods

A questionnaire-based survey to assess the uses of MO and MS was conducted in S. ETH in April 2015 and various localities of KEN in July 2015. Prior to conducting the survey, research ethics approval was obtained from the University of Nottingham, School of Biosciences Research Ethics Committee (SB REC), approval number: SBREC140117A. A purposive sampling approach was pursued by identifying households that cultivated MO and MS, with emphasis on their utilization as a dietary source for human beings. Staff of the Kenyan Forestry Research Institute in KEN and an agricultural expert working for a local Non-Governmental Organization in S. ETH assisted to identify and select MGHs and to translate the questionnaire to local languages during the interviews.

A semi-structured interview was conducted with the MGH heads. For each participant, an information sheet (Appendix 6.1) explaining the purposes of the survey, with details of the conditions of the interview and the rights of the interviewee were provided prior to the commencement of the interview.

Respondents provided their consent (Appendix 6.2) either by signature or thumb impression print and the questionnaire (Appendix 6.3) was administered after obtaining the MGH head's consent. The study was carried out on private/communal land with the owners' permission, and it did not involve endangered or protected species. Data collection was carried out using an online data collection system in KoboToolBox (http://www.kobotoolbox.org) using handheld mobile devices. When mobile data connection was unavailable in the field, the KoboToolBox saved the data temporarily on the device and uploaded it to a cloud server once connection to the Internet was re-established. A total of 24 and 56 MGH heads were interviewed in S. ETH and KEN, respectively. Subsequent statistical analysis and visualization was carried out using KoboToolBox and Tableau Desktop Professional Edition 10.

6.4 Results

Summaries of the responses of MGH heads from S. ETH and KEN with regards to general household characteristics, cultivation of *Moringa* and challenges faced, and the dietary and other modes of utilization of *Moringa* are presented as follows.

6.4.1 Southern Ethiopia

6.4.1.1 Household characteristics

The MGHs in S. ETH were from the Derashe and Konso ethnic groups (Fig 6.1). All the households (n = 24) grew MS and 75% of household heads were men, all of whom were married (

Table 6.1). The median age of the MGH heads was 40 yrs and median number of fulltime residents of MGHs was 6 persons (Table 6.2). Fifty-eight percent of the MGH heads were illiterate (

Table 6.3). The roof of the residential houses of 79% and 21% of the households were made from thatched grass and corrugated iron sheets, respectively. The floors

of the residential houses of 96% and 4% of the households were earthen and cemented, respectively. None of the MGHs had electricity power supply or tap water at their residential houses. Potable water was obtained from boreholes (67%) and springs (33%). All MGHs relied on subsistence agriculture as sources of livelihood.

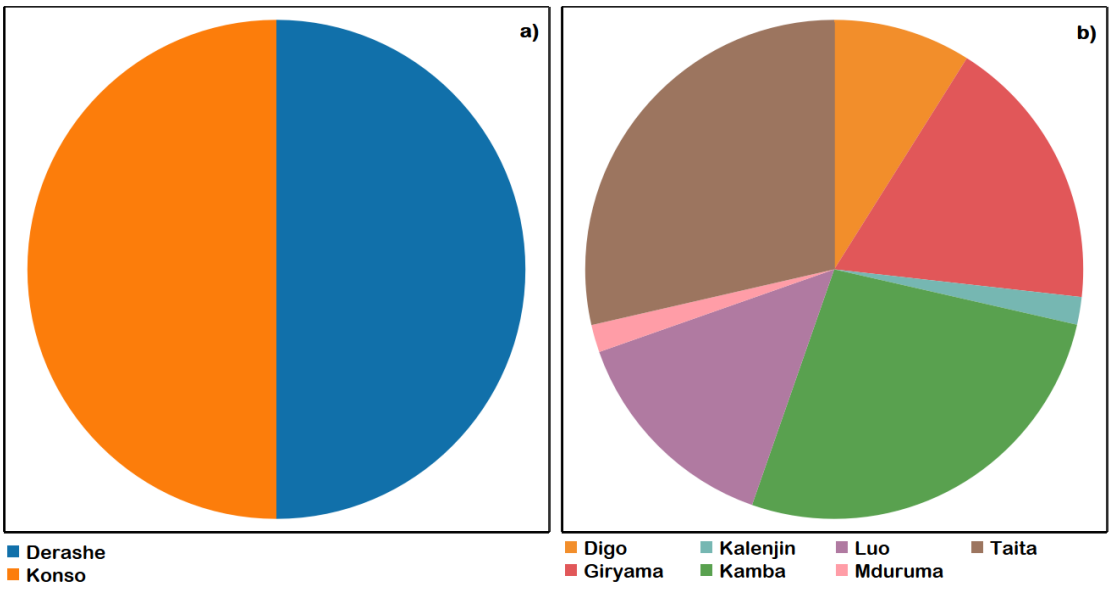


Fig 6.1. Ethnic groups to which the *Moringa* growing households belonged. Southern Ethiopia (a) and Kenya (b). Total number of respondents in southern Ethiopia (n = 24) and Kenya (n = 56).

Table 6.1. Marital status of *Moringa* growing household heads from southern Ethiopia (n = 24) and Kenya (n = 56) as a percentage of total frequency.

Marital status	**S. ETH**	**KEN**
Married	75%	96%
Single	8%	4%
Widowed	17%	

Table 6.2. Descriptive statistics of the age of *Moringa* growing household heads, the number of fulltime residents in the *Moringa* growing household and the number of years for which *Moringa* was cultivated in southern Ethiopia and Kenya.

Statistic	**Age (yrs)**		**Fulltime residents**		**Number of yrs**
	S. ETH	KEN	S. ETH	KEN	KEN
Mean	41	57	6	6	17
Median	40	59	6	6	15
Mode	45	65	5	6	10
Standard deviation	8	13	3	2	12

Table 6.3. Educational level of *Moringa* growing household heads in southern Ethiopia and Kenya as a percentage of total frequency.

Education level	**S. ETH**	**KEN**
Illiterate	58%	20%
Elementary	25%	48%
High school	17%	20%
College		13%

6.4.1.2 Purposes of growing MS

Seventy-one percent of the MGHs had been growing MS as long as they remember. The remaining 29% of the households had grown MS for 2–17 yrs. All MGHs had used MS as a source of food (Fig 6.2), as a source of food and income (42%), as a source of food, income and drink (29%), and as source of food, drink and medicine (21%). All MGHs consumed boiled fresh leaves three times a day (92%) and most days in a week (8%). The quantity of leaves consumed per day were two big and medium bunches (4% each), one big bunch (42%), one medium bunch (29%) and one small bunch (21%). Other forms of consumption of MS included boiled flowers and immature pods, dried and crushed leaves mixed with traditional beverage made from sorghum (*chegga*). While all the households from the Konso ethnic group consumed boiled fresh leaves of MS, those from the Derashe ethnic group consumed boiled fresh leaves, flowers and young pods, and dried and crushed leaf

powder to make tea or mix it with *chegga*. Fifty percent of the MGHs from the Derashe ethnic group reported to have used MS as medicine in the following forms: fresh roots of the tree were crushed and smelled to treat common cold; branches were broken to initiate sap outflow which was used as eye drops to treat eye infections; and fresh leaf juice had been used to treat head lice. Only one MGH head from the Konso ethnic group stated the use of MS as medicine, where the juices from fresh leaves were used to treat gastrointestinal parasites in cattle.

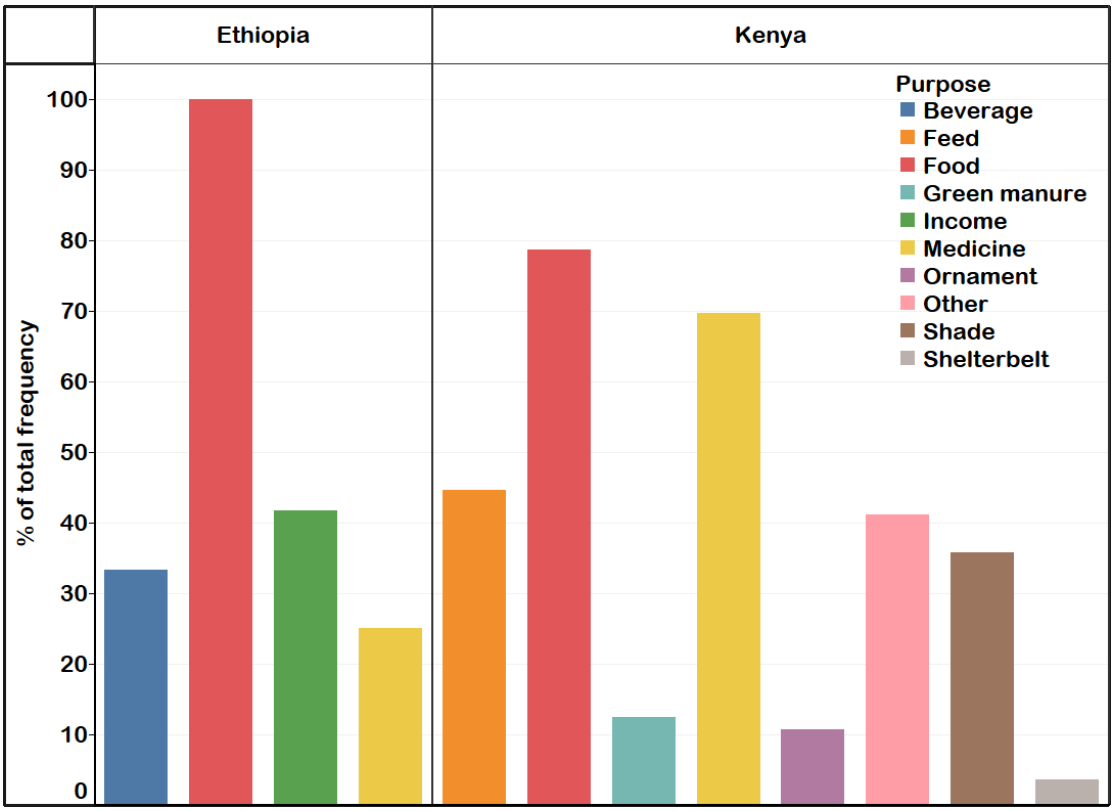


Fig 6.2. Purposes for which *Moringa* was grown in southern Ethiopia and Kenya. Number of respondents: southern Ethiopia (n = 24) and Kenya (n = 56).

