# IMPROVING YIELDS AND ENHANCING DIETARY ZINC AND SELENIUM INTAKE IN THE ZAMBIAN POPULATION THROUGH AGRONOMIC BIOFORTIFICATION OF MAIZE AND WHEAT.

A thesis submitted for the degree of Masters of Agricultural Science

School of Agriculture, Food and Wine

Faculty of Sciences

The University of Adelaide

Christopher Gondwe

September 2018

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

## APPENDICES

## ABSTRACT

Deficiencies of zinc (Zn) and selenium (Se) are major causes of malnutrition-related health problems affecting the world's population. The problem is most serious in developing countries such as Zambia because cereals, which are the principal source of calories, often have low micronutrient concentrations and other dietary sources of Zn and Se are limited. The problem may be exacerbated by low supplies of nitrogen (N) and sulphur (S) because they may play a role in the uptake and deposition of micronutrients in the grain. Soils in the major grain producing areas in Zambia have low N and Zn, while S deficiency is also widespread. In this scenario, grain nutrient concentrations and intakes of Zn and Se in Zambia are likely to be low.

Little attention has been given to Se nutrition in Zambia largely because Se is not essential for plant growth. However, Se is an essential micronutrient for humans and animals and Se deficiency afflicts at least a billion people worldwide, especially in developing countries. There is evidence that many soils in Zambia are low in plant available Se and based on surveys from neighbouring countries, it is likely that the Se concentrations of grain are not high enough to meet consumption requirements of the population.

Despite the important role of Se and Zn in human health, information on their concentration in the grain of crops in Zambia is limited. Two surveys were conducted to document the nutrient concentrations in the grain of maize and wheat grown and consumed in Zambia. One survey sampled grain from farms and the second was based on samples from the market place. All samples of maize (n=67) were deficient in S (median concentration=1030 mg/kg), while 75% were deficient in N (median concentration=1%) and 97% were deficient in Zn (median concentration=19 mg/kg). The survey of wheat was much smaller (n=6), but revealed moderate values of S (median concentration=1335 mg/kg) and Zn (median concentration=26 mg/kg) but adequate N (median concentration=2%). All the samples of both crops were very low in Se (median concentration=16 µg/kg in maize and 8µg/kg in wheat) and based on this an intake of 5 µg Se per day per person was estimated. This is slightly lower than that obtained in Malawi (7 µg

Se per day per person) and much lower than the daily recommended intake of 50-70 µg Se per day. These low concentrations and intakes of Se and Zn are likely to be a health risk contributing to low resistance to infectious diseases and high mortality rates in Zambia which require measures to address the problem.

Two growth room experiments were used to investigate the role of N and S on the vegetative and grain concentrations of N, S, Se and Zn in maize and wheat. Nitrogen application increased the vegetative yields and yields were higher when the N:S ratio was in balance. Sulphur and N nutrition enhanced dry matter and grain yield. No yield reductions were observed due to addition of Se applied as sodium selenate. The concentrations of N, S, Se and Zn increased under adequate S application. Sulphur concentration was also strongly correlated with K and Mo concentrations, nutrients which were also deficient in the maize and wheat samples from Zambia. Selenium concentrations of above 300µg/kg (more than adequate to satisfy dietary requirements) were easily achieved with a low rate of Se of 0.02 mg/kg applied to the soil at planting. The Zn concentration was significantly correlated with N in both experiments and with S in the vegetative tissue in maize, while there was also a significant positive correlation between S and Se in maize. These results suggest that N and S may have a role in Zn and Se uptake and remobilisation from the vegetative parts to the grain.

These studies showed that not only is maintaining a sufficient amount of plant available Se and Zn in the soils a pre-requisite to ensure sufficient uptake of Se and Zn, but also adequate supplies of N and S are important to improve the impact of Se and Zn fertiliser applications. The results of both the surveys and experimental work further suggest the importance of S nutrition in enhancing yields and that inadequacy of S could be a limitation to agronomic biofortification of wheat and maize with Se and Zn. Agronomic biofortification with Se could easily be achieved with soil applications of small quantities of Se as sodium selenate. Therefore, increasing N, S, Se and Zn concentration and content in maize and wheat is a food systems strategy that could improve the intakes of these nutrients for the entire population.

# DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Christopher Gondwe and to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for the loan and photocopying, subject to the provision of the Copyright Act 1968.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue, the Australasian Digital Theses program (ADTP) and the also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Christopher Gondwe

# ACKNOWLEDGEMENTS

A number of people need special mention for their time and effort in assisting me with the completion of this thesis. I would like to especially thank my supervisors, Glenn McDonald and Graham Lyons (University of Adelaide) for providing continued contribution and support. An input of technical expertise, experimental guidance, academic advice, editorial direction and comments are some of the numerous roles they performed, all of which are greatly appreciated.

I am grateful for the assistance I received from many researchers, technicians, scientists and farmers from Zambia, the names of whom are too numerous to mention.

A special thanks goes to my parents for their love, my beloved wife Norah and our lovely children (Joshua and Natasha) for their love, continual support, encouragement and enduring my long absence whilst undertaking the study.

I also wish to thank the Governments of Australia and Zambia for the scholarship and for granting me study leave to take up this study. The University of Adelaide, School of Agriculture, Food and Wine is also acknowledged.

# DEDICATION

This thesis is dedicated to Norah, Joshua and Natasha who have been an exceeding joy and encouragement to work hard. God richly bless you.

# CHAPTER 1

# INTRODUCTION

Micronutrient malnutrition is prevalent throughout the developing world. Zinc (Zn) deficiency is widespread, and is ranked 5th among the 10 most important health risk factors in the developing world (WHO 2002), while low selenium (Se) intakes have been reported to cause several cancers and reduced immunity to infectious diseases (Lyons et al. 2004). Overall dietary diversity in many developing countries is low and cereal staples comprise a high proportion of daily caloric intake among the poor (FAO/WHO 2000). Maize and wheat are the most consumed cereals with maize being the staple for most Sub Saharan countries (SSA) and most developing countries (Graham et al 2001, McGuire 1993) but these grains have low densities of Zn and Se.

The dependency on maize and wheat in the diets of the people necessitates approaches in which food systems are improved to not only meet basic caloric needs, but also supply the dietary Zn and Se requirements. Conventional remedies for nutrient deficiencies in human populations have been food fortification and supplementation programmes (Welsh and Graham 2004), but improving the nutritional quality of cereals through agronomic biofortification can be a sustainable and cost-effective component of a more holistic approach (Welsh and Graham 2004). Nitrogen (N) and sulphur (S) nutrition of plants appear to enhance micronutrient uptake and improving yields (Cakmak 2008; Dev et al. 1979) and a balanced nutritional program may be required to improve Zn and Se uptake by crops in crop biofortification programs.

In sub-Saharan Africa (SSA), most soils under cereal production are inherently low in nutrients and consequently the grain produced has low mineral concentrations (Sanchezi 2002). Cereal production has been on the increase in SSA as more land is cultivated. However, yields are lower than those in the developed world and appear to be constrained by poor soils and relatively low fertiliser use (Table 1.1). Addressing micronutrient deficiencies through addition of deficient nutrients to most soils under cultivation by resource poor farmers who have narrow food choices

Table 1.1. Cereal production in some Sub-Saharan African countries

| Country | Land under cereal production (000) ha | | Fertiliser consumption[a] kg/ha | Cereal yields[b] Kg/ha | |
|---|---|---|---|---|---|
| | 1990-1992 | 2008-2010 | | 1990 | 2010 |
| Kenya | 1,804.8 | 2542.9 | 32.4 | 1562 | 752 |
| Malawi | 1430.2 | 1831.2 | 26.6 | 992 | 2206 |
| Namibia | 195.2 | 309.9 | 1.6 | 457 | 373 |
| Tanzania | 3253.6 | 5018.9 | 8.7 | 1507 | 1332 |
| Zambia | 797.8 | 1216.2 | 27.3 | 1352 | 2547 |
| Zimbabwe | 1168.5 | 1901.8 | 28.0 | 1625 | 752 |

Source: World Bank, Development Indicators 2012 Tables 3.2 and 3.3

[a] Average world fertiliser consumption 122kg/ha, [b] FAO estimate 2756 Kg/ha, world estimate 3568 Kg/ha

could improve yields and intake of micronutrients. Maize and wheat are the most important cereal crops in SSA (FAO, 2009) and of these two cereals consumption of maize is higher (Table 1.2). However, wheat is becoming an important food source and production has been projected to increase (FAO, 2004). Both crops are currently targets of biofortification programs

The rising incidence of micronutrient malnutrition, the so-called hidden hunger, and its impact, especially on women and pre-school children in developing countries, is alarming (UN SCN, 2004). Pfeifer and McClafferty (2007) further state that even mild levels of micronutrient malnutrition may damage cognitive development and lower disease resistance in children and reduce the likelihood that mothers will survive child birth. This could be a direct contribution to relatively high proportion of stunting, low resistance to infectious diseases and maternal deaths in Zambia (World Bank, Development Indicators Report 2012).

Both Zn and Se have demonstrated catalytic, structural and regulatory functions in cells and there is an abundance of evidence in the literature that links low Zn and Se intake to poor health outcomes (Cakmak et al. 2010; IZiNCG 2004; Koivistoinen and Huttunen 1986). Stunted growth and a high prevalence of infectious diseases are proxy indicators of the extent of Zn and Se deficiency. These problems are significant in SSA (Table 1.3). The high national prevalence of child stunting and immunological disorders as well as other morbidity symptoms (e.g. diarrhoea) which are associated with micronutrient malnutrition, indicate chronic malnutrition and poor intake of essential micronutrients across much of southern Africa. Zinc deficiency is thought to be an underlying cause for maternal mortality, and studies in Malawi and Egypt reported a low Zn status for pregnant women (Long et al. 2004).

**Table 1.2. Importance of maize and wheat as a source of calories in selected SSA countries**

| Country | Crop | Production (000tonnes) | Food supply quantity kg/capita/year | Food supply kcal/capita/year |
|---|---|---|---|---|
| Kenya | Maize | 2439 | 77.2 | 672 |
| | Wheat | 219 | 25.0 | 194 |
| Malawi | Maize | 3583 | 133.1 | 1158 |
| | Wheat | 3 | 6.4 | 48 |
| Namibia | Maize | 57 | 68.4 | 562 |
| | Wheat | 12 | 23.4 | 177 |
| Tanzania | Maize | 3324 | 58.1 | 519 |
| | Wheat | 92 | 16.7 | 127 |
| Zambia | Maize | 1887 | 110.2 | 928 |
| | Wheat | 195 | 12.8 | 102 |
| Zimbabwe | Maize | 700 | 110.4 | 876 |
| | Wheat | 40 | 32.4 | 254 |

**Table 1.3. Prevalence of malnutrition percentage of children under 5 and infectious diseases e.g. HIV in the populations in selected countries in SSA.**

| Country | Population | % of children under 5 years stunting | | Prevalence of HIV[b] | | | | |
|---|---|---|---|---|---|---|---|---|
| | (millions) | Males [a] | Female [a] | % of population ages 15-49 years old | | Female | Youth 15-24 years old % of population | |
| | | | | 1990 | 2009 | total of population with HIV | Male | Female |
| Kenya | 40.5 | 37.3 | 33.1 | 3.9 | 6.3 | 59 | 1.8 | 4.1 |
| Malawi | 14.9 | 51.8 | 44.1 | 7.2 | 11.0 | 59 | 3.1 | 6.8 |
| Namibia | 2.3 | 32.0 | 27.1 | 1.6 | 13.1 | 59 | 2.3 | 5.8 |
| Tanzania | 44.8 | 45.9 | 39.2 | 4.8 | 5.6 | 59 | 1.7 | 3.9 |
| Zambia | 12.9 | 48.8 | 42.9 | 12.7 | 13.5 | 57 | 4.2 | 8.9 |
| Zimbabwe | 12.6 | 38.6 | 33.1 | 10.1 | 14.3 | 60 | 3.3 | 6.9 |

Source: World Bank, World Development Indicators 2012 tables 2.1 and 2.20, 2.21 and 2.22

[a]. Data are for the most recent year available 2005-2010.

[b]. Data are for the most recent year available 2009.

Despite these health concerns, little information is available on concentrations of Zn and Se in the grain of maize and wheat in SSA and Zambia in particular. Suboptimal intakes are likely, given that most soils are inherently low in plant available Se and Zn (Melse-Boonstra et al 2007). Similar trends having been observed in other work. Zinc and Se deficiency have been reported in Turkey and Finland, where soils have low levels of plant available Zn (Turkey), and Se intakes by the population are low (Cakmak 2008; Lyons et al. 2004). In these instances, agronomic biofortication has had an immediate positive and sustained impact on the nutrition of the general population. Graham et al (2001) concluded that a new agricultural paradigm is needed to address global micronutrient malnutrition.

*"An agriculture which aims not only for productivity and sustainability, but also for balanced nutrition"*

This is what was called the "productive, sustainable, nutritious food systems paradigm" (Graham et al. 2001).

Many crops of SSA suffer from deficiencies of macro- and micronutrients and while the focus has been on enhanced uptake of Zn and, to a lesser degree, Se, the effectiveness of micronutrient biofortification may be limited if other nutrients are in short supply. The overarching objective of the study reported here is to examine the role of N and S in improving Zn and Se uptake in vegetative tissue and in grain. The research program had the following objectives:

1. To determine the Se and Zn concentrations of maize and wheat grain produced and consumed in Zambia.
2. To estimate the intake of Zn and Se derived from maize and wheat
3. To document the concentration of other essential nutrients in maize and wheat grain in Zambia
4. To study the effect of S and N fertilisation on yield, Se and Zn concentration of wheat grain and maize biomass.

To achieve these objectives, three studies are reported in this thesis. A survey of the grain produced in Zambia was first conducted, which documented the extent of low concentrations of Zn and Se in grain and also highlighted the low S concentrations in crops in Zambia. Subsequently two experiments were conducted to examine the influence of S and N nutrition on Zn and Se uptake. Difficulties in growing maize to maturity with the facilities available meant that the experiment with maize was restricted to responses in the vegetative growth, while the experiment with wheat was able to look at grain yield and nutrient concentration.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1. Introduction

A review of literature on the agricultural production constraints in SSA in general and Zambia in particular is presented. The importance of a balanced supply of N, S and Zn to crop nutrition and of selenium Se in the agronomic biofortification of maize and wheat is then discussed.

## 2.2. Agro ecological diversity and production constraints in Sub-Saharan Africa (SSA)

Sub-Saharan Africa has a total land area of 2.455 billion hectares of which 41% is classified as agricultural land. The sub humid zone accounts for 38% of the total land area of SSA (FAO 2001). The region is characterised by a diverse range of agr- ecological zones and differentiated production and farming systems.

The sub humid zones of the Southern, East and West Africa have variable growing season lengths of between 180 and 270 days. The rainfall pattern in the Southern African region is unimodal with a range of 800-1200 mm per annum, while in the East and Central Africa, it is bimodal, characterised by short and long rainy seasons. In unimodal sub humid areas, the rainfall pattern is highly variable in terms of the start of the season and amount of total rainfall received in the season (Mafongoya et al 2006). This variability in rainfall is associated with mid–season droughts and variable crop yields, especially for maize, and this is arguably a major constraint to production. The Southern Africa region is commonly referred to as the “bread basket of Africa” and is characterised by high variability in soil types, rainfall, altitude and climate (Decker 1993). Zambia is located in this part of SSA.

### 2.2.1. Soil fertility constraints

Low soil fertility ranks as the second most important abiotic stress factor, after drought, limiting maize and wheat production in SSA (Banzinger and Cooper 2001). The soil constraints are of two major types: chemical and physical constraints. The chemical constraints include low nutrient reserves, low cation exchange capacity (CEC), aluminium toxicity and low pH. The physical constraints to increased soil productivity are limited rooting depth, low water holding capacity and susceptibility to soil erosion, crusting and compaction (Sanchez and Logan 1992).

The soils in the SSA are typically old and are a result of parent material, past and current climatic conditions. They are generally leached and have inherently low nutrient levels and consequently are characterised by low N and phosphorus (P) availability and their high acidity is often associated with deficiencies of calcium (Ca), magnesium (Mg) and K and toxicities of aluminium (Al) and manganese (Mn) (Sillanpaa 1982). Deficiencies of other essential nutrients (secondary nutrients) such as S and micronutrients would appear to be widespread and may also limit crop production (Sanchez and Logan 1992). These are however, less frequently reported.

The major soil types in the sub humid zone of East, Central and Southern Africa are shown in Table 2.1. Sandy and sandy loam soils derived from granite, with low organic matter of less than 0.5% and low cation exchange capacities, are widespread in southern Zambia, Zimbabwe, and western and southern Mozambique. Nitrogen deficiency is ubiquitous on these soils, while deficiencies of P, S, Mg and Zn are also common (Grant 1981, cited in Kumwenda et al. 1996). Macronutrients and micronutrients including Zn and boron (B) are reported to be chronically deficient on sandy loam and clay loam soils in Malawi (Wendt et al. 1994), while Zambia has large areas of acidic soils ($pH \leq 4.5$) with high concentrations of free aluminium ($Al^{3+}$) and iron oxide.