6.4.2 Kenya

6.4.2.1 Household characteristics

All the households in KEN planted MO. The respondents were members of seven ethnic groups (Fig 6.1). Seventy percent and 30% of the MGH heads were men and women, respectively. Ninety-six percent of the MGH heads were married (

Table 6.1). Median age of the MGH head was 59 yrs and the median number of fulltime residents in a MGH was 6 (Table 6.2). In terms of the MGH head educational level, 48% had attended elementary school (

Table 6.3). The land tenure was private (84%), communal (13%) and other (3%). Land holdings were 0.4 – 1.2 ha (68%), 1.3 – 4 ha (30%), and 4.1 – 6 ha (2%). Seventy-one percent of the households depend on subsistence agriculture as the source of their livelihood. Potable water sources of the MGHs were tap water (45%), river (32%), borehole (9%) and lake (7%). Roofs of the residential houses were made from corrugated iron sheets (86%), grass thatch (3%) and other (11%). Floors of the MGH residential houses were earthen (55%), cemented (43%) and tiled (2%). Only 16% of the MGHs had access to electricity power supply at their residential houses.

6.4.2.2 Purposes of growing MO

The period for which the MGH heads had been cultivating MO in various parts of KEN ranged between 1–59 yrs (Table 6.2). Planting of MO was conducted by direct seeding (84%), cuttings (11%) and seedlings (5%). Cuttings and seeds were obtained from neighbours (73%) who already had established MO trees while seedlings were purchased from nearby Department of Agriculture nurseries and Kenya Forestry Research Institute research stations. Nine percent of the respondents reported that a private company which promised to buy MO leaves,

immature pods and seeds from farmers had distributed seedlings to some MGHs but did not fulfil the promise.

The three main purposes for which the MGHs plant MO were food, medicine, and feed (Fig 6.3). Those MGHs that did not cultivate MO as a food source planted it for feed, medicine, shade, agroforestry, shelterbelt or other purposes. Many of the MGHs cultivated MO for multiple uses. Thirty-two percent of the MGHs cultivated MO for food, medicine, and feed; while 20% of them had been cultivating MO for food, feed, medicine and shade. The MO plant parts used for food, feed or medicine is indicated in Fig 6.3.

Among the respondents reporting that MO edible parts were used as food, 57% used the fresh leaves as a vegetable, and the remaining 22% used leaves as tea and leaf powders mixed with other foods, and young shoots and fresh flowers as vegetables. Some respondents reported that fried MO flowers tasted like eggs when fried with oil. Reported medicinal uses included: MO bark and roots boiled in water and the solution used to wash body and legs of diabetic patients to treat numbness and tingling sensations; leaves mixed with other foods or used as tea to treat high blood pressure, joint and general body pain, ulcers, food poisoning, and stomach problems. Some interviewees stated that the leaves, immature pods and seeds of MO were sold either for export or local markets and used as sources of income. Leaves, immature and mature pods were used as a source of feed mainly for goats.

6.4.2.3 Challenges in cultivation and use of MO

Eighty-four percent of the MGHs stated that they had encountered some challenges during the cultivation of MO. These include: pest and disease (82%); rotting of trees grown on lands vulnerable to flooding; parasitic plants (Fig 6.4); low demand for

MO products (7%); unknown dosage of MO edible parts used as medicine; uncertainty about the nutritional and medicinal values of MO.

Pest attacks have been reported during dry spells (50%), at the onset of the rainy season (32%) and all year round (2%). Insect larva mostly fed on the leaves (79%) and sometimes bored into the pods (18%). Larva foraging on the leaves reduced leaf biomass production and damaged leaves are unappealing for use as human food. Seeds in the pods bored by larva were damaged and became unviable for seeding and seedling production, and for other uses. Parasitic plants growing on MO were also observed in the field and reported as a problem by some MGH heads. The MGH heads were keen for advice on ways to get rid of diseases and pests that hinder the productivity and usability of the trees they cultivate. In areas that experience flooding in the rainy season, MO trees rot and die due to waterlogging, showing that MO prefers well drained soils (Lim 2012).

Another challenge facing MGHs was the unclear and unreliable evidence regarding the medicinal and nutritional values of MO. They seek reliable evidence in regards to the uses of MO. Besides, for better access to secure markets, MGHs want support from government development and extension agents in providing education on the uses of MO to people who do not currently grow it. In KEN, MGH heads were asked whether they would like to obtain further information about MO. Eighty-six percent of the respondents wanted to get information about the medicinal and the nutritional values, for example, mineral micronutrients in edible parts of MO.

6.4.3 Tree management

The planting arrangements and management of the MO and MS trees were noted during the study. In S. ETH, trees were generally found in sorghum/maize fields,

both on flat silty soils in Derashe and the sandy upland soils of the Konso terraces but some were found in household compounds. Coppicing was practiced widely with trees cut back in the early rainy season, although some were not coppiced. Tree coppicing is conducted to control the height growth (i.e., to keep leafy growth within reach) and to reduce shading when MS trees were intercropped with sorghum and maize. In KEN, MO trees were scattered within homesteads, as fences and hedges around homesteads, woodlots, and intercropped with staple crops such as, cassava, maize and sorghum. The most common method of tree management was pollarding (i.e., cutting back the canopy/branches of the tree) (Fig 6.4a). Some households practiced coppicing and lopping on the trees they cultivated. Weeding was conducted by some households who intercropped MO with maize and sorghum.

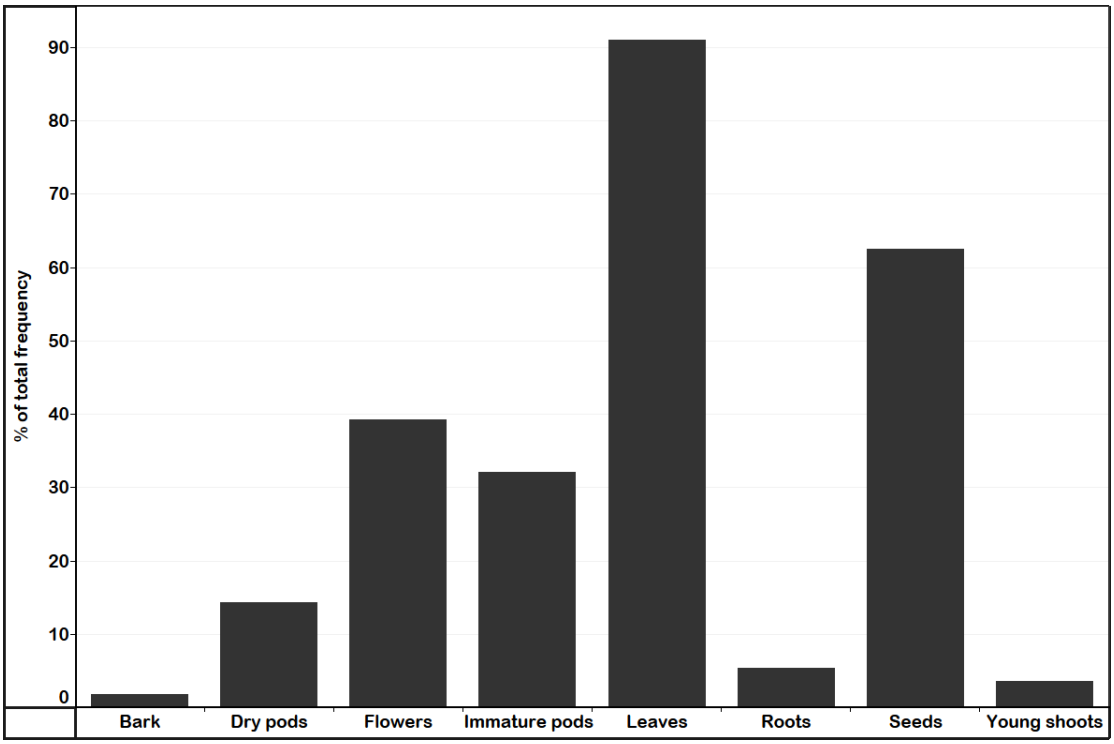


Fig 6.3. Parts of *M. oleifera* used by *Moringa* growing households in Kenya (n = 56).