It would appear that intensified agricultural production has contributed to this declining soil fertility. In Zambia, it has been found that the largest aggregate nutrient losses from soils are seen in areas where a fallow period has disappeared or the length of the fallow period has

**Table 2.1. Distribution of major soil types in the sub humid zone of Sub-Saharan Africa**

| FAO classification | USDA Taxonomy | Area in $Km^2$ | %Coverage of total Area | Areas of major occurrences |
|---|---|---|---|---|
| Ferralsols | Oxisols | 1847898 | 32 | Democratic Republic of Congo (DRC), Zambia, Rwanda, Burundi, Uganda, Southern Sudan |
| Acrisols | Ultisols | 530603 | 9 | Subhumid, West Africa, Southern, Guinea, Togo, Benin, Nigeria, Cameroun, Coted'lvoire |
| Lixisols | Alfisols | 1666151 | 29 | South-East Africa, Madagascar |
| Nitisols | Paleustults/Paleulstaf | 435931 | 8 | Ethiopia, Kenya, Tanzania, Ethiopia, East DRC |
| Arenosols | Psamments | 580433 | 10 | Sudan, Tanzania |
| Vertisols | Vertisols | 301258 | 3 | Sudan, Tanzania |
| Others | Various | 158785 | 9 | Limited occurrences |

Source: Decker 1993

decreased to a point where it is often insufficient to maintain soil fertility due to increased cropping intensity (Mafongoya et al. 2006). The reduction in fallow periods has been due to increased population and the need to intensify production to achieve greater production from the cropping systems. However, the intensification has not been matched by a proportionate increase in nutrient inputs, either from organic manures or fertiliser.

### 2.2.2. Production characteristics of maize and wheat in SSA

Cereal grain production is the dominant agricultural system in SSA, with maize (*Zea mays* L) accounting for over 50% of the cropped area and the calories consumed in many countries in the region (Byerlee et al. 1994; Silesi et al. 2010). The importance of maize in the region makes it a strategic commodity whose cropping intensity has expanded even into marginal areas. This has led to average yields of maize stagnating at around 1-2 t/ha in the region (FAO 2008), although the demand for maize in this region keeps rising.

Rosegrant et al (1996) estimated that demand for maize in SSA would rise from 21.3 million tonnes per year in 1990 to over 52 million tonnes in 2020. However, low and variable yields have led to food insecurity for 6-9 months because production has not kept pace with consumption, despite the cultivation of improved varieties (hybrids) and better agronomic practices. This has resulted in countries in the region importing up to 10 million tonnes of maize each year (Cassman 2007). A plausible reason for slow increases in productivity is that the smallholders, who produce most of the maize, grow it on low fertility soils that are subject to erosion and loss of organic matter (Silesi 2010).

Many countries in SSA grow a small amount of wheat and the region contributes less than 2% of all wheat in the developing world. However, there is a growing demand for wheat and production is projected to double from 2.6 million tonnes in 1997 to 5.1 million tonnes in 2020, due to increases in in area and yield (CIMMYT 2004).

Most of the soils in SSA appear to be extremely depleted of nutrients and will only sustain crop production with judicious regular additions of nutrients. Until recently, little attention has been given to balanced crop nutrition and soil fertility management, especially micronutrients, because the focus has been on N, P, K and gains in production in maize from improved yields as a result of hybrid seeds (Weil and Mughogho 2000, Kumwenda et al. 1996). The nutrient imbalances may contribute to low yields and nutrient concentration levels in the grain. Improvement of micronutrients in cereal based diets of most developing countries, including Zambia, is essential, given that micronutrient malnutrition is a major contributing factor to health problems and maternal death in the region. Dealing with these problems requires exploring means of producing abundant quantities of food containing sufficient quantities of bioavailable micronutrients. Therefore, strategies are needed to optimise nutrient use efficiency if enough food is to be produced and alleviate both “classical” and “hidden” hunger in the region.

### 2.2.3. Importance of soil fertility to human health

As discussed above, the inherently poor soil conditions over much of SSA are a contributing factor to food insecurity and malnutrition, which are the biggest risk factor for human illness and disease in the region (Sanchezi 2002; Sanchezi and Swaminathan 2005). This is because the primary source of all nutrients for people is plant products, either consumed directly or via animals. Therefore, low contents of mineral nutrients in the diet could be attributed to deficient levels in edible parts of staple crops or indirectly to animal products which could have been fed on plants with low concentrations of micronutrients (Bouis and Welch 2010).

The consequences of insufficient intake of nutrients have been described in several reports in the literature as being sickness, poor health, impaired development in children and large economic costs to society (Branca and Ferrari 2002; Golden 1991; Grantham-McGregor and Ani 1999; Ramakrishnan et al. 1999; Welch and Graham 2004). Equally, the extent of the widespread deficiencies has been cited in White and Broadley (2005): of the world’s human population, 60–80% are Fe deficient, >30% are Zn deficient, 30% are iodine deficient and about 15% are Se deficient.

This scenario is likely to be worse in SSA given the diets primarily consist of staple cereal crops (maize, rice, wheat, sorghum, and millet) that may not meet protein and energy demands (Stein 2010), with children under the age of five and women being most severely affected. Poor diet as a result of low intake of micronutrients can significantly contribute to compromised immune function and to the high incidence and prevalence of infectious diseases with important implications for the HIV/AIDS ravaged SSA (Table 1.3).

The current situation in the developing countries indicates that nearly two-thirds of all the deaths of children are associated with nutritional deficiencies, mainly from micronutrient deficiencies (Caballero 2002). Micronutrient, protein and energy malnutrition are of at alarming proportions in many developing countries, including Zambia, probably because of reduced crop yields and low concentration of trace elements (St.Clair and Lynch 2010).

## 2.3. The Zambian agriculture sector

The Zambian environment is suited to both rain fed and irrigated cereal crop production with maize and wheat (*Triticum aestivum* L) dominating production in the summer and winter, respectively. These crops play an important role in satisfying daily calorie and protein needs in Zambia and individual yields of up to 10 t/ha of maize and 6 t/ha of wheat are possible. However, many Zambian soils have low fertility and wide variations in yields across climatic and soil conditions occur (Ministry of Agriculture Crop Forecast Survey 2004-2015).

### 2.3.1. Climate

The high plateau on which Zambia is located ensures that the country has a moderate climate. Two climatic factors are important to the Zambian agriculture system, temperature and rainfall, and the annual variation in these defines three distinct seasons in Zambia: 1) a rainy season in summer from November to April 2) a cool dry winter from May to August and 3) a hot dry season in September and October. Summer temperatures rarely exceed 35°C, but rainfall is unevenly distributed throughout the year, with the majority concentrated in the six months from November to April. Nevertheless, the Zambian climate is favourable for agricultural production with abundant arable land receiving 650 mm of annual rainfall in the southern part of the country and above 1000 mm in the north of the country. There is great variation in rainfall, elevation, mean temperatures, vegetation and soils among these agro-ecological zones and within the zones themselves. The main features of the agro ecological zones in Zambia are summarised in Table 2.2.

The varied nature of these environments makes it possible to grow a wide range of crops throughout the country. Consequently, Zambia is divided into 36 agro-ecological zones which are grouped in three major zones, mainly on the basis of rainfall (Figure 2.1). Zone 1 is characterised by low rainfall, a short growing season, and high temperatures during the growing season, and a high risk of drought. Zone 3 is characterised by high rainfall, long growing season, low probability of drought, and cooler temperatures during the growing season. Zone 2 is intermediate between Zones 1 and 3 for most climatic variables. This region is further distinguished into 2a and 2b based on the soil distribution. The predominance of rain-fed cultivation means that the agricultural sector's performance is strongly correlated with the rainfall pattern and the country's agricultural production is extremely vulnerable to fluctuations in annual rainfall. Rainfall is variable and most of the time it is poorly distributed over the growing period, especially in the southern part of the country.

**Table 2 2. Location and major characteristics of Zambia's agro ecological zones**

| Zone | Location | Elevation (m) | Average rainfall (mm/year) | Growing season rainfall (mm) | Drought risk | Occurrence of frost in dry season | Minimum monthly temperature (Dec-Feb) (ºC) |
|---|---|---|---|---|---|---|---|
| Zone 1 | Major valleys e.g. Gwembe, Lunsemfwa and Luangwa as well as southern parts of western and southern provinces. | 300-900 | 900-1200 | 80-129 | Medium-high | Risk on plateau areas | 19-21 |
| Zone 2a & 2b | Sandveld plateau of Central, Eastern, Lusaka and Southern Provinces, including Kalahari sand plateau and Zambezi flood plains of Western province | 900-1300 | 800-1000 | 100-140 | Medium-Low | Risk on the Central plateau | 17-18 |
| Zone 3 | Part of the Central African Plateau covering Northern, Luapula, Copper belt, North western Provinces and northern parts of Serenje and Mkushi Districts. | 1100-1700<br>(< 1000 in Luapula) | >1000 | 120-150 | Almost nil | Some risk in the south west | 14-16 |

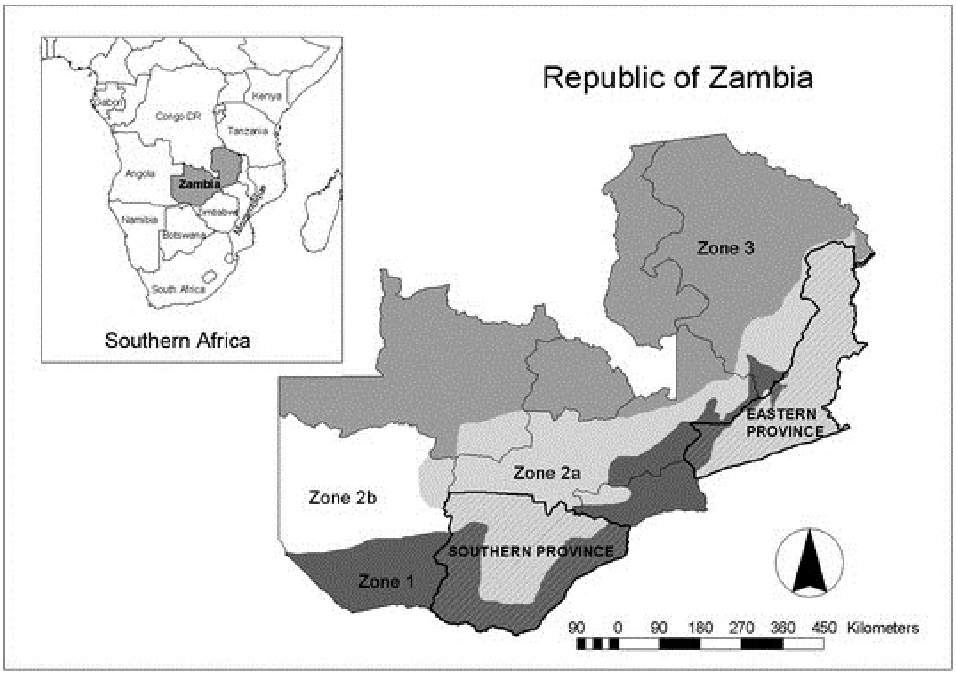

Figure 2.1. Location map of Zambia with agro ecological zones. Source: Springer Images, www.springerimages.com/images

### 2.3.2. Soils and crop distribution

The soils in Zambia are variable with 21 different aggregated groups. They can broadly be categorized into Zones that have tended to dictate the farming systems practised. Figure 2.2 shows the major soil group classification and distribution and Table 2.3 summarises their characteristics and limitations to crop production across the country. These important soil groups influence the cropping systems in the three agro ecological zones as briefly described below:

Zone 1: Consists of Haplic Luvisols and Haplic Solonetz on the flat land and Dystric Leptosols on the hills and ridges (FAO 1973). The limitation of these soils is that they are highly erodible. Arable production is concentrated on pearl millet (*Pennisetum glaucum*), sorghum (*Sorghun bicolour* L Moench) and livestock. Given the environmental limitations in this zone, it would appear that cropping of drought tolerant crops is the only viable cropping system. Food security concerns predominate due to recurrent food shortages caused by the low and variable yields.

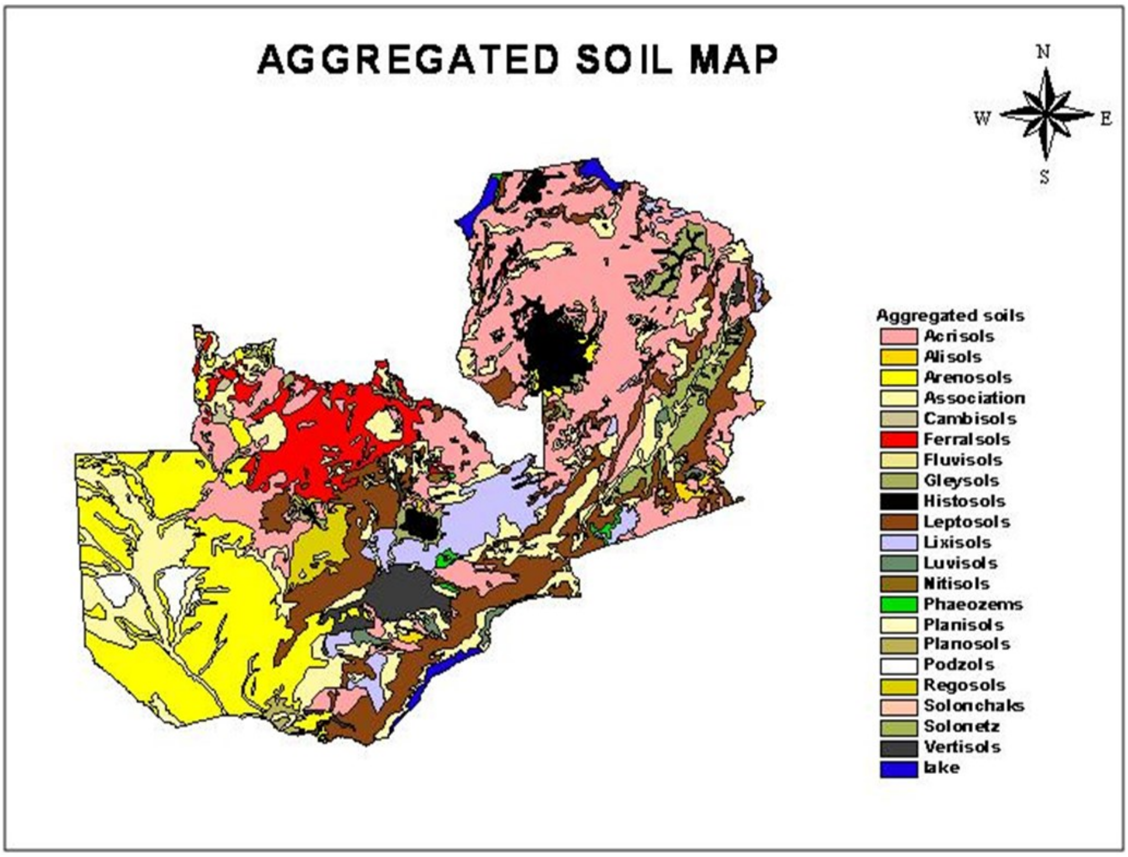

Figure 2.2. Soil group classification of agro ecological Zones. Source: K. Munyinda, University of Zambia, School of Agricultural Sciences (2011)

Zone 2a: The soils are mainly Haplic Lixisols, Haplic Luvisols and Haplic Acrisols (FAO 1973). These soils are more productive with few limitations, and permanent cultivation of sorghum, maize, groundnuts (*Arachis hypogaea* L), and cow peas (*Vigna unguiculata* L). A range of cash crops is also grown including tobacco (*Nicotiana tobacum* L), sunflower (*Helianthus annuus* L), irrigated wheat, soybean (*Glycine max* L) and a range of horticultural crops.

**Table 2.3. A description of the major soils and their limitations in the agro ecological zones of Zambia**

| Zone | General description of soils | Limitations to crop production |
|---|---|---|
| Zone 1 | Loamy and clay with fine tops | Slightly acidic to alkaline, minor fertility limitation |
| | Reddish coarse sand soils | Low pH, available water & nutrient capacity reserves |
| | Poorly drained sandy soils | Severe wetness, acidic & low fertility |
| | Shallow &gravel soils in rolling to hilly areas including escarpment zones | Limited depth & unsuitable for cultivation |
| Zone 2 | Moderately leached clayey to loamy soils | Low nutrient reserves & water holding capacity |
| | Slightly leached clayey soils | Slight to moderate acidity, difficult to work due to heavy textured soil. |
| | Coarse sandy loams in large valley dambos | Imperfectly drained limitation due to wetness |
| | Sandy soils on Kalahari sand | Medium to strong acidity, coarse textured top soil, low water holding capacity and nutrient reserves |
| Zone 3 | Red to brown clayey loamy soils | Very strong acidity and strongly leached |
| | Shallow and gravel soils in rolling hilly areas | Limited depth |
| | Clayey soils, red in colour | Moderately to strongly leached, fewer limitations |
| | Poorly to very poorly drained flood plain soils | Variable texture and acidity |
| | Coarse sandy soils in the pan dambos on Kalahari sand | Very strong acidity |

Source: A.Bunyolo, B.Chirwa and M.Muchinda. Cited in Stephen W.Muliokela (ed) (1995)

Zone 2b: This is the aggraded Western plateau. The soils are Ferrallic Arenosols which are infertile, coarse sands. Cassava (*Manihot esculentum* L), bulrush millet (*Pennisetum glaucum* L) and Bambara nuts (*Vigna subterranean* L) predominate on the upland with some maize and sorghum and in the flood plain rice (*Oryza sativa*), maize and sorghum are grown.