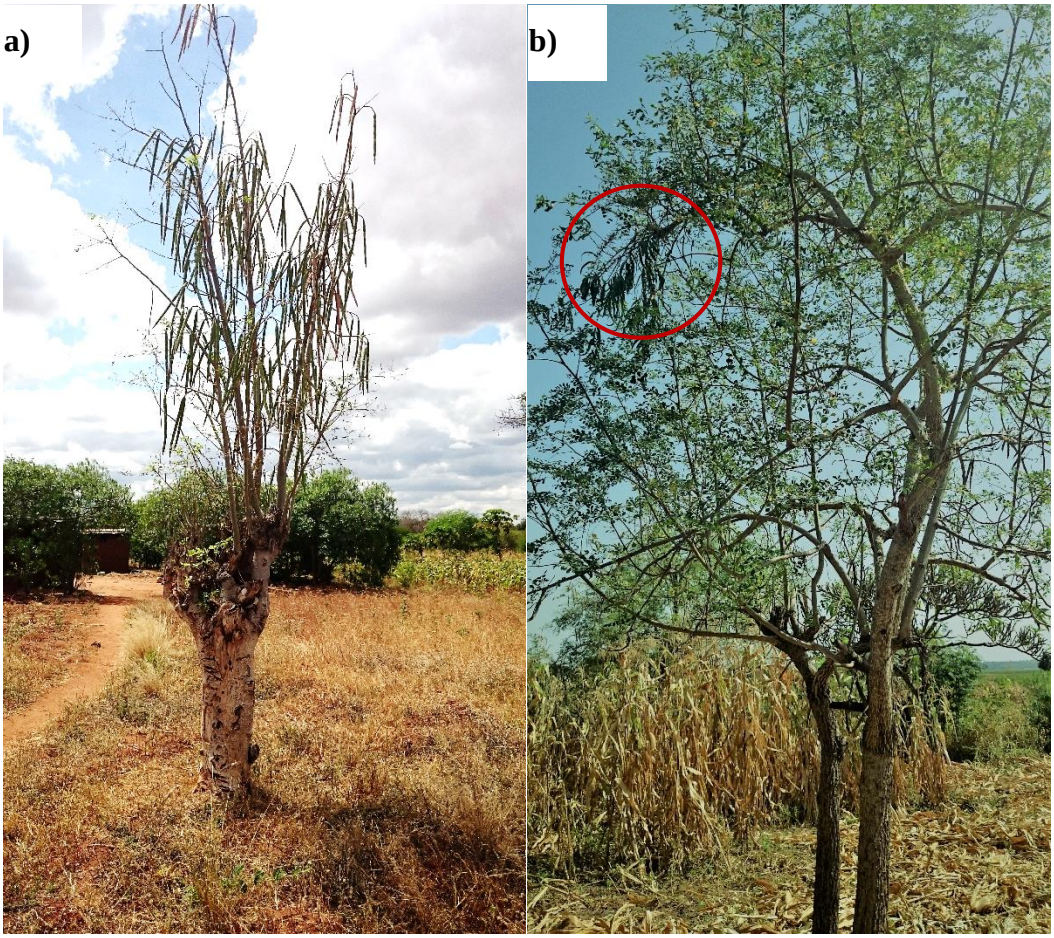


Fig 6.4. Pollarded *M. oleifera* tree in full pod at Kibwezi (a) and *M. oleifera* intercropped with maize at Ramogi (b) with parasitic plant growing on the branch (circled red), Kenya.

6.5 Discussion

The opportunities arising from on-farm use of *Moringa* products and off-farm sales have been reported in previous studies (Stevens *et al.* 2015, Valdez-Solana *et al.* 2015, Dalvand *et al.* 2016, Gopalakrishnan *et al.* 2016, Zeeshan *et al.* 2016, Zheng *et al.* 2016). The survey reported here has generated useful information on human dietary usage and other common uses of MO and MS by communities in southern Ethiopia and Kenya. It has also identified some of the potential barriers to widening

use of these species, according to current growers. The presence of MGHs with *Moringa* saplings of one year or less indicates that the cultivation of *Moringa* is expanding. However, there are competing interests and challenges with regards to cultivation and usage of *Moringa* that can be ascribed to the multiple uses and services these species provide.

6.5.1 On farm uses and services of *Moringa*

Moringa oleifera and MS produce nutritious flowers, leaves, and immature pods that can be used as human food and livestock fodder (Richter *et al.* 2003, Sanchez *et al.* 2006, Yang *et al.* 2006, Ferreira *et al.* 2008, Melesse *et al.* 2013, Nouman *et al.* 2013, Kholif *et al.* 2015, Melesse *et al.* 2015, Gopalakrishnan *et al.* 2016, Zheng *et al.* 2016). The use of these edible parts of *Moringa* by MGHs and for their livestock had been widely demonstrated in the present study.

More than 78% of the MGHs in S. ETH and KEN had been utilising MO and MS edible parts in their diet and >71% were engaged in cultivating these species for >17 years. This level of dietary usage of *Moringa* was similar to those in Nigeria where 71% (n = 745) of the respondents in ethno-pharmacological survey reported food and nutritional utilization of MO edible parts (Popoola *et al.* 2013). Human dietary usage of *Moringa* spp. was mainly boiled fresh leaves, and leaf powders mixed with other dishes and as tea. Immature pods and flowers were used as vegetables by some MGHs. These modes of dietary utilization were consistent with previous reports (Anwar *et al.* 2007, Lim 2012, TFLI 2014, Stevens *et al.* 2015, Gopalakrishnan *et al.* 2016). Based on the response of >90% of the MGH heads from S. ETH, MS leaves were used in their diet on a daily basis. However, the quantity and nutrient density of the leaves could not be estimated in a consistent

measurement unit from the qualitative bunch size stated by the respondents. Quantifying the dietary nutritional contributions of MS and MO to household nutritional security through a specifically designed dietary survey would be a valuable next step.

A common way of cultivating the *Moringa* trees, in both S. ETH and KEN was intercropping with other staple food crops, for example, cassava, maize and sorghum. Under such type of land use, the *Moringa* leaves shed on the soil serve as green manure to increase soil fertility and boost crop yield (Jahn 1991b, Arancibia *et al.* 2007, Palada *et al.* 2008, Petit-Aldana *et al.* 2012, Nouman *et al.* 2013, Undie *et al.* 2013, Bibi *et al.* 2016, Nasir *et al.* 2016, Nawaz *et al.* 2016). Some interviewees in KEN indicated that hedges of MO shrubs had been used for soil conservation.

Phytochemicals in the edible parts and other tissues of these plants are reported to possess therapeutic properties to treat, for example, anaemia (Amagloh *et al.* 2016), common cold (Saini *et al.* 2016b), diabetes (Semenya *et al.* 2012, Yassa and Tohamy 2014, Kamau *et al.* 2016, Lunyera *et al.* 2016), eye and ear infections (Lunyera *et al.* 2016), hyperlipidaemia (Toma *et al.* 2014), hypertension (Geleta *et al.* 2016, Randriamboavonjy *et al.* 2016), leprosy (Jahn 1991b), malaria (Mekonnen and Gessesse 2004) and typhoid (Ganatra *et al.* 2012, Lim 2012, Stevens *et al.* 2015). They also possess bactericidal and fungicidal properties (Eilert *et al.* 1981, Gopalakrishnan *et al.* 2016). Some of these medicinal values of *Moringa* were stated as useful side benefits by respondents from S. ETH and KEN in the present study. Recent *in vitro* research reports indicated that MO leaf extracts had cytotoxic effect on the A459 lung cancer cell lines (Madi *et al.* 2016) and oesophageal cancer

(Tiloke *et al.* 2016b). The moringin extracted from MO edible parts has been reported to have a beneficial role in preventing cancer (Michl *et al.* 2016). Furthermore, MO can be used in the production of gold nanoparticles that are used in cancer therapy (Tiloke *et al.* 2016a).

6.5.2 Off-farm benefits, challenges and opportunities

The seed oil from the *Moringa* is sought after in the soap, and fragrance industry because of its ability to absorb and retain fragrances (Morton 1991, Foidl *et al.* 2001), in the energy sector to manufacture biodiesel (Tsaknis *et al.* 1999, Anwar *et al.* 2005, Ejigu *et al.* 2010, Ayerza 2011, Eloka-Eboka and Inambao 2016, Fotouo-M *et al.* 2016, Saini *et al.* 2016b, Zeeshan *et al.* 2016), and for water purification as a natural coagulant (Ndabigengesere and Subba Narasiah 1998, Ahmed *et al.* 2010, Degefu *et al.* 2013, Popoola *et al.* 2013, Alsharaa *et al.* 2016, Dalvand *et al.* 2016, del Real-Olvera *et al.* 2016, Hamid *et al.* 2016, Kumar *et al.* 2016, Mageshkumar and Karthikeyan 2016). Although these various uses that are derived from the *Moringa* seeds can be an off-farm opportunity to raise household incomes, lack of access to markets was one of the challenges that was faced by the MGHs.

Difficulties with a reported failure of an international buyer for MO by some KEN households shows the importance of secure markets which allow the producer to develop this perennial crop: these MGHs had then struggled to find local markets to sell the *Moringa* products. Although those MGHs were disappointed because the economic gain did not materialize, this resource, for example, could still be used to fulfil the mineral nutritional requirements of their household and/or livestock especially during the dry season and at the onset of the rainy season when other vegetables and forage crops are scarce (Lockett *et al.* 2000, Kubitzki 2003, Diouf

et al. 2007, Rakotosamimanana *et al.* 2015). Raising community-wide awareness on the multiple uses of *Moringa* is required to create market demand and maximize resource utilization.

Other reported challenges were parasitic plants, disease and pests. The diseases and pests reported by the interviewees was in agreement with documented entomological and pathological information on MO. In their review, Kotikal and Math (Kotikal and Math 2016) categorized insect pests associated with MO in India as defoliators, sap feeders, and bark, pod and seed borers, and have listed non-insect pests. Yusuf and Yusif (Yusuf and Yusif 2014) confirmed the presence of MO leaf feeding insect larvae (*Ulopeza phaeothoracica*) in Nigeria. *Moringa* leaves browsed and shredded by insect larvae are less appealing for human dietary consumption. Furthermore, disease, pests and parasitic plants lead to decrease in foliage biomass production and in extreme cases kill the trees. These suggest a need for pathological and entomological research efforts to identify the pests and diseases, and devise control measures that do not contravene with the dietary usage of the edible parts.

6.6 Conclusion

Moringa oleifera and MS are hardy multipurpose trees/shrubs and grow well in tropical and subtropical regions under marginal environmental conditions where other crops struggle to survive (Morton 1991, NRC 2006, Kasolo *et al.* 2010, Nouman *et al.* 2013, Gopalakrishnan *et al.* 2016). Aside from their hardiness, they produce nutritious edible parts and their geographical distribution overlaps with the regions where there are high risks of mineral micronutrient deficiencies hence can play a vital role in tackling *hidden hunger*.

This study has shown that multiple uses and services can be derived from *Moringa*. It is clear from our results that there is a high level of awareness of multiple roles of *Moringa* among MGHs but less in the wider community. Although the multiple uses and services that can be derived from *Moringa* spp. is an opportunity, maximization of the potential benefits requires research, extension and developmental priority setting in consultation with the stakeholders to better understand if this is viable. Various production package options need to be formulated for the different stakeholders considering the objectives that should be achieved.

For instance, for MGHs that want to fulfil their household nutritional requirements and use *Moringa* as a source of income, cultivation of *Moringa* as a household leafy vegetable can be integrated with the production of seeds for bioenergy and fragrance industries. These entail liaising among the different stakeholders and set out management plan that optimizes leaf and seed yields. To this end, areas for research needs include, firstly, selection of *Moringa* landraces with better desirable dietary characteristics (e.g., high mineral nutrient concentration, low concentration of phytate, good taste, etc.), and varieties with good quality and high seed yield. Secondly, silvicultural research to determine the effect of defoliation of *Moringa* trees for human dietary consumption on the pod and seed yield is required. Third, propagation methods (i.e., sexual or asexual) that can help to retain the desirable characteristics of the mother trees needs to be researched. Finally, *Moringa* growers' indigenous knowledge needs to be shared to guide the selection of landraces preferred for human consumption and other purposes.

Moringa growing households also expressed a desire to know more about the nutritional benefits of the edible parts and the dosage for the various ailments that are treated by these species. Several studies have indicated that *Moringa* contains a high concentration of many essential macro- and micro-nutrients, for example, Olson *et al*. (Olson *et al.* 2016) and Kumssa *et al*. (Kumssa *et al.* 2017). However, research findings are scanty on the bioavailability of these nutrients when ingested by humans and livestock. A few studies on bioavailability of some nutrients from *Moringa* leaves indicated variation between nutrients. For example, an *in vitro* study showed that iron bioavailability from MO leaves was very low while beta-carotene bioavailability was 100% (Amagloh *et al*. 2016) which was consistent with Nambiar and Seshadri (Nambiar and Seshadri 2001). Similarly, folate bioavailability study using Wistar rats showed >80% of the folate from MO leaves was bioavailable (Saini *et al.* 2016a).

The perennial nature, multiple uses, and resilience to drought of *Moringa* species make them a suitable target for more agro-silvicultural research. A comprehensive, integrated and multidisciplinary research effort, and links with development and extension agents are required on these multipurpose tree species to develop them not only as crops to contribute to the alleviation of *hidden hunger* but to potentially develop a commodity crop that can improve some the multifaceted socioeconomic problems in tropical and subtropical developing countries.

Appendix 6.1. Information Sheet for Participants

"Why do people in various regions grow *Moringa* spp.?"

(Ethical Approval number)

Invitation

You are being invited to be involved in a research study; before you decide whether you want to take part, it is important for you to understand why the research is being done and what your participation will involve. Please take time to read the following information carefully and discuss it with other people if you wish. Please contact me if anything is unclear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of this study?

The aim of this study is; to establish why people in various countries grow *Moringa* spp. and to assess the potential of this species as multi-nutrient dietary sources for human being.

Why I have been chosen?

You are randomly selected because you have planted *Moringa* spp. There is no specific reason other than that you are one of those who grow *Moringa* to whom the study is relevant.

What will participation involve?

You will be asked a number of questions with your general household, *Moringa* spp. planting, and the products/services you draw from this species. Your responses to the questions will be written down on a mobile phone or tablet.

What if I decide that I don't want to take part?

You are free to decide that you don't want to take part in the study and can:

1. Refuse to answer any questions that you don't want to
2. Decide to stop the interview at any time
3. Remove your consent for the data collected to be used.

Will I be paid for my time?

There is no payment for taking part in this study.

Will I be anonymous, and who will know my identity?

If you agree to take part in an interview, a Participant Number will be generated for you, and that's the only thing that will be used to identify you. Your identity will only be known by the interviewer, and will not be found in any record. Hard copy and electronic data will be stored on the University of Nottingham's Network: this will be deleted after 5 years, or if you withdraw your consent (whichever is sooner).

What do I do next?

If you'd like to be involved in this study we will interview you when we visit your *Moringa* spp. that you have planted. At the interview you'll be issued with one of these information sheets, and will be asked to sign a consent form.

Who shall I contact with any questions?

To be part of this study, or to ask any questions, then please get in contact with the Principal Investigator, Diriba Kumssa:

Email: stxdbk@nottingham.ac.uk

Tel.: +447446119038

Appendix 6.2. Consent Form

You have been invited to take part in a research project; in order to go forward with your participation, it is necessary for you to give your consent.

By completing this form you are consenting to take part in this research project; you can withdraw your consent at any point. To withdraw your consent, please either mention that to the Interviewer during the interview or contact Mr Diriba Kumssa by email before 20 July 2015. Before signing this form, please read the following statements and indicate that you agree with them by initialling next to them.

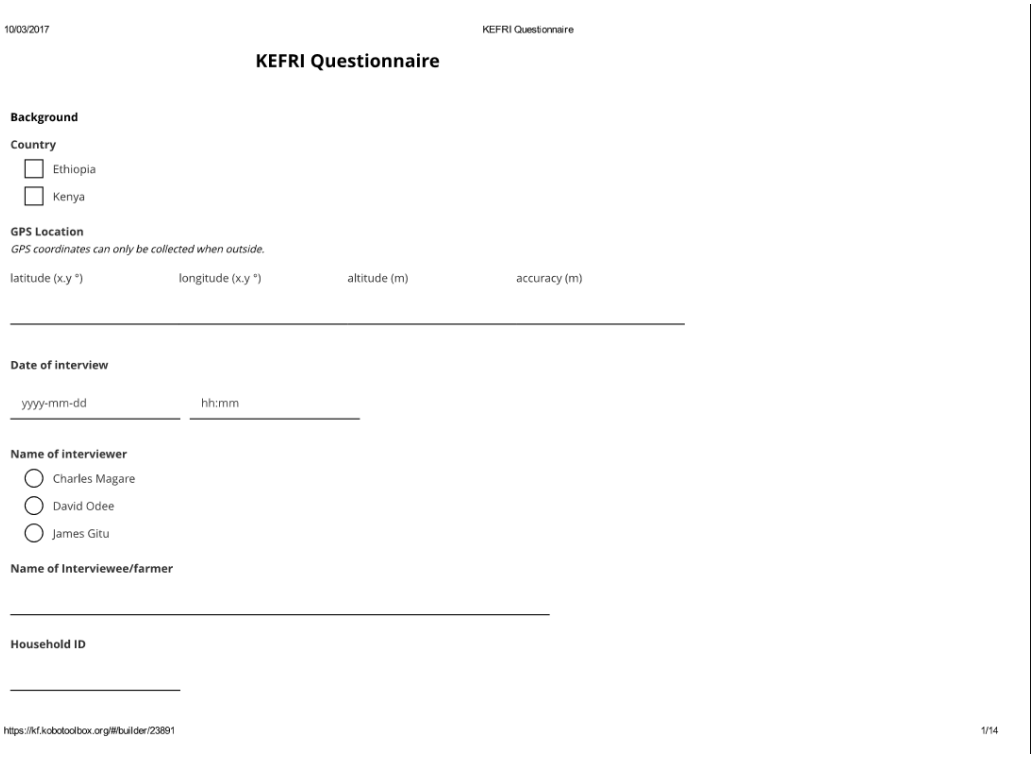
	Initial here
I have been issued with a Participant Information Sheet	
I have been informed what the purpose of this research is, and the nature of the study	
I have been informed how the data that are collected within the research will be handled and stored.	
I have been informed that I can remove my consent at any time either during, or after the interview (up to the 20 April 2015), and that withdrawal of consent will not harm me in any way.	
I have been informed that the interview will be written down on mobile phone/tablet	

I have been informed that my anonymised quotes may be used within the reporting of this research.	
I agree to take part in this study	

Signed by……. Date……

Consent received by…….Date……

Appendix 6.3. Questionnaire used to collect data on the use of MO and MS.



10/03/2017 KEFRI Questionnaire

KEFRI Questionnaire

Background

Country

- ☐ Ethiopia
- ☐ Kenya

GPS Location

GPS coordinates can only be collected when outside.

latitude (x.y °) longitude (x.y °) altitude (m) accuracy (m)

Date of interview

yyyy-mm-dd hh:mm

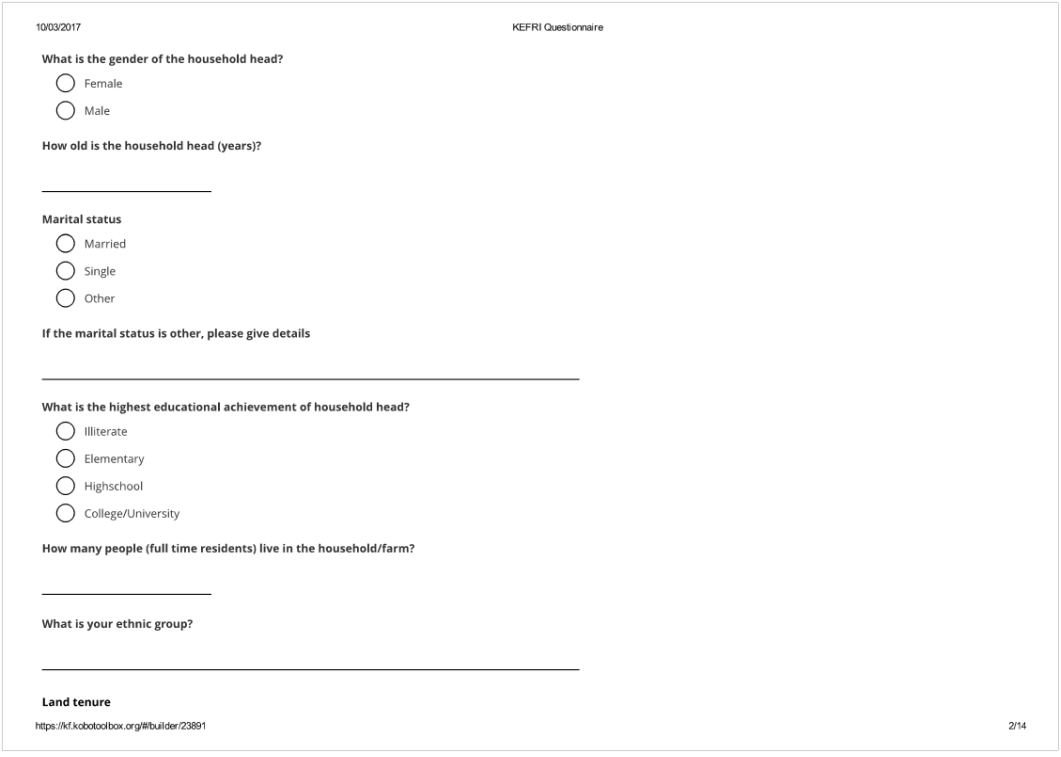
Name of interviewer

- ◯ Charles Magare
- ◯ David Odee
- ◯ James Gitu

Name of Interviewee/farmer

Household ID

https://kf.kobotoolbox.org/#/builder/23891 1/14



10/03/2017 KEFRI Questionnaire

What is the gender of the household head?