Zone 3: The soils are mostly Haplic Acrisols which are highly leached and acidic. Traditional farming systems are based on slash and burn. The main crops are finger millet (*Eleusine coracana* L), beans (*Phaseolus vulgaris* L) and cassava. Cash crops include maize, sunflower, coffee (*Coffea arabica* L), tea (*Camelia sinensis* L), tobacco, irrigated wheat and soybeans.

In general, in the Western plateau the Kalahari sands cover the degraded, more heavily textured soils which, in combination with unreliable rainfall patterns, substantially reduce the capability for arable crops. These parts of Zones 1 and 2 are largely grasslands and mainly suitable for cattle production in their natural state. Maize production is better suited to the northern parts of Zone 2 with loamy to clay–loam soils while tobacco and cotton do well in the southern part of Zone 2. However, maize production dominates throughout all the Zones. In drought years, Zone 3, despite having highly leached acidic soils, has proved to be a reliable maize producer and a net exporter to other provinces (Saasa 2003).

Wheat in Zambia is grown in the winter under irrigation, predominantly in Zones 1 and 2. Although, Zambia had historically imported wheat, steady production gains have met the consumption requirements for the country. In recent decades wheat has become increasingly important as a basic staple food, particularly in urban areas where it accounts for higher proportion of the budget than maize (Mason et al 2009).

### 2.3.3. Farming systems

Based on the area cultivated with crop and the production focus, the farmers are categorised as small scale, emerging, medium or commercial (Table 2.4). The physical environment has had an important effect on the nature of the farming systems practised throughout the country. However, smallholder and emerging farmers make up approximately 70% of the total farmers and produce more than 30% of the marketed maize. The commercial farms are few and are normally concentrated along the railway line. All the wheat in the country is grown by this category of farm. The increase in wheat production in Zambia has depended mainly on development of infrastructure such as electrification and installation of irrigation systems (centre pivots) on the vast cleared tracts of land. The potential for further increased production of wheat still depends on continued infrastructure development (Mbumwae and Riddell 2002).

As discussed earlier, many soils in Zambia have low macro and micronutrients and this affects yield and grain nutrient levels. According to Jha and Hoijjhat (1993), early research work showed that the major nutrient deficiency in the soil is N and recommended P and K levels were essentially perceived as for maintenance. However, in the recent literature large areas of the major soils in Zambia have been reported to have low to deficient levels of S, Se and Zn (Tsuji et al 2005, Melse-Boonstra et al 2007 and Chirwa and Yerokun 2012). Further discussion is given in Section 2.4.

A review of world literature on soil micronutrients shows that Zn deficiency is the most serious constraint to crop production in the tropics, including Zambia, and is becoming as important as the deficiencies of N, P, K, S and Ca (Allaway 1986; Bouis and Welch 2010). It appears too that the use of NPK fertilisers increases the crop demand for micronutrients (Kannwar and Youngdahl 1985), consequently their balance and supply should be equally important. However there has been relatively little recent work in Zambia that has documented the extent and severity of the nutritional problems.

**Table 2.4. Characteristics of Zambian Agriculture**

| Characteristics | Farm category | | | |
|---|---|---|---|---|
| | Small scale | Emergent | Medium | Large scale |
| Total area (ha) | 05-9.0 | 10-20 | 20-60 | Over 60 |
| Crops grown | Food crops | Food/cash | Food/cash | Cash crops |
| Production focus | Subsistence | Commercial and subsistence | Commercial and subsistence | Commercial |
| Means of production | Hand hoe | Hand hoe/oxen | Oxen/tractor | Tractor |

Source: Ministry of Agriculture and Cooperative, 2005

Promoting NPK fertiliser use has been an important element of Zambian agricultural strategy since the 1950s, although the rates of application have been low with current rates fluctuating between 6-12 kg per hectare per year (FAO stats 2010). The use in Zambian agriculture is highly skewed to maize production with nearly 90 % of Zambia's total fertiliser being applied to maize (Jha and Hoijjihat 1993).

The current recommendation is to apply 200 kg/ha of D compound (10-20-10) and 200 kg/ha of urea to maize (Jha and Hoijjati 1993), which delivers 112 kg N/ha, 40 kg P/has and 20 kg K/ha. The recommendations are based on smallholder cultivation with yield expectations of 3-4 tons of maize per hectare. The maize and wheat recommendations for commercial farmers are higher with 300 kg of compound D and 300 kg of urea or ammonium nitrate for expected yields of 6.5 tons per hectare or higher of maize and wheat. To meet these nutrient needs a number of types of commonly used fertilisers are available in Zambia (Table 2.5).

**Table 2 .5. Composition of commonly available fertilisers in Zambia**

| Fertiliser | Nutrient | | | |
|---|---|---|---|---|
| | N | P | K | S |
| Compound D | 10 | 8..8 | 8.3 | 9 |
| Compound X | 20 | 4.4 | 4.2 | 9 |
| Urea | 46 | 0 | 0 | 0 |
| Ammonium nitrate | 36 | 0 | 0 | 0 |
| CAN | 26 | 0 | 0 | 0 |

Source: Extracted from profitability study of maize and wheat, Michigan State University, 2007.

The above set of recommendations is not the only one available to extension agents and farmers. In other work, Gumbo (1988) proposed three levels of fertiliser application based on the initial soil fertility status and recognising that higher fertility land needs lower levels of inorganic inputs (Table 2 6). Another bulletin recommended the following rates for maize on well rotated land 160 kg N/ha, 31 kg P/ha and 29 kg K/ha (Department of Agriculture Zambia 1989).

**Table 2.6. Fertiliser recommendations (kg/ha) for maize based on initial soil fertility status**

| Fertility status | N | P | K | $S^A$ |
|---|---|---|---|---|
| Low | 160-180 | 31-44 | 29-33 | 20 |
| Medium | 120-140 | 18-26 | 8-17 | 20 |
| High | 80-100 | 9-13 | 0 | 20\ |

$^A$ the rate of S is the minimum amount needed and higher rates can be used.

Source: Gumbo 1988 cited in “Framework and initial analysis of fertiliser profitability in maize and cotton in Zambia”, downloadable at http://www.aec.msu.edu/agecon/fs2/Zambia/index.htm

The above recommendations may appear universal and focus mainly on the N, P, and K and also include other management practices that would improve yields for the farmer, but little attention has been paid to the minor nutrients such as S and the micronutrients, such as Zn and Se which have been reported to be nutritional problems in plants and/or the human population in the other regions of Southern and Central Africa.

The current concentrations of N, S, and Se and Zn of individual crops is difficult to estimate because these are not regularly measured in Zambia. Therefore, the poor documentation of soil and plant nutrient concentrations in Zambia means there is insufficient information that can be used to predict yields and nutrient concentrations of produce from the farms and nutritional quality of grain that is consumed by the general population, especially in rural areas.

It would appear that site specific recommendations would be difficult to implement given the soil fertility and climatic variability in the zones and within the zones themselves. The potential increases in productivity and grain quality on these poor soils however, will only be achieved by balanced nutrition between macro and micronutrients. In addition, balanced crop nutrition will greatly aid in improving the micronutrient concentration of these important crops and benefit the resource poor rural population, whose diet is principally cereal-based (McDonald et al. 2008; Yilmaz et al. 1998).

## 2.4. Zinc nutrition in plants and animals

Micronutrients such Zn have important functions in living organisms and are essential for humans, animals and plants. Deficiencies of micronutrients may also cause imbalances in the normal functions of the living organism. The role of Zn in plants and animals respectively will be discussed below.

### 2.4.1 Zinc and crop productivity

Zinc is an essential micronutrient for plant growth and development (Marschner 1995). Among crop plants increased growth of wheat, oats, maize, lupin and peas due to Zn application was first reported as early as 1914 by Javilier (cited in (Thorne 1957). The essential nature of Zn as a fertiliser was accepted when Sommer and Lipman (1926 cited in Brown et al. 1993) demonstrated that Zn was essential for plant growth and reported increased yields of barley, sunflower, buckwheat, beans and vetch due to Zn application. By the early part of the 20th century the agricultural significance of Zn had been recognised, but it was not until the mid-1930s that the first case of Zn deficiency was reported in the field (Chandler 1937).

The native Zn pool in the soil is the dominant factor determining grain Zn concentration followed by genotype and fertiliser treatments (Wissuwa et al. 2008). Maintenance of an adequate amount of available Zn in soil or high Zn concentrations in seed ensures good root growth and contributes to protection against soil-borne pathogens (Alloway 2008). Plants emerging from seeds with low Zn have poor seedling vigour and field establishment on deficient soils (Yilmaz et al. 1998). Therefore, increased amounts of Zn in seed could enable reduction in seeding rates due to improved seedling establishment, more vigorous plants and higher yields (Welch 1999), which could result in substantial economic benefits to resource-poor farmers and the to the country. Most small-scale farmers in Zambia grow both open pollinated varieties and hybrid maize varieties and the likelihood of re-sowing given the scale of production is high. Consequently, when seed with low concentrations of Zn is re-sown, the ability of the new crop to withstand environmental stresses at the early growth stages is greatly impaired.

### 2.4.2 Extent and degree of zinc deficiency in Zambian soils

Zinc deficiency is acknowledged as a worldwide problem and has become a significant constraint to crop production, particularly in cereal crops produced on calcareous soils of the arid and semi-arid regions (Cakmak 1988a). In Zambia, Zn nutrition is becoming significant for crop production and quality as well. It appears that depletion of soil Zn reserves due to intensive cultivation may have increased Zn deficiency in soils and it is now recognised as one of the most widespread micronutrient deficiencies in the country.

According to a study by Sillanpaa (1982), irrespective of the methods used for determining extractable soil Zn, the distribution of Zambian soil and plant Zn contents are much alike and correlations are good. The soil and plant Zn values are within the “normal” international level, but soils at several locations showed such low Zn concentrations that disorders due to Zn deficiency are likely. All of the soils submitted for that study came from Zones 1 and 2 of the agro ecological zones.

In addition, work by Banda and Singh (1989); found low available soil Zn for maize in Zone 3 of the country. Their work appears to agree with the above observations of Silanpaa (1982) and, depending on the extraction method, critical levels for major soils in the high rainfall areas of Zambia were found to be 0.7, 2.0 and 1.5 mg Zn/kg using 0.005M DTPA, 0.1N HCL and NH4OAc-EDTA methods respectively. Most soils in the country fail short of these critical levels except from well fertilised arable land.

Although limited literature on the degree of deficiency is available on Zn in Zambia, based on soil properties and cropping history, large areas are likely to be low in available Zn and Zn deficiency will more likely manifest as hidden hunger and contribute to low crop productivity. There have not been consistent visual observations of wide-spread Zn deficiency in crops grown on these soils arguably due to the higher likelihood for deficiencies of N, P, K and S to appear earlier.

### 2.4.3 Physiological importance of Zn

Zinc is taken up by plants predominantly as $Zn^{2+}$ and mainly functions as a divalent cation by coupling enzyme with corresponding substrates and forming tetrahedral chelates with different organic compounds such as peptides (Brown et al. 1993). Zinc acts as a functional, structural or regulatory cofactor and is a constituent of many enzyme systems.

Zinc-dependant enzymes are involved in macronutrient metabolism and cell replication, (Arinola et al. 2008; Hays and Swenson 1985). Zinc is also necessary for the production of chlorophyll and carbohydrates. Zinc deficiencies in plants first appear in the young leaves because of its low mobility in the plants and in maize it is often referred to as “white tops” because the young leaves at the top turn white or light yellow during early growth. Leaves may develop broad yellow bands (chlorosis) on one or both sides of the midrib, while other symptoms include bronzing in rice and legumes, little leaf and rosetting of subterranean clover, fruit trees and severe stunting of maize and beans. Zinc is also important in maintaining the integrity of cell membranes.

In humans, Zn deficiency can result in disease or symptoms including hypogonadism, growth failure, impaired wound healing, and decreased taste and smell acuity. Zinc is also necessary for optimum insulin action (Murray et al. 2000), cell replication and gene expression (Soeten et al. 2010), Vitamins A and E metabolism and bioavailability also depends on Zn status (Szabo et al. 1999). It is ultimately necessary that due to the above vital functions of this trace element it is important to monitor its intake to improve immune function in the general population at large and especially the women and children.

### 2.4.4 Diagnostic criteria of Zinc deficiency in maize and wheat

Plant tissue analysis has become widely recognised as an effective tool for the diagnosis of the nutrient status of crop plants and the information provided is used as a guide to nutrient management for optimal plant production. The critical deficiency concentration for a particular nutrient has been defined as the nutrient concentration in the tissue where there is a 10% reduction in the yield due to nutrient deficiency (Ulrich and Hills 1967). In practice, the critical deficiency concentration is not a single value but rather a narrow range of nutrient concentrations above which the crop is adequately supplied with nutrients and below which the crop is deficient (Dow and Roberts 1982).

Critical levels are defined for specific plant parts e.g. youngest emerged blades (YEBs), whole shoot and for defined growth stages. In wheat, the critical deficiency has been reported to be 16-18 mg/kg dry weight, based on the Zn analysis of the YEB at both the seedling stage and anthesis (Riley et al. 1992; Wilhelm et al. 1993). However, under some conditions critical concentration may be as low as 11 mg/kg as found by Brennan (1992) using YEBs sampled at the six-leaf stage. For diagnosis of grain Zn approximately 23-30 mg/kg should be adequate (Graham et al. 2001). In maize critical deficiency based on mature leaves 56 days after sowing has been found to be 15 mg/kg (Banda and Singh 1989) and adequate levels at 50% silking blade opposite and below the cob (BOBC) of 11-50 mg/kg (White et al 1987).

### 2.4.5 Interaction with Nitrogen and Sulphur

As discussed above, soil fertility is a manageable soil property and its management is of utmost importance for optimising crop nutrition, in both the short and long-term to achieve sustainable crop production. The macro elements such as N and S are essential in meeting these demands. However, low soil levels of these nutrients are wide-spread in Zambia and an important factor limiting crop production.

#### 2.4.5.1 Nitrogen

Nitrogen appears to affect Zn status of the crops by promoting both plant growth and by changing the pH of the root environment. In many soils N is the most limiting factor on growth and yield and not surprisingly improvements in yield have been found through positive interactions by applying both N and Zn fertiliser. Crops may respond to application of N and Zn together and not Zn alone (Alloway 2008). The application of N in the absence of Zn can lead to Zn deficiencies through a dilution effect brought about by an increase in growth due to N. This can also result in deficiencies of other micronutrients such as copper if they are also of marginal status in the soil (Kirk and Bajita 1995). This is the case in many Zambian soils. Low N supply leads to low protein in seed and plant vegetative parts which may also limit the concentration and bioavailability of Zn (Kutman et al. 2011).

It is important to note that very little plant-available N is present in the soil in its natural state, as most of the N is contained in the soil organic matter. The total amount of N in the soil therefore depends on the organic matter concentration in the soil. Most soils in Zambia have low soil organic matter and N fertiliser addition is necessary to achieve meaningful yields. Nitrogen fertilisers, such as ammonium sulphate and ammonium nitrate, can have acidifying effects and can lead to an increase in Zn availability, but care should be taken for maintenance liming. Nitrates also remain in the soil solution and are therefore subject to leaching, particularly in the sands and other free draining soils in high rainfall areas.

### 2.4.5.2 Sulphur

Sulphur is an essential element for plant growth and is an important limiting factor in crop production including maize and wheat. Generally, sandy soils with low organic matter content have been identified as being responsive to fertiliser S (Pangani and Echeveria 2011).

The need for S is closely related to the amount of N available for crops. The role of S in the activity of the enzyme nitrate reductase, whose low activity depresses soluble protein levels, is important. Low activity of nitrate reductase can inhibit seed formation in sensitive crops and impact negatively on yield. Therefore, S cannot be ignored when evaluating N use efficiency.