- ◯ Female
- ◯ Male

How old is the household head (years)?

Marital status

- ◯ Married
- ◯ Single
- ◯ Other

If the marital status is other, please give details

What is the highest educational achievement of household head?

- ◯ Illiterate
- ◯ Elementary
- ◯ Highschool
- ◯ College/University

How many people (full time residents) live in the household/farm?

What is your ethnic group?

Land tenure

https://kf.kobotoolbox.org/#/builder/23891 2/14

How is the land owned?

- ◯ Own
- ◯ Rental
- ◯ Communal
- ◯ Others

If Others, please specify

_____

What is the approximate size/acreage of your farm?

- ◯ 1-3 acres
- ◯ 4-10 acres
- ◯ 11-15 acres
- ◯ 16-20 acres
- ◯ > 20 acres

Are you engaged in any other off farm income generating activities?

- ◯ Yes
- ◯ No

If 'yes', what's the activity/ profession?

- ◯ Formal
- ◯ Self-employed
- ◯ Casual labour
- ◯ Others

If 'Formal', please specify

_____

If 'Others', please specify

_____

What crops do you grow on the farm? (Rank in order of importance)

_____

Do you keep any livestock?

- ◯ Yes
- ◯ No

If 'Yes', list/rank in order of importance

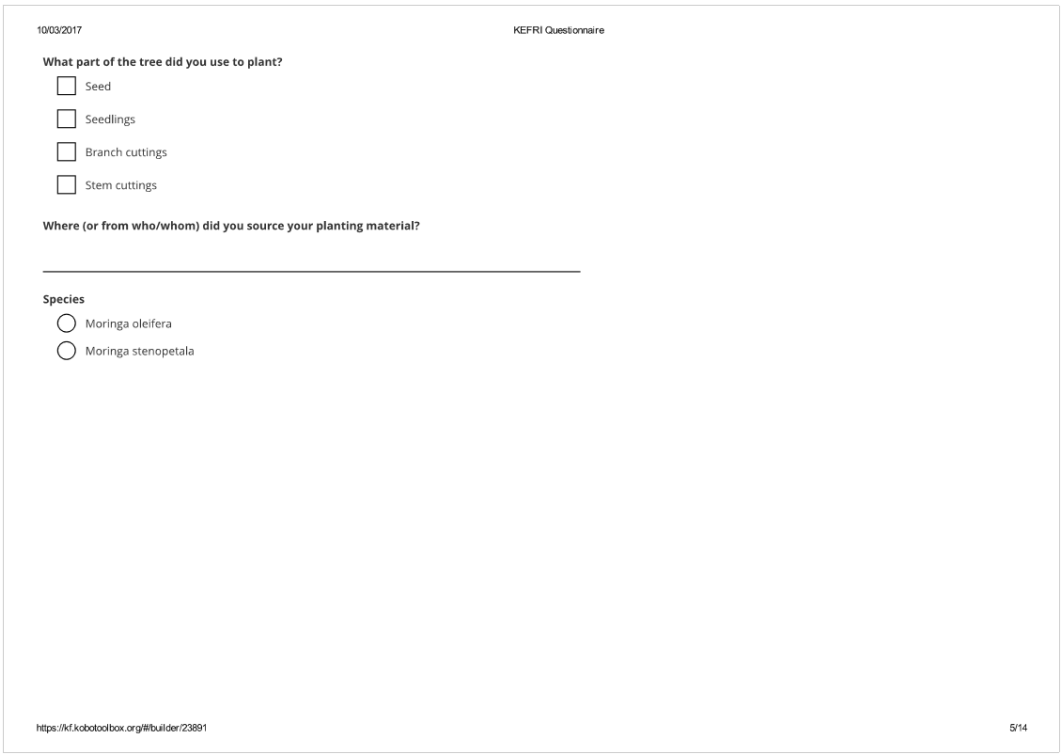
_____

For how long have you grown Moringa (years)?

_____

Why do you plant Moringa?

- ☐ Food
- ☐ Medicine
- ☐ Shade
- ☐ Ornament
- ☐ Shelterbelt
- ☐ Feed
- ☐ Green manure
- ☐ Other



10/03/2017 KEFRI Questionnaire

What part of the tree did you use to plant?

- [ ] Seed
- [ ] Seedlings
- [ ] Branch cuttings
- [ ] Stem cuttings

Where (or from who/whom) did you source your planting material?

_____

Species

- ◯ Moringa oleifera
- ◯ Moringa stenopetala

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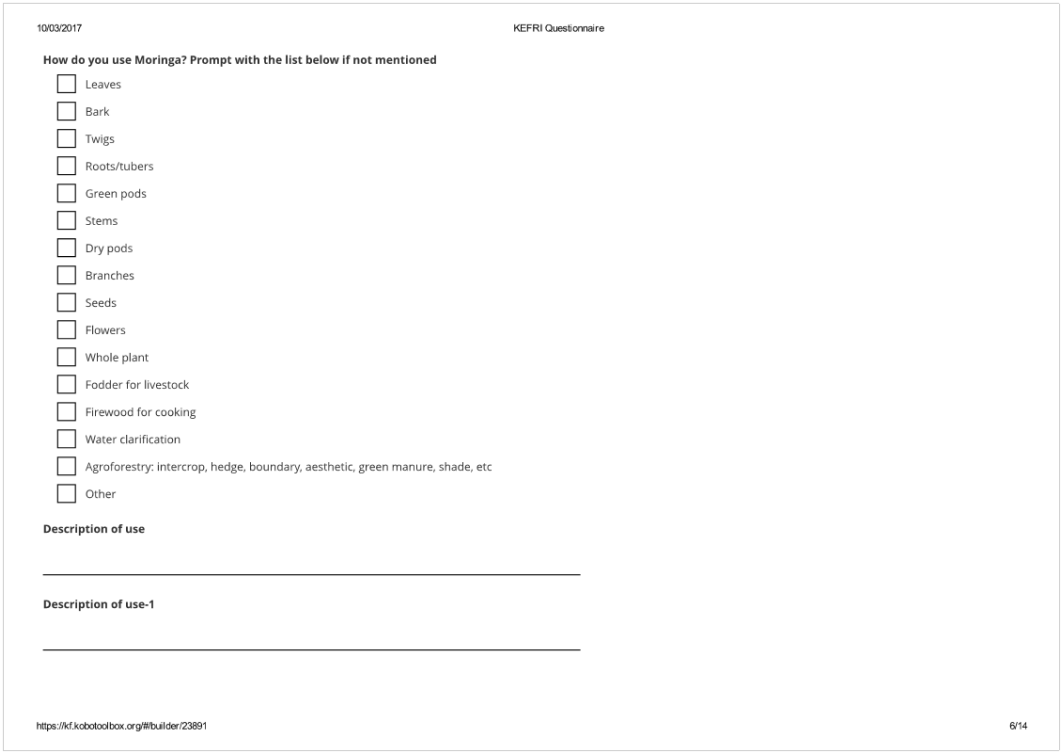

10/03/2017 KEFRI Questionnaire

How do you use Moringa? Prompt with the list below if not mentioned

- [ ] Leaves
- [ ] Bark
- [ ] Twigs
- [ ] Roots/tubers
- [ ] Green pods
- [ ] Stems
- [ ] Dry pods
- [ ] Branches
- [ ] Seeds
- [ ] Flowers
- [ ] Whole plant
- [ ] Fodder for livestock
- [ ] Firewood for cooking
- [ ] Water clarification
- [ ] Agroforestry: intercrop, hedge, boundary, aesthetic, green manure, shade, etc
- [ ] Other

Description of use

_____

Description of use-1

_____

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Description of use-2

_____

Description of use-3

_____

Description of use-4

_____

Do you sell any of the Moringa plant parts or products?

- ◯ Yes
- ◯ No

If 'Yes', list Moringa plant parts/products, value and buyers (namely green leaves, dry leaves, greed pods, dry pods, seeds, fresh flowers, dry flowers, branches, stem roots, tubers, firewood

_____

If 'Yes', list Moringa plant parts/products, value and buyers (namely green leaves, dry leaves, greed pods, dry pods, seeds, fresh flowers, dry flowers, branches, stem roots, tubers, firewood

_____

Management

How often do you harvest or extract Moringa parts for use or sale?

- ◯ Monthly
- ◯ Quarterly
- ◯ Bi-annually
- ◯ Annually
- ◯ Other

If 'other', please specify

_____

What is the estimated number of Moringa tree/saplings on the farm?

- ◯ 0-30
- ◯ 30-60
- ◯ 60-100
- ◯ > 100

Have you experienced any problems with pest, disease or livestock/wildlife damage? Moringa tree?

- ◯ Yes
- ◯ No

If yes, describe the causal agent, type and part of the tree damaged

- [ ] Leaves
- [ ] Roots/Tuber
- [ ] Stems
- [ ] Green pods
- [ ] Dry pods
- [ ] Branches
- [ ] Seeds
- [ ] Flowers

Description of the damage

_____

Description of the damage

_____

Description of the damage

_____

If pest or disease attack, what period, season or time of the year when it most severe?

_____

What intervention or control methods did you use?

_____

Do you have any questions or comments about Moringa or general comments?

_____

Would like to receive or know more information regarding Moringa?

- ◯ Yes
- ◯ No

Observations to be made by the interviewing team (planting configuration, location, regeneration, management)

_____

What is the tree arrangement on the farm?

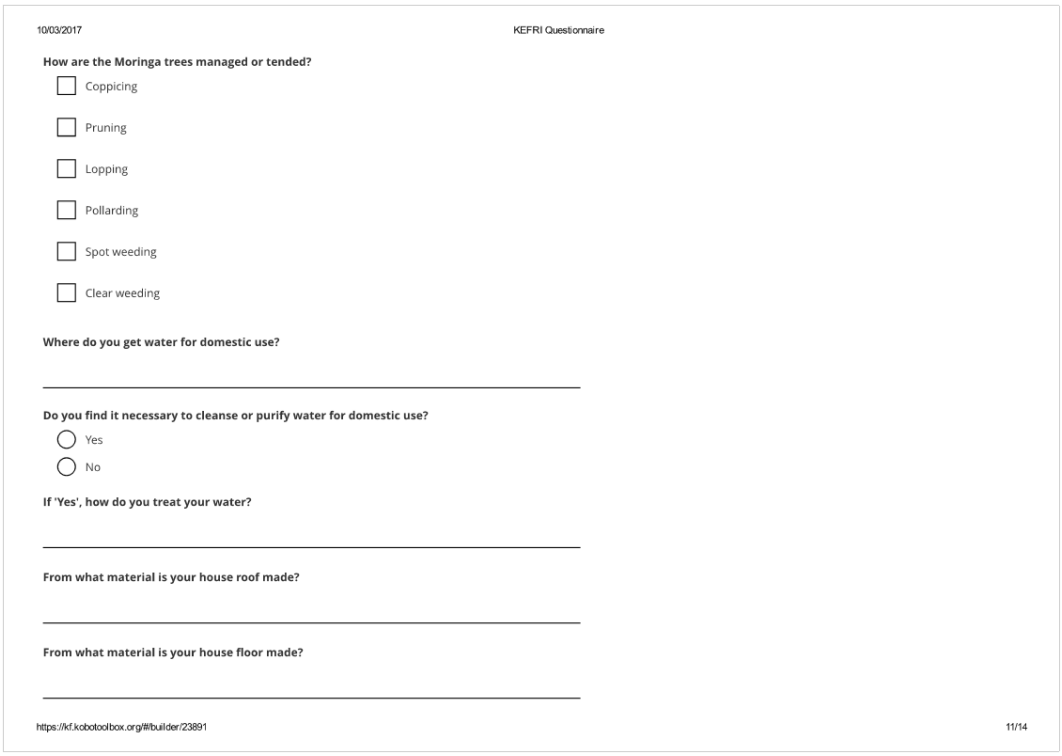
- [ ] Scattered
- [ ] Isolated
- [ ] Lines
- [ ] Woodlot

Where on the farm the trees are planted/located?

- [ ] Farm/home boundary
- [ ] Hedge
- [ ] Intercropping with other crops

Are there any signs of natural regeneration or fresh plantings?

- [ ] Young seedlings
- [ ] Saplings
- [ ] Sprouting
- [ ] None



10/03/2017 KEFRI Questionnaire

How are the Moringa trees managed or tended?

- [ ] Coppicing
- [ ] Pruning
- [ ] Lopping
- [ ] Pollarding
- [ ] Spot weeding
- [ ] Clear weeding

Where do you get water for domestic use?

_____

Do you find it necessary to cleanse or purify water for domestic use?

- ◯ Yes
- ◯ No

If 'Yes', how do you treat your water?

_____

From what material is your house roof made?

_____

From what material is your house floor made?

_____

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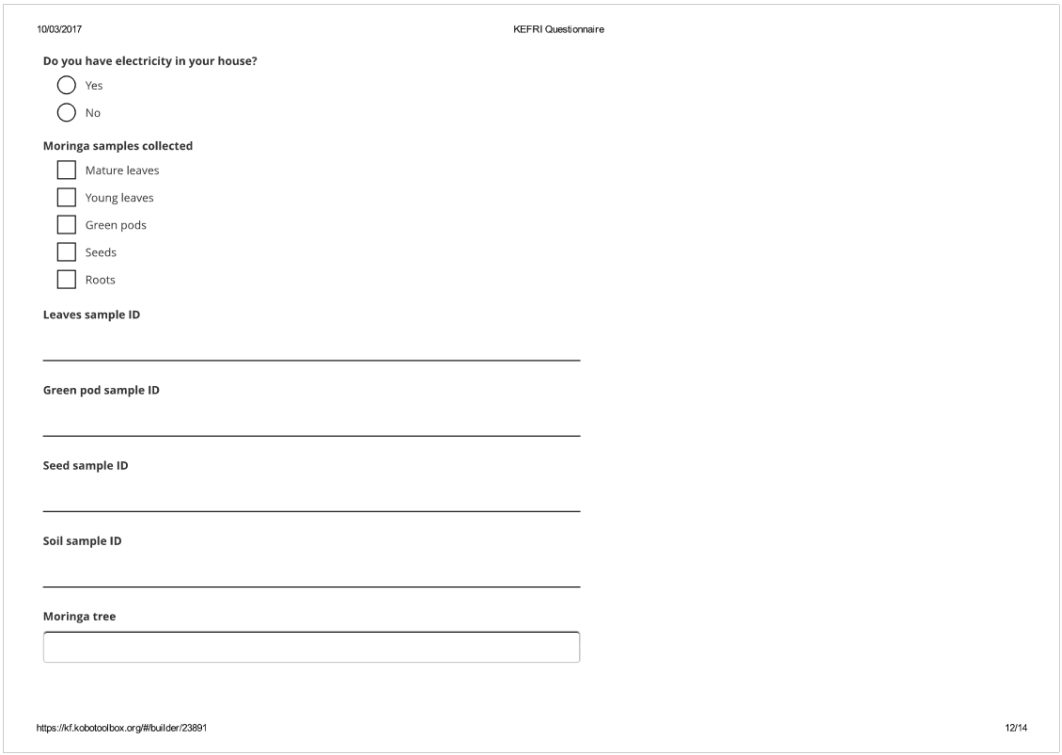

10/03/2017 KEFRI Questionnaire

Do you have electricity in your house?

- ◯ Yes
- ◯ No

Moringa samples collected

- [ ] Mature leaves
- [ ] Young leaves
- [ ] Green pods
- [ ] Seeds
- [ ] Roots

Leaves sample ID

_____

Green pod sample ID

_____

Seed sample ID

_____

Soil sample ID

_____

Moringa tree

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Moringa leaves

Moringa green pods

Moringa seed

Root samples

Soil samples

What in-the-field treatment is applied to the sample? (e.g., washing with water)

What is the approximate height of the tree? (metres)

What is the approximate diameter at breast height of the tree? (centimetres)

At what depth was the soil sample taken? (centimetres)

Estimate the distance between soil sampling point/s and the base of the Moringa tree (metres)

Thanks for completing the questionnaire!

CHAPTER 7. GENERAL DISCUSSION

7.1 Estimating dietary mineral nutrient intakes and deficiency risks

To understand historical global trends in human mineral nutrition and to aid future agricultural, health and nutrition policy planning, human dietary Ca, Mg and Zn supply between 1992 and 2011 were estimated for 145 countries using data from various sources. National daily per capita food availability from the FAO FBS was used as a proxy for food consumption with an individual coefficient of variation of 25% in food intake (Joy *et al.* 2012, Wessells *et al.* 2012a). The concentration of the mineral nutrients in the food items were determined by matching with food composition tables from the USDA nutrient database SR26 (USDA 2013). The daily per capita supply of Ca, Mg and Zn were calculated by adding up the amount of these nutrients derived from individual food items that were available for consumption. Daily per capita supply was compared with population-weighted estimated average requirements to estimate the risk of dietary deficiency of each mineral.

This supply-based approach utilized readily available secondary food supply and composition, and demographic structure data to generate useful global human dietary mineral nutrient information. The caveats and uncertainties in this type of approach have been discussed in Chapters 2 and 3. For example, there was shortage of disaggregated food consumption data, and comprehensive local food composition tables to account for variation in soil and varieties of food crops grown and consumed at various locations in different countries. The influence of phytoavailable soil nutrients on plant edible part nutrient concentration is a well-

established fact. Besides, there is intra- and inter-specific variation in the absorption, translocation and accumulation of nutrients in edible parts of plants (Watanabe *et al.* 2016, White 2016, Yamunarani *et al.* 2016).

The importance of using appropriate food composition tables is illustrated of Se in Fig 7.1, for which transfer from soil to crop strongly controlled by soil properties. In a ratio of estimates of Se intake by Kumssa *et al.* (Unpublished) and Joy *et al.* (2014) in 41 African countries are presented. Joy *et al.* (2014) used regional food composition tables while Kumssa *et al.* used the USDA-SR26. In most of the countries, usage of the USDA-SR26 Se composition data overestimated dietary Se intakes (Fig 7.1). This can be ascribed to the high Se concentration in the food crops produced in Northern America with higher soil Se, and transfer of Se to crops, as compared to most African soils (Combs 2001, Murphy and Cashman 2001, Broadley *et al.* 2006, Christophersen *et al.* 2013). Hence, the use of USDA-SR26 food composition table was not appropriate to assess and map global level dietary Se.

Due to this, the global dietary mineral nutrient studies in this thesis focused on three mineral elements, Ca, Mg and Zn, for which spatial variation in food composition was generally less extreme than for Se. Seasonal variation in the type and quantity of available food, food wastage, supplementations, and fortification were also not captured using supply-based approaches. Nonetheless, the current national level estimates of dietary deficiency risks of Ca, Mg and Zn can be used as a baseline to plan agriculture-centred food, nutrition, public health and agricultural policies especially in less developed countries where most of the population rely on

smallholder agriculture to produce foods, and information on dietary nutritional intakes are limited.