Sulphur is primarily absorbed as the sulphate ($SO_4^{2-}$) anion but can also enter plant leaves from the air as sulphur dioxide ($SO_2$) gas. Sulphur is part of every living cell and a constituent of 3 of the 21 amino acids that form proteins, cysteine, cystine and methionine.  Also, thiamine and biotin (members of the vitamin B complex) and coenzyme A contain S in their molecules (Malhotra 1998). Sulphur is also needed for S amino acids that are precursors of nicotianimine that is involved in the transport of micronutrients including Zn and Fe (McDonald and Mousavvi-Nik 2009; Zeng et al. 2010).

Plants deficient in S show a pale green colour in the younger leaves, although the entire plant can be pale green and stunted in severe cases. Sulphur deficiency symptoms are similar to those of N as both are constituents of proteins. An important distinguishing factor in the early stages of deficiency is that N deficiency is more severe in older leaves because N is a phloem mobile plant nutrient and moves to new growth. Sulphur on the other hand is less mobile in the phloem, so new growth suffers first when S levels are not adequate to meet crop need.

As discussed above, there is little information on the extent and severity of S deficiency in Zambian crops. However, given the low to deficient S levels in most major soils, it is likely that many crops grown on these soils are low to deficient in S partly due to repeated trash burning over a long period of time and inadequate deliberate addition of this nutrient.

#### 2.4.5.3 The N:S ratio

The use of ratios in the interpretation of plant analysis results involves the evaluation of two essential elements together recognising the effects of one element on the other. The ratio between N and S is important to measure and understand because of the impact it has on N use efficiency, plant vigour, water use efficiency, phosphate use, carbohydrate production and utilisation, rate of grain fill, maturity and many other plant factors.

The N: S ratio primarily reflects the complementary relationship that N and S have in producing plant proteins and grain fill. If the ratio of the sample is higher than 15:1, additional S is required to effectively use the N in the crop. If the ratio is below then no additional S is required but, there may be a need for additional N. In reality the plant doesn’t assimilate nitrogen well if S is limiting and crops show little or no response to N fertiliser until the S deficiency is corrected.

## 2.5 Selenium

Selenium has an atomic weight of 78.96 and an atomic number of 34. It lies between S and tellurium in Group 6A and between arsenic and bromine in Period 4 of the Periodic Table. This position accounts for many of its biological relationships with S, arsenic and P (Lyons et al. 2004). Despite its rarity in the earth’s crust Se plays an important role in animal and human nutrition.

However, it was not until the work of Schwarz and Foltz (1957), that Se was recognised as an essential nutrient for humans; it was known mainly for its toxicity and considered a carcinogen. This led to the objections by the US Food and Drug Administration to its use as an additive in livestock feed (Oldfield 1981). It is thus ironic that Se now arouses most interest as an anti-carcinogen (Lyons et al. 2004).

### 2.5.1 Selenium in soil

The ultimate source of all Se in plants is the rocks and soils of the terrestrial environment in which it is ubiquitous but unevenly distributed. Soil concentrations range from less than 0.1 to more than 100 mg/kg and total soil Se of 0.1 to 0.6 mg/kg is considered deficient (Melse-Boonstra et al 2007), but total soil Se concentrations may not be a reliable predictor of Se availability.

Granite soils, particularly in humid, high rainfall areas are likely to be deficient but availability of Se to the plants is influenced by soil pH, aeration and presence of iron oxides. Selenium is poorly available in acidic and poorly aerated soils occurs because it occurs as insoluble selenides and elemental Se. In lateritic soils, which have a high Fe content, Se binds strongly to DFe oxides to form poorly soluble ferric hydroxide-selenite complexes (Cary and Allaway 1969). Elemental Se, although stable in soils can be slowly oxidised, particularly at high pH (Geering et al. 1968). Selenium is available in aerated acid or neutral soils where selenites form and in aerated alkaline soils in the selenate form (Cary and Allaway 1969). Selenate is highly soluble and the dominant species in slightly acid to alkaline conditions and is easily taken up by plants (Melse-Boonstra et al.2007).

In Zambia it is expected that the predominantly highly weathered soils would be quite deficient in Se. A survey of some selected soils for Se levels in the country showed that the Se levels in the sampled locations fell below the reported range of mean worldwide values for this element of 0.5-1.27 mg/kg (Melse-Boonstra et al. 2007). Consequently, the low Se status of the soil is not likely to result in any significant accumulation of the element in the crops that grow on it.

### 2.5.2 Selenium availability and uptake in plants

The Se content of plants varies according to available soil Se and species. Although lower plants such as algae require Se for growth (Lindstrom cited in Lyons et al. 2004), it is not considered to be an essential nutrient for higher plants (Terry et al. 2000). Rhizosphere processes play an important role in the availability of Se for plant uptake. In particular, ascorbic and gallic acids and manganese oxides can increase oxidation of selenites to selenate (Blaylock and James 1994).

Surveys indicate that wheat is the most efficient accumulator of Se among the common cereals such as rice, maize, barley and oats (Lyons et al. 2005); however, these Se levels appear to be influenced by soil Se status. Therefore, it is likely that room exists for improvement of Se status in Zambian crops with Se application and consequently, there is considerable potential for improving the status of certain segments of the population with inadequate Se intake. As noted by Lyons et al. (2005), a substantial increase in the population’s Se intake may result in decreased rates of several important cancers, cardiovascular disease, viral diseases progression and a range of other conditions that involve oxidative stress and inflammation.

### 2.5.3 Selenium and human health

Selenium has a role in many respects of the immune response to infections and its contribution to the integrity of the immune system is a major feature of its nutritional functional role discussed below.

Selenium is a constituent element of the entire defence system that protects living organisms from harmful action of free radicals (Soeten et al. 2010). It is a constituent of the antioxidant enzyme glutathione peroxidase, whose concentration is monitored in the blood or liver to determine whether animals are at risk from selenium deficiency. Selenium deficiency results in white muscle disease, an illness that causes high mortality in young calves and lambs.

It is also important in the prevention of diseases closely associated with oxidative stress e.g. cardiovascular disease, Alzheimer’s disease, Parkinson’s disease and many other diseases (Pollack and Leeuwenburgh 1999). It appears Se is also of significance to people with HIV/AIDS as it is an antioxidant that increases immune function and it has been shown that RNA viruses e.g. HIV, Hepatitis B, C, measles, influenza become more virulent in a low Se environment (Beck 2007).

Selenium deficiency is a significant predictor of HIV-related mortality and viral load ((Baeten et al. 2001; Baum et al. 1998; Campa et al. 1999). It is thus important that strategies to increase Se intake as a major public health issue through development of effective and sustainable options are addressed. One such strategy is through crop production, as the case with the Finnish experiment has demonstrated that agriculture can safely, effectively, easily and cost efficiently raise the Se levels in a human population (Graham et al 2005).

### 2.5.4 Selenium interaction with Nitrogen

Research on N and Se interaction is limited, but a number of reports in the literature indicates strong positive association with protein level. For instance, in a survey of wheat and bread in the UK, Barclay and MacPherson (1992) found soft wheats to contain less Se (0.02-0.13 mg/kg) compared to hard wheats (0.05-1.09 mg/kg). Gissel-Nielsen (1979) also found that a high N level strongly increased the Se concentration in maize roots exposed to selenite, but decreased translocation and increased the proportion of selenoamino acids in the xylem sap.

### 2.5.5 Selenium interaction with Sulphur

The literature indicates that soil sulphate level is an important determinant of Se uptake and transport in plants. Studies on crop and pasture plants show that increasing soil sulphate level decreases Se uptake and transport (Hopper and Parker 1999; Ylaranta 1990). The effect can be strong at high sulphate levels where Se concentration and content decreased by more than 90% in perennial ryegrass and strawberry clover (Hopper and Parker 1999).

Similarly, when pasture yields responded to S topdressing, the Se concentration in the legumes present was reduced by up to 50 % (Pratley and McFarlene 1974). This can be explained partly by a dilution effect caused by a growth response of the plant to the applied S (Lyons et al. 2004).

Like S, Se can exist in five valence states: selenide (-2), elemental (0), thioselenate (+2), selenite (+4 e.g. $Na_2SeO_3$) and selenate (+6 e.g. $Na_2SeO_4$). Selenium forms many inorganic and organic compounds that are similar to those of S (Greenwood and Earshaw 1984). Therefore, observed reduction in plant Se level due to increased S is largely due to competitive inhibition as sulphate and selenate use the same S transporter (Lauchli 1993; Lyons et al. 2004). These interactions have implications on soil fertility management and agronomic biofortification with Se by crops given that Zambian soils have both low/deficient S and Se levels.

### 2.6 Biofortification

Biofortification can be defined as genetic or agronomic. Exploiting the genetic variability in crop plants for micronutrient density (genetic biofortification) or approaches to enhance micronutrients through use of fertilisers (agronomic biofortification) can be effective methods to improve the nutrition of entire human populations. Agronomic approaches will be the focus of work reported in this thesis but first an introduction to genetic biofortification is provided.

#### 2.6.1 Genetic biofortification

Genetic biofortification been defined as the development of micronutrient-dense staple crops using the best traditional breeding practices and modern biotechnology (Nestel et al. 2006). Breeding new genotypes for high micronutrient concentration is the most cost effective and long-term strategy to address the problem. However, genetic variation for Zn concentration in maize grain is moderate and is mostly within the range 15-35µg/g (Banzinger and Long 2000; Long et al. 2004; Sinyinda and Mwala 2010; Welch and Graham 2004) which constrains this option. The small range in Zn concentration in maize grain suggests that breeding needs to be supplemented with agronomic biofortification, at least in the short to medium term. In contrast to maize, substantial variation in Zn density in wheat accessions grown together have been demonstrated (Graham et al. 2001), and screening for efficient types makes breeding feasible, but it appears there has been no clear evidence of genetic variability between wheat cultivars for grain Se density (Lyons et al. 2004). However, substantial variability exists within cereal crop varieties for Zn, Fe and other nutrients and these findings could suggest that it should be possible to breed cultivars with enhanced Se uptake and /or retention.

Improving grain micronutrients (i.e. Se and Zn) and concentrations using transgenesis may be feasible, but this strategy is a long-term option and the deployment of transgenic cultivars is compounded with complicated regulatory and biosafety concerns. Because of this, CIMYYT is no longer breeding for micronutrient efficient cultivars in maize and is pursuing only a modest improvement for Zn concentration (Ortiz-Monasterio et al. 2007).

It would appear that there are no published studies of Se and Zn fertilisation of maize and wheat in Zambia. However, given that the size of plant-available pools of micronutrients (Se and Zn) in the soil is low this may greatly affect the capacity of these efficient cultivars to take up and accumulate micronutrients in the grain.

### 2.6.2 Agronomic biofortification

The intentional addition of micronutrients to macronutrient fertilisers (agronomic biofortification) is a quick and effective approach to increasing grain concentrations (Alloway 2009; Cakmak 2008; Rengel et al. 1999) and thus an excellent complementary tool to the breeding strategy for successful biofortification of cereal grains (Cakmak 2009). Agronomic biofortification could also have multiple benefits for the population. This has worked in Finland and Turkey where soil Se and Zn are low, respectively. This leads to suboptimal intakes of essential nutrients by consumers as was the case with Se intake in Finland, which may contribute to increased risk of cardiovascular diseases and some forms of cancer (Cakmak 2008; Graham et al. 2005; Koivistoinen and Huttunen 1986).

As discussed above Se and Zn intake in humans is determined mainly by the level of availability in the soil on which their food is grown and by dietary composition. Combs (2001) suggests that the vast majority of the world’s population have suboptimal Se intakes and Cakmak (2008) also notes worldwide Zn deficient problems contributing to major disease burden.

It is not likely that the current food systems of Zambia deliver optimum micronutrients like Se to maximize the expression of selenoenzymes. The impact of this deficiency and sub optimality is difficult to quantify but is likely to be enormous given the high prevalence of various cancers, cardiovascular diseases and viral diseases (including HIV/AIDS, hepatitis etc)

### 2.6.3 Agronomic biofortification with Se and Zn

The use of Se as a soil amendment in fertiliser is practised in Finland (by law from 1984) where it is currently added to NPK fertiliser at a rate of 10 mg/kg (Eurola et al. 1990) and Zn has been extensively applied with N fertiliser in Turkey (Cakmak 2008). Zambia may not have to legislate to improve the nutrient levels but may need to pursue similar deliberate programmes to address the high levels of the prevailing deficiencies. Micronutrient deficiencies in humans are common in Zambia (Famine Early Warning Systems Network 2006; Gitau et al. 2005) and this could be primarily linked to low micronutrient density of staple food crops.

Although Zambia has a track record of successful implementation of universal fortification of sugar with vitamin A and salt with iodine (Serlemitsos and Fusco 2001), individuals who are at risk of nutrient deficiencies (frequently women and children) often rely on cereal-based staple foods for most of their energy requirements and lack the money to improve their diet. Hence it is important to fortify the staple maize and other cereals with various micronutrients in the field to meet fortification levels in food consumption for both adults and especially children. Children have fast growth rates yet often low nutritious food intakes.

The Se levels in major food classes usually occur within the following ranges: 0.10-0.60 mg/kg (fish), 0.05-0.60 (cereals), 0.05-0.30 (red meats) and 0.002-0.08 (vegetables and fruits) (Combs 2001). However, most of these sources may be beyond reach for most of the population and increased intakes may appear to be mainly supplied from maize and wheat (cereals) to achieve HarvestPlus targets for Zn and general Se biofortification levels for the general public.

### 2.6.4 Bioavailability of Se and Zn in diet

In human nutrition terms, bioavailability can be defined as the amount of a nutrient in the meal that is absorbable and utilisable by the person eating the meal (Van Campen and Glahn 1999). Selenium is well absorbed (generally 73-93%) from most sources, often being more bioavailable from plant forms than from animal foodstuffs (Bugel et al. 2002; Combs 1998). The form of Se appears to be important with selenomethionine (form in which Se mainly occurs in cereals) and selenate usually absorbed more efficiently than selenite. Studies have demonstrated serum Se increases in a dose-response manner in the high bioavailability wheat–Se by feeding trials (Meltzer et al. 1992).

On the other hand, Zn bioavailability appears to be low in the presence of high P (Hotz and McClafferty 2007) and so adding P to crops that may reduce the bioavailability of Zn. Phytate is a P storage molecule in cereal grains and is a strong chelator of minerals including Zn (IZiNCG 2004). Phytate cannot be digested or absorbed by the human intestine tract, consequently minerals bind to it pass through the intestine unabsorbed (IZiNCG 2004). Myo-inositol hexaphosphate (phytic acid) consists of a ring of six phosphate ester groups and phytate is Mg, Ca or K salt of phytic acid. The phytate: Zn molar ratio of the diet has been used to estimate the proportion of absorbable Zn (Hotz and Brown 2004).

Generally, keeping the phytate:Zn ratio lower than 15-20 by increasing Zn or reducing phytate concentrations improves bioavailability of Zn in the human body (Donovan and Gibson 1995). This could possibly be achieved through balanced crop nutrient requirements in the food production system in Zambia. Elsewhere, it has been shown in field trials that soil and foliar application of Zn reduced the shoot and grain P concentration with a corresponding reduction in phytate-to-Zn ratio (Erdal et al. 2002), hence presenting a potential benefit of increasing bioavailability of Zn in human diets.

## 2.7 Conclusion

The review of literature presented here examined the strategies of the agricultural systems to supply Se and Zn to improve intake in the Zambian population and the SSA community at large.

The major constraints appear to be infertile soil and climatic conditions. The role of balanced nutrients in crop production and human nutrition has been identified as important in improving the current scenario.

The agronomic biofortification approach (as a complementary approach to genetic biofortificaton) offers a relatively easy, efficient and safe strategy to improve productivity and nutrient concentration of maize and wheat grain. Cereals are generally low in micronutrients compared to other food crops and thus cereal-dominated food systems, like those found in Zambia, are prone to produce grain low in micronutrients.

The Zambian agricultural system would need to focus on producing healthy foods that meet energy and nutrient requirements of the entire population especially from commonly consumed foods. Moreover, diets in Zambia are less diverse than those in most developed countries leading to deficiencies in micronutrients especially iron, Zn, iodine, Se and vitamin A.

This Review of Literature has identified a number of gaps in the knowledge that may slow improvements in Zn and Se concentrations in grain by agronomic biofortification:

*(i)* There is little information on the extent and severity of Zn and Se deficiency in Zambia. To address this a survey of crops and grain from market places will be conducted.

*(ii)* Nitrogen and S deficiency are common in Zambia, so to improve the effectiveness of agronomic biofortification programs, it is also important to understand how N and S nutrition affects Zn and Se uptake. Experiments under controlled conditions will be conducted to examine the influence of N and S nutrition on micronutrient uptake.

# CHAPTER 3

# The Zambian grain surveys

## 3.1. Introduction

The survey results of the Zambian summer and winter crops are reported here with particular focus on maize and wheat grain Se and Zn concentrations. Wheat is grown under irrigation in winter following maize which is harvested in May to June. Where this rotation is practised farmers grow two crops in the year, otherwise only maize is grown as a rain fed crop with the start of the rains in November.