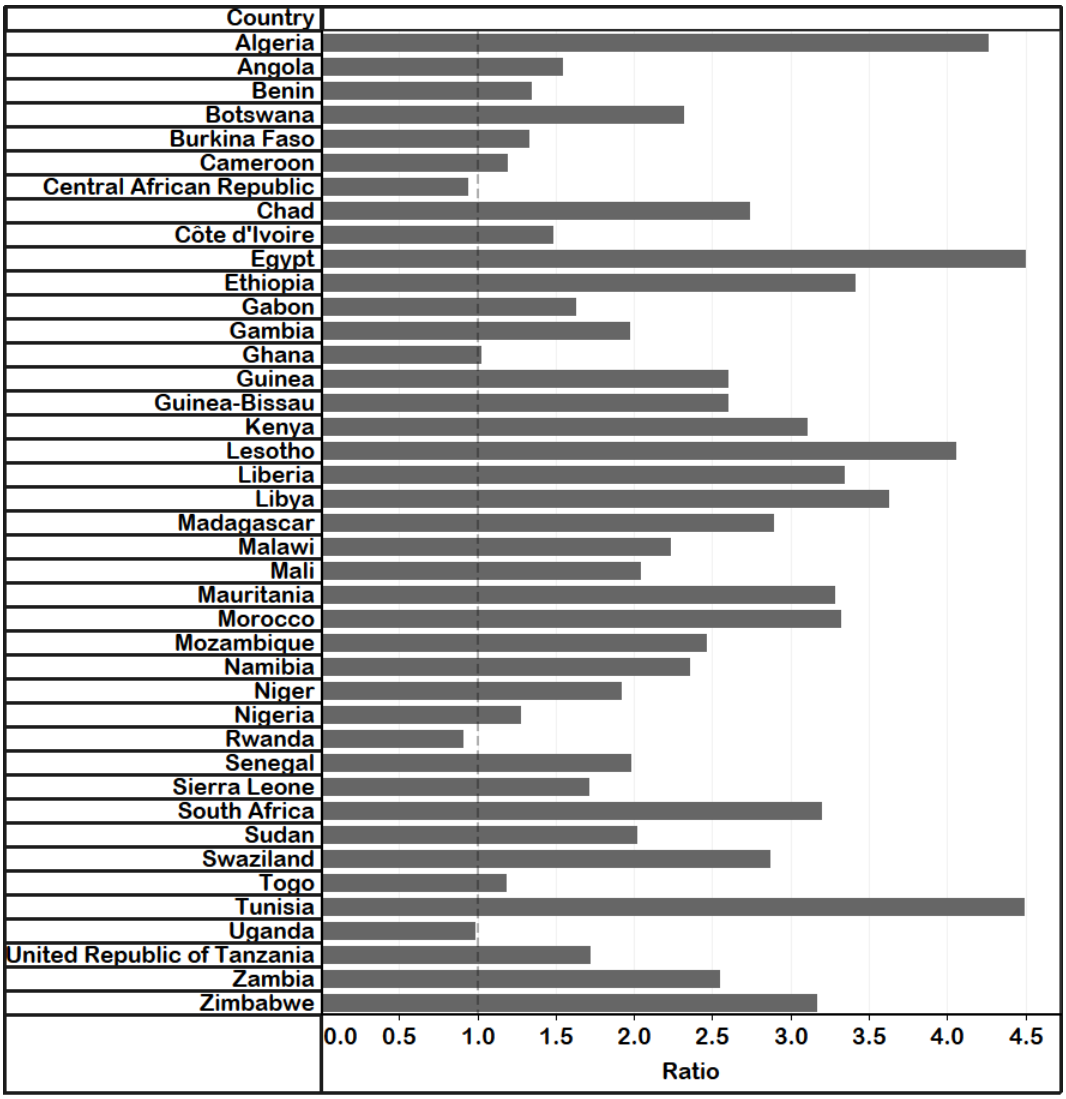

Fig 7.1. The ratio ([Kumssa *et al.*, unpublished]: [Joy *et al.*, 2014]) of dietary Se supply using different food composition tables in some African countries. Kumssa *et al.* used the USDA-SR26, while Joy *et al.*, 2014 used regional food composition tables. The vertical broken line represents the ratio = 1.

A disaggregated high resolution case study was undertaken in Malawi to estimate dietary energy, Ca, Cu, Fe, I, Mg, Se and Zn deficiency risks at EPA level (Chapter 4). Household food consumption data based on 7 d dietary recall (n = 12117) from

the Malawi IHS3, and local food composition table (Joy *et al.* 2015a, Watts *et al.* 2015) matched with the soil types of the surveyed households were used to make estimates of nutrient intakes, and deficiency risks by comparing intakes with population weighted estimated average requirements. There was broad agreement between the national level low resolution estimates and the high resolution EPA level estimates of dietary mineral nutrient consumption and deficiency risks for Malawi population (Joy *et al.* 2015b). A comparison of estimates obtained from supply-based approaches at a national level, and intake-based assessments from dietary recall is discussed in Chapter 4, Section 4.6. Discrepancies in the estimates of the MNDs can be attributed to the use of more relevant local food composition data, and inter-household and cross-seasonal variation in food consumption data in the IHS3. However, there are uncertainties in using dietary recalls to estimate household food consumption. For example, unintentional/intentional under/over reporting of food consumption as discussed in Chapter 4.7.1 is a major potential weakness of this approach.

7.2 Role of underutilized crops in alleviating human mineral nutrient deficiency risks

In 2011, about half of the world population was at risk of one or more mineral micronutrient deficiencies (Kumssa *et al.* 2015a). Developing countries in Africa and Asia share ~90% of this risk. Depending on the scale and urgency of essential micronutrient deficiencies, various options can be pursued to resolve human MND problems: multi-nutrient supplementation in pregnant and lactating women, and children up to five years; micronutrient fortification using various food vehicles to

tackle widespread MNDs in populations (e.g., salt iodization); biofortification (e.g., Se & Zn) and dietary diversification using underutilized crops are some examples.

Here, the potential role of dietary diversification using underutilized MO tree leaves in alleviating mineral micronutrient deficiency risks in tropical and subtropical developing countries where foods are mainly produced and consumed locally can be illustrated by a simple simulation exercise, using Se as an example in Malawi. With a national median Se supply of 25 μg d^{-1} AME^{-1}, 81% of the households (Fig 7.2 and 7.3) surveyed during the Malawi IHS3 in the year 2010-11 had Se intake below the RNI (Joy *et al.* 2015b) indicating high Se deficiency risk. For comparison, the RNI for an adult man is 55 μg d^{-1} (IOM 2000a). With an average Se concentration of 0.86 μg g^{-1} of fresh MO leaves (Kumssa *et al.* 2017), the impact of MO leaf dietary usage to reduce the proportion of the Malawian population with Se deficiency risk was modelled at a step of 5 g daily per capita intakes and results presented below.

Based on the 2010-11 Malawi IHS3 food consumption data, daily consumption of 50 g $capita^{-1}$ of fresh MO leaves would raise the daily Se intake from 25 to 69 μg AME^{-1} and would reduce the proportion of the Malawian population with Se intake below the EAR and RNI to 1% and 9%, respectively (Fig 7.3 and 7.4). And, an intake level of 65 g $capita^{-1}$ d^{-1} of fresh MO leaves would match the RNI for Se of the Malawian population (Fig 7.5). Besides, from 50 g fresh leaves of MO, an individual would obtain 183, 0.07, 0.002, 2.02, 53.7 and 0.36 mg of Ca, Cu, I, Fe, Mg, and Zn, respectively. Similarly, at an intake level of 65 g of fresh MO leaves,

an individual can obtain 238, 0.09, 0.003, 2.63, 69.7 and 0.46 mg of Ca, Cu, I, Fe, Mg, and Zn, respectively (Kumssa *et al.* 2017).

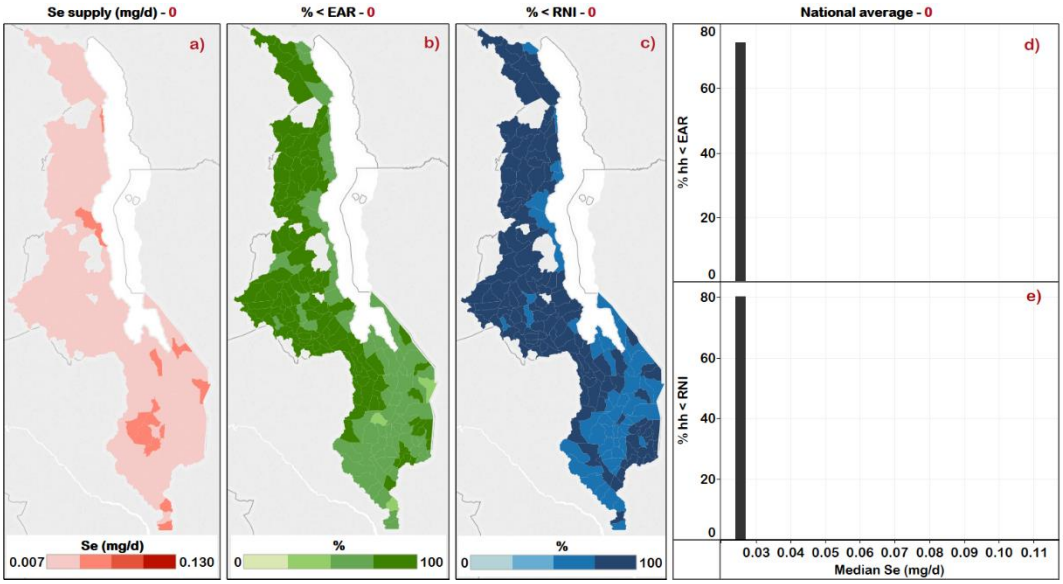

Fig 7.2. Extension Planning Area (EPA) level per capita Se supply (a), percent of population with median Se intake < EAR (b) and < RNI (c), and national level aggregated percent of population with daily median Se intake < EAR (d) and < RNI (e) without MO leaf consumption (0 intake).

Extension Planning Areas (EPAs) are the administrative units at which various development plans are set and implemented in Malawi. As demonstrated in Fig 7.2, 7.4 and 7.5, the household intake of Se varied between EPAs. The possible causes of these variations include, the soil type, the vicinity to lakes where the population can have access to fish. These highlights the importance of increasing the spatial resolution at which MND assessments and interventions should be made to effectively address local dietary MND problems. Towards this end, generating local food composition data for accurate assessment of existing dietary mineral nutrient intakes, and devising interventions that can be sustainably and easily implemented is crucial.

Moringa oleifera grows in Malawi and there are some commercial farms that produce MO leaf powders for use as tea and antioxidant. Nonetheless, out of the >12000 households which participated in the IHS3, only 9 reported that they had used *Moringa* in their diet in the past 7 days. This is an indication of the presence of low level of awareness about the nutritional uses of MO. A concerted effort is required from the agriculture, health, and education ministries of Malawi to tap the multiple uses and services this hardy plant species can offer, especially as a perennial household vegetable crop both in times of need and as a regular source of dietary mineral nutrients.

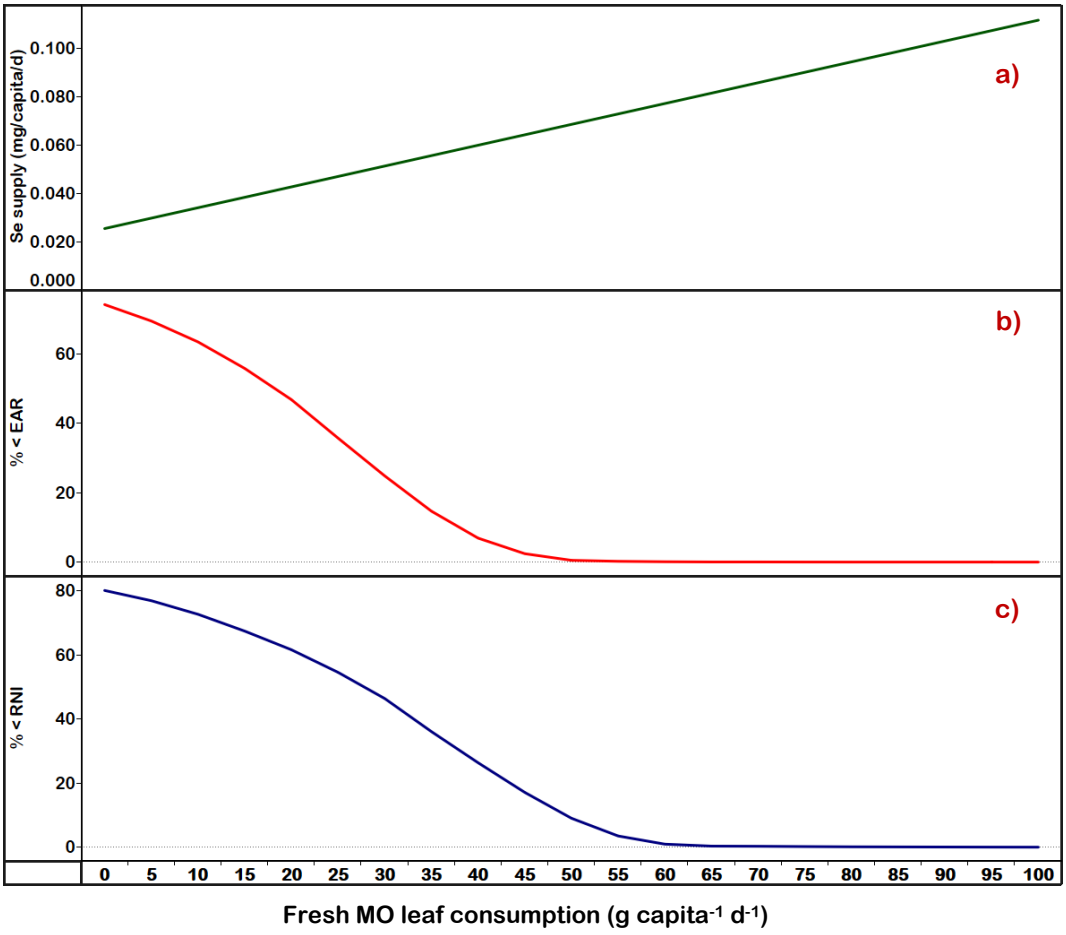

Fig 7.3. The impact of fresh MO leaf consumption on dietary Se intake (a), percentage of population with median Se intake < EAR (b) and < RNI in Malawi.

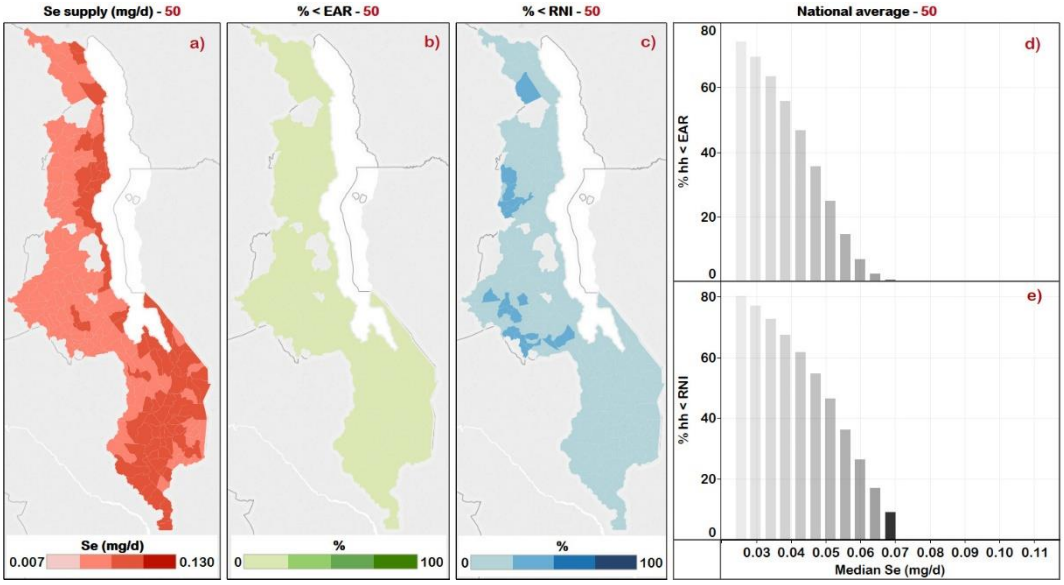

Fig 7.4. Extension Planning Area (EPA) level per capita Se intake (a), percent of population with median Se intake < EAR (b) and < RNI (c), and national level aggregated percent of population with daily median Se intake < EAR (d) and < RNI (e) at 50 g MO fresh leaf consumption.

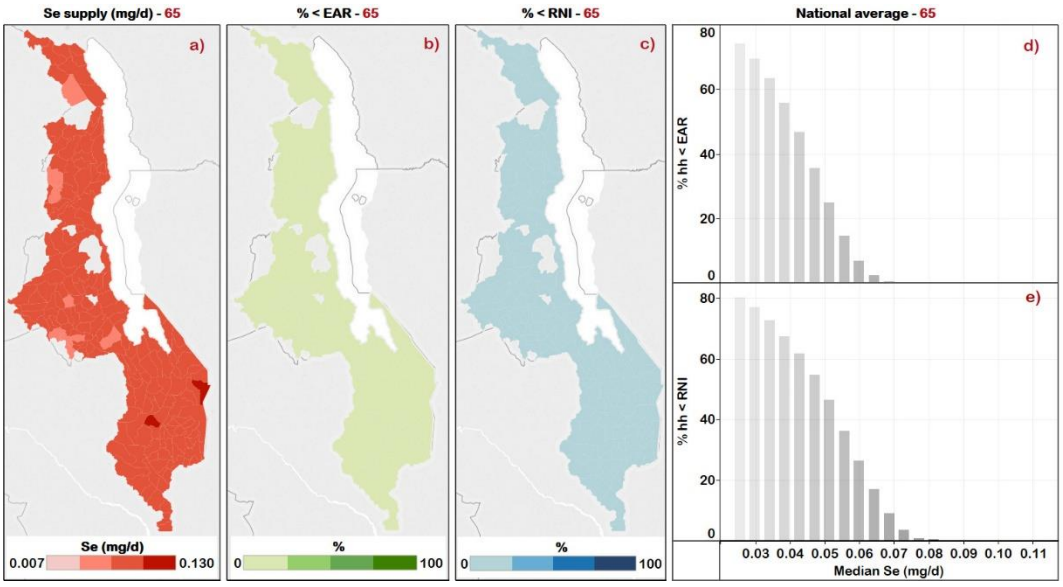

Fig 7.5. Extension Planning Area (EPA) level per capita Se intake (a), percent of population with median Se intake < EAR (b) and < RNI (c), and national level aggregated percent of population with daily median Se intake < EAR (d) and < RNI (e) at 65 g MO fresh leaf consumption.

7.3 Conclusion and recommendation

Diet-based human nutritional deficiency analyses in the global food systems between 1992 and 2011 indicated that there had been widespread dietary Ca and Zn deficiencies, the prevalence being higher in less developed countries of Asia and Africa. A sub-national case study using high resolution food consumption and composition data in Malawi confirmed these findings. In less developed countries, most of the population live in rural areas. For example, in 2014, 84% of the population of Malawi, 67% of those in south Asia, and 63% of those in sub-Saharan Africa lived in the rural areas. During the same period, there were 84 countries where ≥ 50% its population resided in the rural areas (The World Bank 2014). Due to the poor road and market infrastructure in the rural areas in less developed countries, the population depend on locally produced food (Zerfu *et al.* 2016). In such population, diet-based nutritional deficiency assessment making use of food composition data, for example, from high input agricultural production systems in developed countries affects the reliability of the population nutritional information. Therefore, compilation and development of local food composition tables, and regularly updating it with changes in the agricultural production system is vital to produce a reliable nutritional information, and devise appropriate food-based nutritional interventions. Besides, data on household food wastage, seasonal variation in food supply, the usage of fortified foods, for example, iodized salt etc. need to be documented to increase the accuracy of nutritional deficiency risk estimates.

In this thesis research, the potential roles of underutilized *Moringa* tree leaves in alleviating or reducing multiple human dietary mineral MNDs, especially Se, has

been demonstrated. Besides, the agro-ecologies in which *Moringa* spp. grow overlap with the regions where the prevalence of mineral MNDs is high. However, cultivation and dietary utilization of these hardy and multipurpose species is not common in comparison with its high potential as a human dietary source. Furthermore, those households which cultivate and utilize the *Moringa* spp. face several challenges, such as disease, and lack of improved varieties, nutritional information and markets to sell *Moringa* products. In localities where these species grow vigorously, a concerted research, development and extension effort is required to overcome these challenges and exploit the potential of *Moringa* spp. in alleviating human mineral MNDs. The food taboo associated with underutilized crop/tree species that limit their usage to times of need should be broken by raising community awareness through educational interventions, so that those readily available food resources are used efficiently.

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