Maize is the major staple grain in Zambia and is extensively grown by small scale and commercial farmers, who produce 70% and 30% of the national production, respectively, but it is susceptible to deficiencies of Zn and other minerals (St Clair and Lynch 2010). On the other hand, wheat is a minor crop and production is still relatively small, but it is increasing in importance as a source of calories and nutrients for the Zambian population. Wheat production is mainly concentrated along the railway line in zone 2 where it is cultivated under irrigation in winter by commercial farmers.

An inadequate intake of micronutrients and the development of micronutrient deficiencies in the population as a result of over-dependence on staples such as maize, rice wheat and cassava that are low in micronutrients, have been reported in several studies but the problem is most common in developing countries (Bouis and Welch 2010; Cakmak et al. 2010; Lyons et al. 2004; Ortiz-Monasterio et al. 2007). Most Zambian people depend on maize, and increasingly on wheat, and therefore it is important to know the nutrient composition of the grain they consume to assess the current intakes of these nutrients in the diet.

Suboptimal intakes and low dietary diversity can lead to ‘‘hidden hunger” with serious consequences being poor health, including impaired brain function and mental development, diarrhoea, and reduced immunity to deadly infectious diseases, along with large economic costs to society (Branca and Ferrari 2002; Golden 1991; Grantham-McGregor and Ani 1999;

Ramakrishnan et al. 1999; Stein 2010; Welch and Graham 2004).

Although evidence suggests that children under the age of five and women are most severely affected and nearly two-thirds of all the deaths of children in the developing world are associated with nutritional deficiencies, mainly from micronutrient deficiencies (Caballero 2002), it appears there has been little interest in micronutrient nutrition of crops in southern Africa, and Zambia in particular. Consequently, information on the nutrient status of staple crops is scarce. Both Zn and Se deficiency in the population are related to high consumption of cereal based foods with low concentrations of these micronutrients. It appears these deficiencies are related to the capacity of soils to supply adequate nutrients to the plant and subsequently to translocate nutrients to the grain (St.Clair and Lynch 2010). Many soils in Zambia have poor fertility, and Se is poorly available and Zn deficiency is common (Chirwa and Yerokun 2012; Melse-Boonstra et al. 2007). Therefore, it is likely that crops grown on these soils have low grain nutrient concentrations and yield (St.Clair and Lynch 2010). Micronutrient deficiencies in humans are reported to be common in Zambia (Gitau et al. 2005). To that effect, efforts to correct some of these deficiencies have been directed towards universal fortification of sugar and salt with vitamin A and iodine respectively (Serlemitsos and Fusco 2001).

However, there is a challenge to increase micronutrient intakes through a cost-effective strategy to improve the health and development of the general population, especially rural and low-income segments of the population. Agriculture offers various promising and cost-effective strategies to contribute to the solution of Zn and Se deficiency problem in the country, but there has been no attention given to Zn and Se.

The survey reported here was conducted to investigate the nutrient concentrations in maize and wheat grown and consumed in Zambia with a specific focus on Zn and Se concentrations of grain. The purpose of the survey was to gain an understanding of the degree and extent of nutrient deficiency in crops by using the grain nutrient concentrations as indicators of nutritional status, as well as the role of N and S in enhancing adequate grain loading which may improve the intake in the population. The study consisted of two related approaches. First, a survey was conducted in which maize and wheat grains were sampled from the farms, referred to as "farm survey". The second survey was of maize grain from the market place, called the "market survey." The latter survey was conducted to provide information on the nutrient levels of grain bought and consumed by local populations.

## 3.2 Materials and Methods

A survey of maize and wheat crops in Zambia was carried out in the three agro-ecological zones in 2011 and 2012. Supporting information was collected which included production (yield) levels, climatic factors, soil reaction (pH) and management and farmer perceptions of productivity trends and constraints. Maize and wheat grains were sampled from randomly selected farms and a voluntary survey of production practices conducted through a questionnaire (Appendix 3.1). Maize was also collected from some open maize markets in the zones.

The data on grain samples were collected from farmers' fields in May 2011 and January 2012 for the market place. The farms surveyed included small and commercial scale farmers and farmers were interviewed to determine management practices used. All fields sampled grew hybrid maize and had been fertilised according to the Ministry of Agriculture recommendations. Cobs of maize (between 2 and 5) were sampled individual plants from at least 20 metres inside the borders of the fields, hand shelled, bulked together and sub-samples of grains were taken from the bulked grain for analysis. To minimise contamination, sampling sites close to roads or other dusty places were avoided. The pH of the plough layer (0-15 cm) was measured in the field from the sampling sites using a field pH testing kit. Contamination of the grain sample was minimised or avoided by handling with clean hands and sealing in envelopes. Wheat samples (approximately 100g) were sampled from bags in storage sheds. The number of bags sampled varied with the farm. The areas covered during the survey are shown in Figure 3.1.

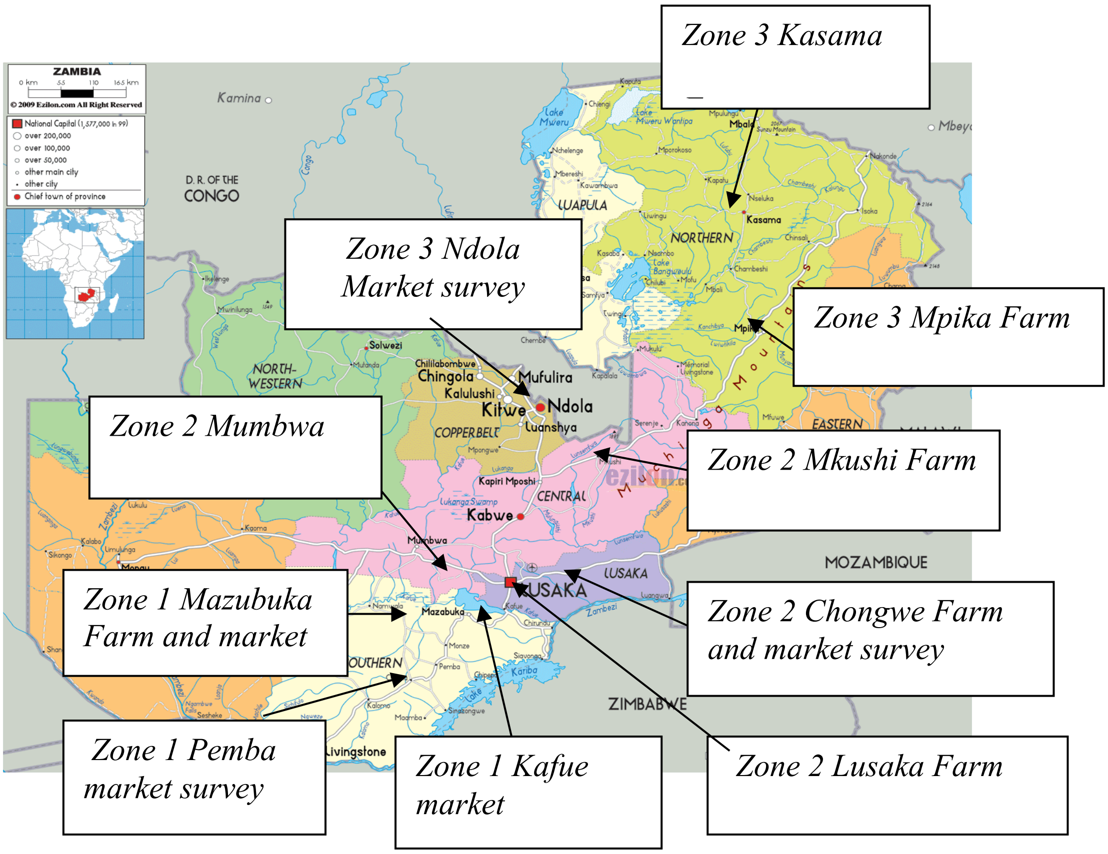

Figure 3.1 Map of Zambia indicating areas covered in the farm and market surveys in 2011 and 2012.

Maize grains from markets were sampled from bags of maize publicly displayed for sale with the consent from the marketeers. It was thought that grain sold within the market place would reflect the composition of grain consumed by rural populations as well as the general population in the agro ecological zones.

The grain samples were imported to Australia for analysis and were irradiated with UV light by quarantine authorities. The grain nutrient analysis was done by Waite Analytical Services, University of Adelaide, South Australia by the methods outlined in Wheal et al (2011). Briefly, the samples were dried in an oven for 48 hours at 80°C before they were ground and analysed using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) for macro and micronutrients. Selenium was analysed using ICP-Mass Spectrometry and N was analysed by the Dumas Combustion Technique using an Elementar Analyser. Nutrient measurements were checked by using certified standard reference materials. The results are presented in mg/kg dry matter or percentage units.

### 3.3 Statistical analysis

The calculations of means and standard error of the means for the farm survey were performed and comparisons of mean concentrations for grain samples collected from the farm surveys and market places were performed using the Student T-test and Kruskal-Wallis one way analysis of variance to determine whether they were significant differences in crops and in the zones using Genstat.14th edition. Correlations were also performed on the samples for the relationships between pairs of nutrients.

### 3.4 Results and discussion

#### 3.4.1 Farm survey

The survey aimed at randomly collecting 50 maize and a few wheat samples since wheat was out of season at the time the surveys were conducted. A total of 42 samples were collected of which 36 were maize and 6 were wheat samples. A number of farmers were unwilling to volunteer information on farming practices and so that aspect of the survey is incomplete.

The main results from the on-farm survey regarding agronomic practices among the farmer categories are summarised in Table 3.1. Reliance on hand-hoeing, limited use of farm machinery and ability to buy inputs were the main limitations to area under crop and yields and which differentiated the categories of the farming community. These differences in area under cultivation also reflected the level of management of the crops in terms of planting practices and weeding (level of investment of financial resources). The use of herbicide was a common feature on the medium and commercial farms, whereas hand-weeding was the used on the small-scale farms.

All the sampled fields had received inorganic fertilisers except one where organic fertiliser were being used. For this field, it was difficult to quantify how much N, P and K that was contained in the manure tea that was used. This field also grew open pollinated varieties. There were no deliberate additions of the manure, use of agroforestry trees to improve fertility (e. g Gliricidia sepium and sunhemp) or use of organic residues observed on the rest of the farms, nor was deliberate addition of micronutrients such as Zn and Se reported for any field or crop. Crop residues were routinely burned annually to facilitate land preparation.

**Table 3.1. Characteristics of the areas used in the survey in 2011 indicating annual rainfall, soil pH and mean yields of maize and wheat crops in three agricultural zones of Zambia. Number of farmers in each category is shown in parenthesis.**

| Zone | Farmer category | Mean Rainfall (mm/yr) | pH[A] | Maize | | Wheat | |
|---|---|---|---|---|---|---|---|
| | | | | Grain Yield[B] (t/ha) | Area[C] (ha) | Grain Yield[B] (t/ha) | Area (ha) |
| 1 | Small scale (3) | 660 | 5.5 | 2.5 | 3.0 | | |
| 2 | Small scale (6) | 966 | 5.3 | 2.0 | 0.5 | | |
| 2 | Medium (2) | 966 | 5.5 | 4.0 | 50.0 | 2.5 | 5.0 |
| 2 | Commercial (3) | 966 | 5.8 | 8.0 | 3160 | 6.0 | 2500 |
| 3 | Small scale (4) | 1133 | 5.0 | 2.5 | 1.0 | | |
| 3 | Medium (1) | 1004 | 5.8 | 4.8 | 60.0 | | |

[A] Based on field pH measurements.

[B] Based on estimated average yields by farmers surveyed.

[C] Based on typical area cultivated (farm).

Note: Wheat is grown in winter under irrigation by commercial farmers.

Rainfall data for the season 2010/2011 were collected from the Zambia Meteorological Department as most farmers did not keep a record of the annual precipitation on their farms. The pH ranged from 5.0 to 5.8 in the surveyed areas. The farmers rating of crop yields was low for the medium and small-scale farmers and they attributed the low yields to poor soil fertility, late distribution of fertiliser from the government, weed infestation and poor rainfall distribution within the growing season. The rainfall and pH trends are consistent with those reported in zones and most soils of Zambia, respectively.

It appears that these conservative yield estimates were influenced by the availability and ability to purchase inputs such as hybrid seeds and fertiliser as well as area cultivated. Consequently, small scale farmers' estimates are lower than those of either the medium (emerging) or a commercial farmer. The hybrid cultivars grown are selected based on duration of the growing season and rainfall. It appears that potential yield of the available maize cultivars is rarely obtained even in the best of seasons. The likely cause of these lower than estimated yields could be attributed to several factors but is likely to be mainly due to suboptimal nutrition status of the crops during the growing season.

Studies in Zambia have shown that decline in soil fertility and yields could be as a result of continuous cropping, with most attention being focused on the macronutrients, mainly N (Lungu and Dynoodt 2008). The problem is widespread and increasing on intensively cultivated lands and requires to be counteracted with a more balanced plant nutrition approach for sustainable crop production.

There was no intentional application of S and soil testing is only rarely done by medium sized and commercial farmers. Liming of the fields is also practised on some medium and commercial farms to increase soil pH, although not regularly. The typical rates of lime application are 0.25-1.08t/ha, but maintenance liming at the rate of 500 kg/ha every after three years is recommended (Lungu and Dynoodt 2008; McPhilips 1986).

This situation is likely to affect nutrient concentrations, especially among small scale farmers who are unable to carry out soil analysis over a long period of time. Therefore, imbalance/deficiencies in nutrients are likely to affect yield and quality of staple crops grown. This category of farmers mainly depends on the government support with subsidised inputs (fertiliser and seed) meant for the production of 1 ha of maize. Ideally, yields of 4-5t/ha is possible as most varieties have yield potentials of more than 6 t/ha (Mukuka 2013).

The government inputs support to the farmers is meant to encourage maize production as a way of reducing rural poverty and ensuring adequate supply of the staple throughout the year. These inputs are distributed throughout the three agro ecological zones based on the Ministry of Agriculture recommendations. Sometimes seeds and fertiliser have not been distributed on time in the season due to poor road infrastructure during the rainy season, leading to either use of retained seed or delayed planting and late application of fertiliser.

### 3.4.2 Mineral concentration analysis

Means for the mineral element concentrations across the agro ecological zones and production categories from the farm surveys are summarised in Tables 3.2 and 3.3 for both crops. The analysis for N, S, Se and Zn of these cereal samples showed significant differences in the concentrations of minerals in maize and wheat grain using the Kruskal-Wallis test. There were variations in the concentrations among and within the zones in N, S, Se and Zn grain concentration, although these were not significant.

**Table 3.2; Nutrient concentration in maize grain grown by small (n=19), medium (n=13) and commercial (n=4) farmers in the surveyed area. Data are shown as the means ± SD and the range of values is shown in parentheses. The critical concentration for grain is also shown**

| Zone | Farmer category | Nutrient concentration in maize grain | | | |
|---|---|---|---|---|---|
| | | N<br>(%) | S<br>(mg/kg) | Zn<br>(mg/kg) | Se<br>(μg/kg) |
| 1 | Small scale | 1.5 ± 0.2 | 1092 ± 89 | 21.9 ± 3.3 | <2 |
| | | (1.2-1.6) | (940-1160) | (16-25) | |
| 2 | Small scale | 1.2 ± 0.2 | 930 ± 85 | 16.9 ± 1.0 | <2 |
| | | (1.1-1.3 ) | (870-990) | (13-25) | |
| 2 | Medium scale | 1.5 ± 0.3 | 1100 ± 71.0 | 18.2 ± 2.0 | 28 |
| | | (1.4-1.7) | (1050-1150) | (15-16) | |
| 2 | Commercial scale | 1.4 ± 0.2 | 1032 ± 98.2 | 18.4 ± 4.0 | 4 |
| | | (1.2-1.7) | (850-1170) | (13-20) | |
| 3 | Small scale | 1.4 ± 1.0 | 1085 ± 177.0 | 17.6 ± 1.0 | 8 |
| | | (1.3-1.6) | (960-1210) | (15-24) | |
| 3 | Medium scale | 1.4 ± 0.1 | 1093 ± 64.3 | 19.1 ± 1.4 | 6 |
| | | (1.3-1.6) | (1020-1140) | (15-16) | |
| | Critical conc. | 1.5[A] | 2300[B] | 27[C] | 300[D] |

[A] Adriaanse 1992 [B] Based on Robinson and Reuter 1997, [C] White and Johnson, 2003 [D] Lyons pers.com 2012 Se is not essential for plant growth; there is no critical plant concentration *per se*. However, 300μg/kg can be considered a plausible target of grain Se concentration for optimising Se intake in humans and animals. Only a sub-sample of maize was analysed for Se.

**Table 3.3. Nutrient concentrations of nitrogen (N), sulphur (S), zinc (Zn) and selenium (Se) in wheat grain samples from the farm survey. All samples came from commercial farms (n=6) in Region 2. Data are shown as means ± SD and the range values in parentheses.**

| Zone | Nutrient concentration | | | |
|---|---|---|---|---|
| | N% | S(mg/kg) | Zn(mg/kg) | Se(µg/kg) |
| 2 | 2.0±0.3 | 1327±126.8 | 30±8.7 | 8.5±2.1 |
| | (1.6-2.2) | (1180-1480) | (21-41) | (4.8-14) |
| Crit.conc$^{A}$ | 2.0 | 1200 | 23-30 | 300 |

$^{A}$. Based on Robinson and Reuter, 1997

The N, S, Se and Zn concentrations were lower in the maize than wheat. (Tables 3.2 and 3.3). Generally, wheat had a higher concentration of other nutrients such as iron, manganese, copper, calcium, P and K as well (Appendix Tables 3.2a, 3.2b). The concentrations of most samples also were below grain critical concentrations for K and P (Appendix Tables 3.2a, 3.2b). The results are in agreement with observations in the literature that wheat could be a better source of nutrients and is a better accumulator and source of Se than maize (Lyons et al. 2004).

The Zn concentration levels in the maize grain ranged from 13 mg/kg to 25 mg/kg. These concentrations are consistent with the low ranges obtained in neighbouring Zimbabwe and also in China with mean Zn values of 23mg/kg and 18mg/kg for maize, respectively (Lyons et al. unpublished data 2008). However, the levels are lower compared to typical grain concentration of 27-33 mg/kg Zn in maize in the United States of America (White and Johnson 2003) and much lower than the HarvestPlus final target contents for maize of 38 mg/kg (dry weight) (Bouis and Welch 2010). Harvest Plus baseline Zn concentration for maize is 30 mg/kg, indicating that the concentration of the grain samples is not adequate for adequate human nutrition. Other studies suggest that for adequate Zn nutrition of human populations, grain Zn concentrations should be even higher, for example at least 40 mg/kg (Cakmak 2008).

The N, S and Zn concentration for wheat was within the range of adequacy, being, 23-30 mg/kg Zn, 2 % N and 1200 mg/kg S (Reuter and Robinson, 1997). The analysis also indicates deficiency in K, adequate levels of Ca, P and moderate levels of micronutrients (Appendix Table 3.2b). A grain Se concentration of 300 µg/kg Se is an estimated ideal level for human and animal intake for adequate selenoenzyme activity (Lyons, pers.comm 2012), but all values were well below 50 µg/kg Se desired level in the grain (Lyons et al. 2004). However, as Se is not required by higher plants for growth, there is no critical concentration for it, unlike N, S and Zn.

The deficient-marginal concentrations of N, S and Zn are more likely to be due to soil deficiencies as cultivar differences seem to be minor (White and Broadley 2005) and relatively stable over wide ranges of soil reaction (pH), rainfall, temperature and crop management levels.

The relatively high yields on commercial farms for both maize and wheat grain appear to be as a result of a response to higher levels of N application. In commercial intensive agricultural systems application rates range from 200-400 kg N as top dressing at 6-8 weeks after crop emergence compared to half these rates or zero in small scale agriculture (Lungu and Chinene 1993). There were significant correlations between N and S, in both crops, but S and Zn was only significantly correlated in maize (Table 3.4).

**Table 3.4. Linear correlations between concentrations of nitrogen (N), sulphur (S) and zinc (Zn) in maize and wheat grains from the farm survey (P<0.05), NS = not significant)**

| | S | Zn |
|---|---|---|
| Maize (n=36) | | |
| N | 0.651 | NS |
| S | | 0.395 |
| Wheat (n=6) | | |
| N | 0.991 | NS |
| S | | NS |

Correlations of a sub sample (n=8) that was analysed for grain Se concentration were also determined (Table 3.5) and indicated significant correlations between N and S, S and Zn in both crops but only S and Se in wheat was significantly correlated with a strong negative correlation evident.

**Table 3.5 Linear correlations between concentrations of N, S, Zn and Se in maize and wheat from the farm survey (P<0.05, NS=not significant)**

| | S | Zn | Se |
|---|---|---|---|
| Maize(n=8) | | | |
| N | 0.795 | 0.733 | NS |
| S | | 0.817 | NS |
| Zn | | | NS |
| Wheat (n=4) | | | |
| N | 0.996 | NS | NS |
| S | | NS | -0.907 |
| Zn | | | NS |

The results of the mineral analysis of the grain from the survey suggest S deficiency is widespread and adding S has the potential to be an economical way to improve yield and quality. The deficient S levels suggest that the crop responses to N fertiliser, to which most farmers has access, may be limited by the low supply of S. This is because changes in the S supply affect the N demand and *vice versa* and as such under conditions of S deficiency, the utilisation of nitrogen is reduced and consequently non-protein N compounds including nitrates accumulate (Matula 2004; Schnug 1998). The deficient S status of the crops could also be indicative of low S-amino acids in the grain and hence lower protein quality. The N:S ratio for maize and wheat are shown in Table 3.6.

**Table 3.6. The N:S of maize and wheat grain obtained in the surveyed areas in the agro, ecological zones.**

| Zone | Maize | Wheat |
|---|---|---|
| 1 | 13-14 | |
| 2 | 14-15 | 14-15 |
| 3 | 13-14 | |
| Critical value [A] | 7.5-9 | 16 |

[A] Based on Orman and Ok 2012, Robinson and Reuter 1997, Zhao et al 1999).

The N: S ratio is a reliable indicator of S deficiency and in wheat an imbalance between N and S is indicated at ratios greater than 16 (Zhao et al. 1999). Values for the N:S ratio from the survey for maize and wheat (Table 3.6) are above the critical value for maize, indicating an S deficiency, and just below the critical level for wheat, suggesting the crops are approaching S deficiency. The absence high N: S ratio in maize and wheat grain suggests the poor S supply may be limiting the returns from the investment in N applications.

The widespread S limitation suggested by these results could partly be due to continuous cropping, use of high yielding hybrids, the use of high analysis N, P, K fertiliser, failure to supplement with S and the annual burning of crop residues in the fields. This situation could be expected to rapidly deplete available S supplies in the soil. Potassium deficiency was also apparent (Appendix Table 3.2a) and was probably due to leaching (Weil and Mughogho 2000). Similar trends have also been reported in Malawi (Weil and Mughogho 2000). The same researchers also add that in such situations, the S supply from organic matter mineralisation is inadequate and S fertiliser application would be required.

Sulphur status has also an impact on the uptake and grain concentration of Zn. Sulphur and Zn are significantly correlated; hence, low status could limit grain Zn concentration. McDonald and Mousavvi-Nik (2009) reported that increasing S application (15-55 mg $kg^{-1}$) raised wheat grain

Zn concentration by 40-50%. This is due to various physiological steps in plant tissues where S and N nutrition can act positively to promote high grain accumulation of Zn (Cakmak et al. 2010). In such cases S-Zn interactions can play an important role in plant nutrition and improving the grain Zn concentration.

Sulphur is chemically similar to Se and because of this, plant uptake of S and Se are closely related (Mikkelsen and Wan 1990). Comprehensive reviews of the role of sulphate transporters and S assimilation enzymes and Se-molybdenum uptake and metabolism are given by Terry et al, (1998) and Kaiser et al. (2005). The Se levels in maize and wheat grain samples were low and ranged from less than 2µg/kg (below limit of detection) to 28µg/kg and a significant negative relationship between S and Se in the wheat grain was evident.

The uptake of Se by plants and subsequently in the food chain is influenced by the chemical form and concentration of Se in the soil solution, redox conditions, pH of the rhizosphere and the presence of competing anions such as sulphate and phosphate (White et al. 2004). Given the antagonistic interaction between $SO_4^{2-}$and $Se^{6+}$ at low $SO_4^{2-}$ (Mikkelsen and Wan 1990) one would expect elevated concentrations of Se in the grain under the reported S deficient conditions, but this was not apparent. However, the results of the grain Se concentration suggest the Se status in major soils in Zambia are low as well, and input of Se to the soil is generally non-existent as the fertilisers used do not contain Se. The fertilisation of crops with Se is a cost–effective method of enhancing the concentration of organic Se in the grain in order to increase Se intake of animals and humans (Lyons et al. 2005). Some common fertilisers that contain Se as an impurity include, ammonium sulphate containing up to 36 mg Se/kg, phosphate rock with up to 55 mg Se/kg and single super phosphate up to 25 mg Se/kg (Bisbjerg 1972; Swaine 1962; White et al. 2004). These levels could be utilised for agronomic biofortification with Se especially given the low P levels measured in the survey. However, these fertilisers are not commonly used in Zambia, and lack of investment in the rock phosphate fertiliser production even worsens the scenario. Rock phosphate deposits are abundant in Zambia in Zones 2 and 3 (Chileshe et al. 2000) and their agronomic effectiveness have been reported (Damaseke et al. 1993).

It appears the grain Magnesium (Mg), Ca, K, and P levels in the three agro-ecological zones are marginal to deficient perhaps due to sub-optimal fertiliser application and leaching (Appendix Tables 3.2a and 3.2b). Low nutrient retention is described as a severe constraint in zone III (high rainfall areas) to slightly deficient in zones I and II (low to moderate rainfall respectively) (Kalima and Veldkamp 1985). In addition, Lungu and Dynoodt (2008), observed a decrease in exchangeable bases (Ca and Mg) in the soil as a result of soil acidification and lack of liming. Soil acidity in Zambia increases with increasing rainfall received in the agro ecological zones, but appears to be further enhanced by the use of N fertilisers over a long time without liming. Use of gypsum in this case can be useful in supplying the deficient S and Ca in the farming systems. In general, balanced nutrition of field crops is an important prerequisite of plant production and quality. The knowledge of potential nutrient interactions in the soil and in the proper nutrition of crops would ensure a rational fertilisation programme. The Ministry of Agriculture recommendations indicate 200kg basal dressing of compound D (N=10, P=20, K=10 with traces of S and Zn) and top dressing of 200kg urea fertilisers.

In all of the zones, it is clear that soils are subject to depletion of nutrients, hence N, S and Zn need to be applied for sustained high crop production. Nitrogen and S appear to be the most limiting and have most effect on the grain yield, Zn and Se grain concentrations. In the field S deficiency can be confused with N deficiency due to related functions in the plant, hence it has not received adequate attention.

### 3.4 3 Market surveys

The market survey had 31 samples that were collected from markets indicated in Figure 3.1. The nutrient concentrations of the grain samples were low in N, S, Zn and Se (Table 3.7).

**Table 3.7. Concentration of N, S, Zn and Se from samples taken from selected market places in the three agro ecological zones in Zambia. Values are means ±SD and the range is shown in parentheses.**

| | Nutrient concentration in maize from markets | | | |
|---|---|---|---|---|
| Zone | N<br>(%) | S<br>(mg/kg) | Zn<br>(mg/kg) | Se<br>(µg/kg) |
| 1 (n=6) | 1.0 ± 0.2 | 985 ± 105. 8 | 20 ± 2.6 | 18 ± 7.7 |
| | (1.1-1.5) | (850-1010) | (17-22) | (12-33) |
| 2 (n=23) | 1.0 ± 0.1 | 985 ± 99.7 | 19 ± 2.0 | 26 ± 34.2 |
| | (1.1-1.6) | (700-1090) | (15-23) | (7.1-34.2) |
| 3 (n=2) | 1 ± 0.1 | 1010 ± 127.3 | 20 ± 0.01 | 21 ± 19.8 |
| | (1.3-1.5) | (920-1010) | (19-20) | (6.7-35) |
| Critical Conc. | 1.5 | 2300 | 27 | 300 |

The analysis of grain from the market survey showed a similar trend to the farm survey: low Zn and Se levels were consistent with the values reported earlier from the farm. However, it is not possible to infer the management practices on the farms from which maize on the market came as the exact origin of the grain is not known since the grain can be transported from different farms to the market place; however, the bulk of it comes from small scale farms.

Despite, the variations observed in grain concentrations, there are significant correlations between N and S (Table 3.8). These nutrient interactions are important for crop production and may have a great influence on the grain yield and Zn and Se concentration in the grain.

**Table 3.8. Linear correlations of N, S, Zn and Se in a grain Sample collected from some markets in the three ecological zones of Zambia.**

| | S | Zn | Se |
|---|---|---|---|
| Maize (n=31) | | | |
| N | 0.493* | 0.006ns | -0.042ns |
| S | | 0.111ns | -0.066ns |
| Zn | | | 0.289ns |

Pairwise comparisons of nutrient concentrations among the regions revealed that the average concentrations of N, S, Zn and Se in the grain samples in the 2 surveys were not significantly different from each other and therefore the samples could be considered to have come from the same population using the Kruskal-Wallis test ($X^2$=13.96, $p< 0.001$). Therefore, the maize samples from the farm survey and market survey were combined. Table 3.9 shows quartile values of the concentrations in the maize and wheat crops grown and consumed in Zambia.

**Table 3.9 Quartile values for the Zn, S and N concentrations of combined farm and market maize and wheat grain in the survey areas of the three agroecological zones of Zambia**

| Maize | Nutrient concentration | | | |
|---|---|---|---|---|
| | N (n=67) (%) | S (n=67) (mg/kg) | Zn (n=67) (mg/kg) | Se (n=39) (µg/kg) |
| Q1 | 1.3 | 970 | 17 | 9 |
| median | 1.4 | 1030 | 19 | 16 |
| Q3 | 1.5 | 1100 | 21 | 21 |
| maximum value | 1.7 | 1240 | 26 | 48 |
| Wheat (n=6) | | | | |
| Q1 | 1.6 | 1218 | 25 | 7 |
| median | 2.0 | 1335 | 26 | 8 |
| Q3 | 2.1 | 1423 | 37 | 9 |
| maximum value | 2.2 | 1480 | 41 | 14 |

All the samples were deficient in S, 75% were deficient in N, 97% were deficient in Zn and all were below the reported levels of Se in literature (see TableS 3.2 and 3.3a for critical concentrations). In Zambia, like most countries in Southern Africa, more than 50% of energy intake comes from maize. Based on FAO (2009) a mean energy intake of 1879 kcal/person/day is consumed, equating to 0.316 kg /person/ day and an overall median grain Se concentration of 0.016 mg/kg from the all the samples analysed (n=39), the estimated median Se intake from maize is 5.1 µg Se per person day. However, since food consumption data are based on national *per capita* supplies which will overestimate food intake due to wastage during storage and cooking (FAO 2009), Se intakes are likely to be lower than estimated. In neighbouring Malawi, a country with similar consumption patterns (Chilimba et al. 2011), and where Se from other food sources have been analysed, contribution to the daily diets of the people of such sources were low (Erik 2007) and consequently suboptimal Se intake appears to be widespread in Zambia.

Therefore, given the critical of role Se and Zn in human health and that the soil to grain Se and Zn transfer is primarily determined by soil available Se and Zn, addressing these suboptimal intakes through agronomic biofortification appears to an inexpensive and sustainable intervention with immediate positive impact on the health of the general population (Lyons et al, 2004).

### 3.5 Extent and degree of mineral deficiency in maize and wheat in Zambia

The results of the survey of mineral concentration of the maize and wheat presented in this study show that current nutrient status of the crops is low to deficient for maize and moderate to adequate for wheat for grain Zn concentration. However, both crops are deficient in S and Se concentration. The yields and nutrient concentrations in the grain appear to be affected by low available S content in the soil. In a field trial conducted during the period 1963-1964 a remarkable response to S was observed using the highest rate of 140 kg/ha of ammonium sulphate (McPhilips 1986), but since S fertilisation have not been supplemented over time S available levels in the soil have declined as reflected in grain concentrations and the high N: S ratios, which indicated an N-S imbalance in maize.

The findings are consistent with other reports on the mineral (N, Zn, S, Se) status of most soils in

Zambia. Low S grain concentration is in agreement to the low S reported in the soils in Zambia (Tsuji et al. 2005) and could limit crop productivity. In addition, S deficiency can affect the quality of the produce as S is part of the S amino acids that form protein such as methionine, cysteine and cystine (Malhotra 1998). Furthermore, S amino acids are precursors of nicotianimine that is involved in the transport of micronutrients including Zn and Fe (McDonald and Mousavvi-Nik 2009; Zeng et al. 2010).

Sulphur deficiency appears to be widespread in SSA but has received little attention and in Zambia it is difficult to estimate the extent of S deficiency because the determination of S content in soil was not regularly conducted. However, some early reports have indicated S deficiency and significant responses to S in maize have been reported in western Kenya (Allen 1976), in Zimbabwe and Nigeria (Grant and Rowell 1976; Kang and Osiname 1976) and Zambia (McPhilips 1986). It is not surprising that in Zambia S deficiency is becoming increasingly common on land continuously cropped for food production due to the reasons outlined in the preceding paragraphs.

The Se concentration is lower than the target Se levels reported in literature and is consistent with the reported low Se levels in the country and the Southern African region. For example, Melse-Bonstraa (2007) observed widespread Se deficiency in most Zambian soils and selected crops, while similar results have been reported in Malawi (Chilimba et al. 2011). The findings reported here may have important implications for human nutrition as sub-optimal intakes are likely given the narrow food choices available to the populations; consumption of maize is high and that of animal products (meat, milk and eggs) are typically low, especially for the low-income populations. The Recommended Daily Allowance for an adult person is 15 mg per day for Zn, though the Zambian Food and Nutrition Commission have set this at 12 mg per day. An intake of Se of 50-70 µg per day has been recommended in most countries (Chilimba et al. 2011).

However, in the light of the evidence presented here, and given limited food choices, there may be a problem in achieving this level from the current grain concentrations. Therefore, maize and wheat agronomic biofortification with Zn and Se could have an immediate role in improving intake levels. One of the reasons for the survey was to investigate the limitations to yield and grain Zn and Se loading, and therefore to predict whether there could be a response to N and S.

Therefore, the low-deficient levels of N, S and Se and Zn could indicate that a response may be expected if plant nutrition is improved. In the Zambian context this can be achieved by encouraging the use of mixed fertilisers (e.g. as part of the farm input subsidy program) containing NPKS and Zn/Se by small scale farmers, commercial farmers and organisations. There is a potential for increasingly widespread S limitation to crop production in Zambia. Further research to better define the extent and degree of S limitations where continuous cultivation of the crops is practiced is necessary. Correcting S nutrition may exacerbate the low Se content of the grain and hence a need to balance the nutrients.

Therefore, the growth room experimental work described in the following Chapters was carried out with the main objective to examine the role of N and S and their interactions in the agronomic biofortification of Zn and Se.

# CHAPTER 4

# Effects of N and S nutrition on the uptake of Zn and Se in maize

## 4.1. Introduction

The Zambian grain survey suggested widespread S deficiency, moderate levels of N, and low Zn and Se concentrations which indicated that suboptimal intakes of Zn and Se in the Zambian population was likely. Nitrogen is the major nutrient applied to grain crops in Zambia, with S applied less often. However, the level of N nutrition can influence uptake of S and Zn and there are also well-reported interactions of S with Zn and Se (see Chapter 2). Understanding these interactions will be an important aspect of improving productivity and nutritional quality of grain crops in Zambia. Two experiments were conducted at the University of Adelaide, Waite Campus with a view of evaluating N and S interactions and their influence on the vegetative growth and grain loading of Zn and Se in maize and wheat, respectively. Maize is the main grain consumed in Zambia, however the growth room facilities at the time did not allow maize to be grown to maturity and so it was decided to examine the responses in a short-term experiment. On the other hand, wheat could be grown successfully to maturity, which allowed responses in yield and grain nutrient concentrations to be examined. Therefore, the first experiment looked at uptake in vegetative tissue by maize plants (Chapter 4) and the second experiment (Chapter 5) examined uptake into the grain in wheat.

The nutrient concentrations of Zambian staple food crops need immediate improvement in order to supply adequate amounts of Zn and Se to consumers. Nitrogen and S supply could enhance yield and improve quality of the grain protein which is a sink for Zn and Se (Cakmak et al. 2010; Kutman et al. 2011; Pearson et al. 2009). Nitrogen, S, Se and Zn are part of various proteins and have been documented to share similar transporters (S and protein transporters) and are easily transported through the xylem and the phloem by similar mechanisms (Huang et al. 2000; Uauy et al. 2006). Nitrogen and S nutrition therefore can have a profound influence on the uptake, translocation, remobilisation and grain loading of Zn and Se from the soil and into the plant as well as movement of these nutrients to the edible portions of the plants and/or within the plant. Therefore, vegetative mobilisation and remobilisation of nutrients to the grain is important and essentially the foundation of agronomic biofortification (McDonald and Mousavvi-Nik 2009).

The low dietary intake of Zn and Se is considered to be the major reason for the prevalence of micronutrient deficiencies in human populations (Bouis and Welch 2010; Cakmak et al. 2010; White and Broadley 2009). Most of these deficiencies however, occur in developing countries because cereals like rice, maize, sorghum and wheat, which have low micronutrient concentrations in the grain, dominate the calorie intake and because other dietary sources of Zn and Se are limited. In Zambia, the problem is exacerbated by large areas of infertile soils from which uptake of Zn and Se is low.

The major source of the nutrients for plant growth is the soil, and specifically the amount of plant-available nutrients. However, there is abundant evidence in the literature to suggest that in most soils positive interactions are obtained by applying a balanced combination of fertilisers to obtain favourable yields and adequate grain nutrients concentrations. Crops may respond to application of N and Zn fertilisers and not Zn alone if N is limiting growth (Alloway 2008) and a combination of S and Zn fertilisation may increase concentration of Zn in the grain of cereal crops to enhance and improve human health (Orman and Ok 2012).

Similarly, S is an important determinant of Se uptake and transport in plants. Studies on crop and pasture plants show that increasing soil sulphate levels decreases Se uptake and transport (Hopper and Parker 1999; Ylaranta 1990). However, in the Zambian scenario, where both S and Se deficiency are apparent, application of S to overcome chronic S deficiencies may limit uptake of Se and may exacerbate low Se concentrations in the grain. In this case both S and Se need to be applied to improve uptake.

The experimentation in this Chapter deals with evaluation of maize plant uptake of Zn and Se as well as the role of N and S in the vegetative loading of nutrients and concentration of N, S, Se and

Zn for enhanced yield and improved Se and Zn concentrations. These results may be valuable in understanding the importance of these interactions for a balanced fertiliser recommendation programme to enhance maize grain N, S, Se and Zn concentrations and uptake.

## 4.2 Materials and methods

The experiment was conducted in a growth room equipped with an evaporative cooling system, with the temperatures adjusted to simulate those of the maize and wheat growing areas in Zambia. Plants were grown at 24 $^{o}$ C/15 $^{o}$ C day and night temperatures, while the day-length was maintained at 12 hours. The plants were grown under a light intensity of 500-800 µmol quanta/m$^2$/s using high pressure sodium lamps (Philips SON-T Agro 400W).

### 4.2.1 Soils and nutrient treatments

The soil used in the experiments was obtained from a small cattle farm between Mylor and Echunga, 20km south–east of Adelaide. The farm has an average annual rainfall of 725 mm/year. The soil was chosen for use in the experiment because it was a leached sandy acidic soil similar to those found in parts of Zambia. It was thought to be low in Zn, despite having a history of fertiliser application, including Zn and Se. The field had been under a permanent pasture of mixed grasses and legumes for 10 years. Comparison with one of the benchmark soils at the University of Zambia Farm (a clayey, kaolinitic, isohyperthermic, Oxic Paleustalf; Soil Survey Staff 1975) indicates that the soil from Echunga had a similar pH, lower mineral N, but higher organic C, phosphorus (P), potassium (K) and Zn (Table 4.1).

The experiment was a factorial combination of N, S and Zn+Se treatments each at two levels. The Zn+Se treatment was combined into a single treatment rather than examined separately as they are not likely to interact and Se should not influence growth. These treatments received a basal application of N, P and K as well as trace elements besides Zn (Table 4.2). In addition a control treatment (no additional nutrients) and a basal treatment (only N, P and K) were included. The control and the basal treatments were included to assess the value of the basal nutrients and the Zn and Se on plant growth because of the history of fertiliser use, which had resulted in relatively high levels of some nutrients in the soil. The basal and other micronutrients expressed as equivalent amounts (kg/ha) are indicated in Table 4.2. The N and Zn supply levels referred to as high levels in the study represent normal commercial rates. The low Se level represents a plausible application for agronomic biofortification of cereal grain while the high level is included for comparison and may produce crops with Se levels higher than ideal for human consumption. Before sowing, all the nutrients were applied in solution and homogeneously incorporated into 1.8 kg of soil in plastic lined pots (10 cm diameter).

The composition of the basal macronutrients supplied was: 70.4 mg/pot phosphorus in the form of $Ca_2$ $(H_2PO_4)$. $H_2O$, 80.7 mg/pot $MgCl_2.6H_20$, 84.5 mg/pot $KNO_3$ as basal dressing. As well, a basal micronutrient treatment was applied to pots. These were applied as 3.32 mg/pot $MnSO_4.4H20$, 3.21 mg/pot $CuSO_4.5$ $H_20$, 4.59 mg/pot $H_3BO_3$, 0.138 mg/pot $Na_2MoO_4.H_2O$ and 3.3 mg/pot $Fe_2SO_4.7H20$. Nitrogen was applied as low or high treatments at 88.8 mg/pot and 177.6 mg/pot urea, respectively, S was applied at 110.9 mg/pot and 221.8 mg/pot $CaSO_4$ for the low and high S treatments, while Zn was applied at 0.29 mg/pot $ZnSO_4.7H_2O$ for low or 8.97 mg/pot $ZnSO_4.7H_2O$ for high treatments and 2.4 µg Se for low and 12 µg Se for high treatments were applied as $Na_2SeO_4$ (sodium selenate). The basal and other micronutrients and equivalent amounts per ha are indicated in Table 4.2.

**Table 4.1. Characteristics of the soil used in the experiment compared with a soil from Zambia commonly used for maize production**

| Soil Property | Adelaide soil | Typical soil (Zambia)[A] |
|---|---|---|
| Texture | Sandy-loam | Fine-loam |
| Ammonium Nitrogen (mg/Kg) | 8.5 | 47.0 |
| Nitrate Nitrogen mg/Kg | 19.5 | 38.0 |
| Phosphorus Colwell mg/kg | 69.5 | 29.8[B] |
| Potassium Colwell mg/Kg | 134 | 3.0 |
| Sulphur mg /Kg | 10.1 | - |
| DTPA Copper mg/kg | 1.5 | - |
| DTPA Zn mg/kg | 4.6 | 0.4 |
| DTPA Manganese | 5.5 | - |
| DTPA Iron | 74.0 | - |
| Exch. Calcium cmol$_c$/kg | 5.8 | 4.0 |
| Exch.Magnesium cmol$_c$/kg | 1.7 | 1.2 |
| Exch.Potassium cmolc/kg | 0.3 | 0.2 |
| Exch. Sodium cmolc/kg | 0.1 | 0.1 |
| pH ($H_2O$), | 6.1 | 6.6 |
| pH ($CaCl_2$) | 5.2 | 5.5 |
| Organic carbon (%) | 2.4 | 1.6[C] |

[A] Source: Chinene ( 1984).

[B] Based on Bray 1=critical 10 mg/kg; [C] 0-20 cm depth

**Table 4.2. The equivalent rates of N, P, K, Zn (kg/ha) and Se (g/ha) applied as treatments in the experiment.**

| Treatment | N | P | K | S | Mg | Zn | Se | Fe | Mn | Cu | B | Mo |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | - | - | - | - | - | - | - | - | - | - | - | - |
| Basal | 17.5 | 30 | 40 |  | 25 | - | - | - | - | - | - | - |
| Low N | 50 |  |  |  | 25 |  |  | 2 | 1 | 1 | 1 | 0.1 |
| High N | 100 |  |  |  |  |  |  | 2 | 1 | 1 | 1 | 0.1 |
| Low S |  |  |  | 40 |  |  |  | 2 | 1 | 1 | 1 | 0.1 |
| High S |  |  |  | 80 |  |  |  | 2 | 1 | 1 | 1 | 0.1 |
| Low Zn |  |  |  |  |  | 0.1 |  | 2 | 1 | 1 | 1 | 0.1 |
| High Zn |  |  |  |  |  | 2.5 |  | 2 | 1 | 1 | 1 | 0.1 |
| Low Se |  |  |  |  |  |  | 3 | 2 | 1 | 1 | 1 | 0.1 |
| High Se |  |  |  |  |  |  | 15 | 2 | 1 | 1 | 1 | 0.1 |

### 4.2.2 Experimental layout

The experimental lay out was a completely randomised block design with three replications. Three seeds of the white maize variety Pioneer 3287W were sown in each pot and 5 days after emergence, the plants were thinned to 2 per pot. After prior determination of field capacity, the soil was maintained at 100% field capacity by weighing and watering using deionised (18 MOhms resistivity) water daily. The plants were harvested when seedlings were 4 weeks old.

### 4.2.3. Measurements

Just before the final harvest, photosynthesis and transpiration measurements were conducted on the plants using an infrared, open gas exchange system LI-6400 (Li-Cor, Inc., Lincoln, NE, USA). Measurements were taken on the last fully expanded leaf on two plants per pot at a $CO_2$ concentration of 400µmol/mol and a PAR of 800µmol/m$^2$/s using the inbuilt light source. Measurements were taken 2-4 hours after the lights came on.

At harvest the heights of the plants were measured from the ground to the tip of the longest leaf using a metre rule before they were cut at ground level and then dried at 75$^o$C for 48 hours and weighed. The dried plant material was ground to pass a 2.0 mm sieve before analysing the whole shoots for nutrients. Budgetary restrictions meant that only a subset of samples could be analysed and nutrient analysis was done on two of the three replicates. Mineral concentrations of the shoot were determined by chemical analysis using the ICP-AES methods as outlined in Wheal et al. (2011). Selenium was analysed using ICP-Mass Spectrometry and N was analysed by complete combustion using an Elementar N analyser, which uses the Dumas method to measure total N in plant tissue. Nutrient measurements were checked by using certified standard reference materials. The values were reported as mg/kg units or percentage. The total uptake was estimated by multiplying the concentrations of the mineral nutrients in shoot parts by the dry weight of each treatment.

### 4.2.4 **Statistical analysis.**

Data analysis was performed using Genstat (14th edition) statistical software. The structure of the experiment was a control and basal treatments plus a factorial combination of N, S and Zn+Se treatments. The analysis of variance (ANOVA) compared (a) the control and basal treatments with the N, S and Zn+Se fertilised treatments and (b) the main effects and interactions between the N, S and Zn+Se treatments. All reported values are means of the three replicate pots except the nutrient analysis which were based on 2 replicates. Treatments means, when significant effects were evident were compared using LSD tests. Simple linear correlations were used to examine associations between variables.

## 4.3 Results

### 4.3.1 Photosynthesis and plant growth.

There were few significant treatment effects (Appendix Table 4.1) and these occurred between additional basal fertilisers and main effects of N (Table 4.3). There was significant difference in the photosynthetic rates between the control and basal fertiliser treatments, with additional nutrients from the basal treatment significantly reducing the photosynthetic rate.

**Table 4.3. The photosynthesis rate (mmol $CO^2/m^2/s$) of the last fully expanded leaf of maize grown under different combinations of fertilisers. The fertiliser values are the average of the S and Zn+Se treatments as there was no significant interaction with N. Values are means of three replicates.**

| Treatment | Low N | | High N |
|---|---|---|---|
| Control | | 10.9 | |
| Basal | | 9.2 | |
| Fertiliser | 11.5 | | 15.1 |
| LSD (P<0.05) | | 0.94 | |

However, photosynthesis rates increased with increasing N supply and were greatest at the high rate of N. The transpiration rate and stomata conductance were significantly affected by the addition of N and micronutrients Zn and Se There was no significant difference between the control and basal treatment in both transpiration rate and stomata conductance. However increasing the N and micronutrient supply increased the transpiration rate and stomata conductance (Tables 4.4 and 4.5). A summary of the treatment effects is presented in Appendix Table 4.1.

**Table 4.4. The effects of N on the (a) transpiration rate ($mmol/m^2/s$) and (b) stomata conductance $mol/m^2/s$ of maize grown under different fertiliser treatments. The Fertiliser values are the average of the S and Zn+Se treatments as there was no significant interaction with N.**

| Treatment | Low N | | High N | Low N | | High N |
|---|---|---|---|---|---|---|
| | | (a) Transpiration | | | (b) Stomata conductance | |
| Control | | 1.7 | | | 0.08 | |
| Basal | | 1.5 | | | 0.07 | |
| Fertiliser | 1.8 | | 2.2 | 0.08 | | 0.10 |
| LSD (P<0.05) | | 0.34 | | | 0.02 | |

**Table 4.5. The effects of micronutrients Zn+Se on the(a) transpiration rate (mmol/m$^2$/s) and (b) stomata conductance (mol/m$^2$/s) of maize grown under different fertiliser treatments. The Fertiliser values are the average of the N and S treatments as there was no significant interaction with N.**

<table>
<tr><th>Treatment</th><th>Low Zn+Se</th><th>High Zn+Se</th><th>Low Zn+Se</th><th>High Zn+Se</th></tr>
<tr><td></td><td colspan="2">(a) Transpiration</td><td colspan="2">(b) Stomata conductance</td></tr>
<tr><td>Control</td><td colspan="2">1.7</td><td colspan="2">0.08</td></tr>
<tr><td>Basal</td><td colspan="2">1.5</td><td colspan="2">0.07</td></tr>
<tr><td>Fertiliser</td><td>2.1</td><td>1.8</td><td>0.08</td><td>0.10</td></tr>
<tr><td>LSD (P<0.05)</td><td colspan="2">0.34</td><td colspan="2">0.02</td></tr>
</table>

**Table 4.6. The effect of S and micronutrient treatment on the height of maize. Values are averaged over the N treatments as there was no significant interaction with S and Zn+Se.**

<table>
<tr><th>Treatment</th><th>Low S</th><th>High S</th></tr>
<tr><td>Control</td><td colspan="2">18.9</td></tr>
<tr><td>Basal</td><td colspan="2">19.3</td></tr>
<tr><td>Low Zn+Se</td><td>19.5</td><td>18.2</td></tr>
<tr><td>High Zn+Se</td><td>19.1</td><td>20.3</td></tr>
<tr><td>LSD (P<0.05)</td><td colspan="2">0.57</td></tr>
</table>

There were few significant treatment effects on plant height (Appendix Table 4.2) and the only significant interaction occurred between S and micronutrients (Table 4.6). However, even though they were significant, the effects on height were relatively small. There was no significant difference in height between the control and basal treatments and between the basal and low S treatments. At low S there was no significant effect on height but at high S there was a significant increase in height. The maximum height was attained when S supply was high in combination with high supply of Zn and Se.

**Table 4.7. The effect of N and micronutrient treatment on the shoot dry matter (g/pot) of maize. Values are averaged over bot S treatments as there was no significant interaction**

| Treatment | Low N | | High N |
|---|---|---|---|
| Control | | 4.61 | |
| Basal | | 5.31 | |
| Low Zn+Se | 6.18 | | 7.50 |
| High Zn+Se | 6.97 | | 7.27 |
| LSD (P<0.05) | | 0.435 | |

Applying additional nutrients above the control and basal treatments significantly increased the shoot dry matter of maize and this was the largest influence on dry matter (Appendix Table 4.2). There were no significant effects of S treatment on the dry matter but in general N increased dry matter (Table 4.7), but the response depended on the amounts of micronutrients supplied. When the supply of Zn and Se was low, adding N significantly increased shoot dry matter by 21% whereas at the higher rate of micronutrients supply, there was no significant effect of additional N. Maximum growth was achieved if the supply of N was high.

### 4.3.2 Shoot nutrient concentrations

With the exception of the nutrients of interest (N, S, Zn and Se) the concentrations of the other nutrients were not significantly affected by the treatments and all the values, except copper, were above the critical level (Appendix Table 4.3). The nutrient analysis indicated a number of significant interactions of S and N with micronutrients Zn and Se (Appendix Table 4.4). There were few significant treatment effects of N and the only significant interactions occurred between

N and micronutrients (Table 4.8). There was a significant difference in the concentration of the Control and the Basal fertiliser treatment. Additional nutrients increased N concentration. However, increasing the N supply when the micronutrients Zn and Se were low reduced N concentrations by 24% whereas at the higher rate of micronutrient supply, there was no significant effect of additional N.

**Table 4.8. The effects of N and micronutrient treatment on the whole shoot N concentration (%) of maize.**

| Treatment | Low N | | High N |
|---|---|---|---|
| Control | | 1.21 | |
| Basal | | 1.76 | |
| Low Zn+Se | 1.84 | | 1.39 |
| High Zn+Se | 1.54 | | 1.80 |
| LSD(P<0.05) | | 0.29 | |

The effects of the Basal treatment and the micronutrient effects on the concentration and uptake of S are shown in Figures 4.1 and 4.2. There was no significant difference in the S concentration between the control and basal treatments but applying nutrients above the basal levels increased S concentration in the shoot (Figure 4.1a). The S concentration in the Control and Basal treatments suggested S deficiency.

In addition the rate of Zn+Se applied to the soil increased the S content significantly by over 70% compared to the low rate of S (Figure 4.1b). This effect was not solely due to the additional S applied at the higher rate of Zn (which was applied as $ZnSO_4.7H2O$) as the increase in total shoot S was approximately 7 mg/plant (Figure 4.1b), which was much higher than the S applied in the high Zn+Se treatment.

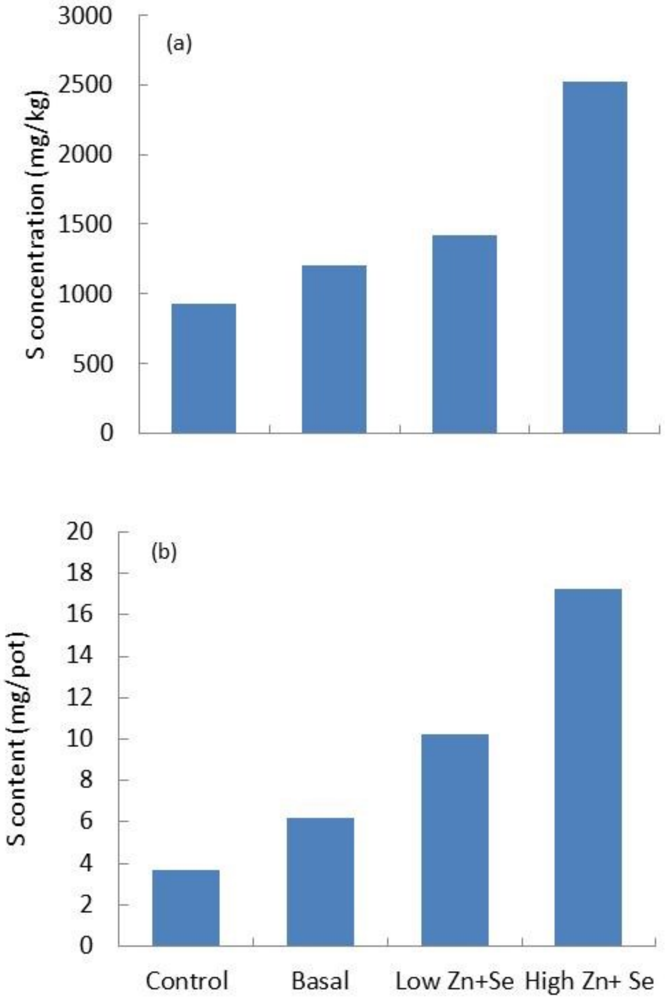

Figure 4.1. The effects of additional Zn+Se on the whole shoot S concentration and content in Maize. LSD (P<0.05) =486 mg/kg and 3.22mg/pot, respectively

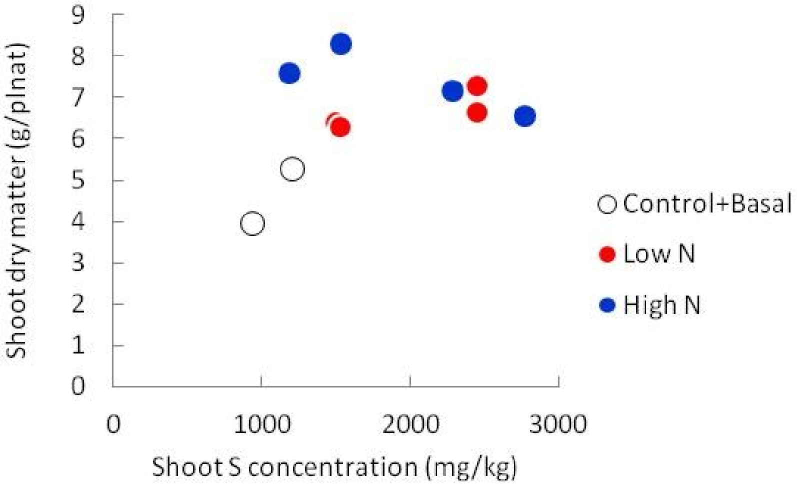

Figure 4.2. The relationship between shoot S concentration and plant growth

Figure 4.2 shows the relationship between shoot S concentration and plant growth. The Control and Basal treatments show low S and in general plant growth responds to increases in shoot S concentrations with maximum growth appearing to occur around a S concentration of 1500 mg S/kg.

The concentration of Se in the whole shoot showed a number of significant interactions with N, S and Zn+Se treatments (Appendix Table 4.4). The effects of N, S and Zn+Se treatments on the concentration of Se are shown in (Table 4.9). There was no significant difference between the Control and Basal treatment in the Se concentration. However, there was a complex interaction between the levels of N, S and the micronutrients Zn+Se on the Se concentration. Increasing S supply above the Basal nutrients when the Zn+Se concentration were low had no significant effect on the concentration of Se. However, increasing S when N was low significantly halved the Se concentrations whereas there was no significant effect of S treatment when N was high. Increasing the Zn+Se supply when the S and N supply were low also resulted in high Se concentration. Equally when S was low and N was high additional Zn+Se increased the Se concentration.

**Table 4.9.The effects of N and S on Se concentration (mg/kg) of whole shoot maize**

| Treatment | Low Zn+Se | | High Zn+Se |
|---|---|---|---|
| Control | | 0.09 | |
| Basal | | 0.07 | |
| Low S, Low N | 0.06 | | 39.50 |
| Low S, High N | 0.09 | | 36.51 |
| High S, Low N | 0.08 | | 18.96 |
| High S, High N | 0.27 | | 36.08 |
| LSD (P<0.05) | | 6.60 | |

There were few significant treatment effects on the N:S ratio (Appendix Table 4.4). There was no significant difference between the N:S ratio of the Control and Basal treatments (Table 4.10). However, the N:S ratio of the treatments was significantly reduced by adding additional Zn+Se .

**Table 4.10 The effects of micronutrient Zn and Se on the N:S ratio. Means are averaged over the N and S treatments as there was no significant interaction.**

| Treatment | Low Zn+Se | | High Zn+Se |
|---|---|---|---|
| Control | | 12.93 | |
| Basal | | 14.86 | |
| Fertiliser | 11.22 | | 6.71 |
| LSD (P=0.05) | | 3.22 | |

There was no significant correlation between N and S, but significant correlations were observed between N and Zn nutrients and between S and Se, S and Zn, and S and N:S ratio, as well as between S and Se content and S and Zn content (Table 4.11). Both Se and Zn concentrations were significantly correlated with S and N content, Se does not promote growth, and uptake will be more strongly be affected by Se concentration than Zn because Zn can affect both growth and uptake as expected.

**Table 4.11. Correlations between maize whole shoot concentrations of N, S, Se and Zn (P<0.05), NS indicate not significant.**

| (N=20) | S | Se | Zn | N:S ratio | Se content | Zn content |
|---|---|---|---|---|---|---|
| N | NS | NS | 0.71 | NS | NS | NS |
| S | | 0.88 | 0.46 | -0.84 | 0.87 | 0.47 |
| Se | | | NS | 0.36 | 0.99 | NS |
| Zn | | | | NS | NS | 0.61 |

### 4.4 Discussion

Even though the fertility of the soil was higher than one would have wished, the soil was still responsive to additional nutrients. This may imply that the nutrients may not have been plant available to meet plant requirements, and may reflect that the plants were grown in small pots where the nutrient demand can be high relative to the supply. The shoot dry matter yield and concentrations reported here indicated positive influences of N, S and Zn to plant growth. The primary function of photosynthesis is to supply the carbohydrates necessary for growth and biomass production of the crop (Hossain et al. 2012). The increased photosynthesis rate which may have resulted in production of high biomass was apparent generally with increasing N. The increases in shoot dry matter yield became more positive when the N rate increased and stimulated greater growth. A similar observation was also reported in other work (Salvagiotti and Miralles 2007).

Photosynthesis provides carbohydrates for growth and influences biomass production, which is positively related to plant height (Li et al. 2009; Malik et al. 2007, Reddy and Matcha 2010). Therefore, in the data described here, highest total biomass and plant height were associated with high photosynthetic rates: these occurred when S, N and micronutrients were high respectively. Nitrogen and Zn+Se interactions resulted in an increase in biomass yield of the shoots; both additional N and S increased shoot dry matter under low Zn+Se, while a high S supply increased plant height under a high supply of micronutrients

Nitrogen and S fertiliser treatments had marked effects on the concentrations of N, S, Se and Zn. No significant interaction between N and S rates was observed, both N and S had a positive effect on either biomass yield or height and concentration respectively, but the response to each fertiliser was independent of the other and was influenced by the level of micronutrients supplied. Therefore S and N are both required to be applied to obtain good yield and concentrations and are in good agreement with work reported in Ercoli et al, (2012). Increasing N supply under low micronutrients Zn and Se resulted in decreased concentration of N. However, increasing the N supply under high

Zn+Se resulted in increased N concentration. Recently, it has been reported that grain concentrations of Zn can be enhanced by N supply and that Zn and N have a synergistic effect on grain N and Zn concentration in durum wheat, but the strength of the Zn-N correlation is dependent on sufficiently high Zn availability to the plants and may be lost under low soil Zn (Kutman et al. 2011). This observation could also explain why there was no significant correlation between grain N and Zn in the grain survey (Chapter 3) in maize and wheat. The insufficient amount of plant available Zn in the soil or plant tissues in Zambia may limit the effects of the enhanced sink activity from the increased protein concentration and consequently the positive Zn-N correlation and the increase in Zn concentration in the whole grain from additional N is not observed.

The positive effect of increasing N supply on Zn uptake were also apparent, supporting earlier observation that N nutrition can enhance Zn uptake (Cakmak et al. 2010). Enhanced Zn uptake is a foundation for increased grain Zn concentration, in addition to remobilisation of Zn into the grain, and essentially should be taken into consideration when both yield and high grain Zn concentrations are targeted (Kutman et al. 2011). This observation is in agreement with the data reported here as significant positive correlations indicated sufficiently high Zn availability to the plant. Although the soil had high background Zn an increase in Zn uptake was apparent with increased application of Zn in combination with N and S. This suggests that N and S could enhance Zn availability. Sulphur concentration significantly increased with the additional S and Zn+Se supply at all rates and was significantly correlated to plant growth (Figure 4.2). The data suggested a critical level for S within the range of 1500-2000mg/kg which is comparable to the reported critical value for growth stage (V4) 28 days after emergence maize of 2100 mg S/kg (Reuter and Robinson 1997).

The concentrations of Se and Zn were significantly correlated to S concentrations in the shoot, while N was significantly correlated to Zn concentration (Table 4.11). The positive correlation between S and Zn, S and Se and N and Zn implies that S and N could play a critical role in enhancing Se and Zn concentrations in plants. Sulphur increased both Zn content and concentration in the shoot dry matter. This may indicate that the level of S can affect the uptake of Zn into vegetative tissue and its subsequent deposition in the grain. This effect is in good agreement with previously published data in which S and Zn concentration has been correlated and has inferred a link between S and Zn and/or protein transporter (McDonald and Mousavvi-Nik 2009). In addition the dominantly positive correlations of S with other nutrients like K and Mo (data not shown) may imply that S play a role in the uptake of other macro and micronutrients and consequently increased density (Rengel et al. 1999). Potassium and Mo were equally deficient in the Zambia survey (Appendix Table 3.1a).

In the case of S and Se it is thought that the competition between S and Se for assimilation into amino acids and proteins may account for the strong correlation between these nutrients (Mikkelsen and Wan 1990). The toxicity of selenate is progressively reduced by the presence of increasing sulphate in the rhizosphere solution (Mikkelsen et al. 1989) because high sulphate concentrations in the rhizosphere reduce selenate uptake and because they lower the Se/S quotient and the relative incorporation of selecysteine and selenomethionine into proteins (White et al. 2004). Based on this data, it appears too that high S may not reduce Se where N is also high. An antagonistic relation between S and Se has been documented in plants (Lyons et al. 2004) and in the low N treatment, increasing S rate significantly halved Se concentration, but this did not occur when the N rate was also high (Table 4.9). The peculiarity of Se compared with other metals is its high chemical/physical similarity to S and for this reason Se compounds follow the same metabolic routes as S compounds (Birringer et al. 2002; Mapelli et al. 2011). Furthermore, White et al (2004) showed a complex dependence on the ratio of selenate to sulphate. They indicated that rhizosphere selenate promotes sulphate uptake, possibly by preventing the reduction in the abundance and /or activity of sulphate transporters by sulphate and /or its metabolites. Perhaps, due to this tight interplay between S and Se metabolism, it might appear that they exert a reciprocal influence on each other.

Our data indicate an increase in S under high Zn+Se supply, i.e. S concentration in the shoot was almost doubled by the high Zn+Se application in high S and even then S is low (Figure 4.1a). The effect may be associated with the increase in Se supply as the response is in agreement with findings reported in barley and rice that shoot S concentration increased with increasing concentrations of solution of Se (Mikkelsen and Wan 1990); however, this synergistic response was mainly found when S was at low concentrations in the treatments. Interestingly, this observation is in agreement with other reported studies (Banuelos et al. 1990; Saurez et al. 2003). Selenium content however was not affected by the presence of S and Se content in the shoots was commensurate with the rate applied, perhaps due to the moderate levels of S and low N in the study. Detailed studies will be required before a complete explanation of this phenomenon can be given.

### 4.5 Conclusion

The results of the study conducted under growth room conditions showed that a sufficiently high N and S supply is a critical factor in increasing concentration of Zn in the vegetative tissue. Therefore may speculate that increasing the vegetative concentrations could ultimately increase grain Zn concentrations. Sulphur uptake may be enhanced by addition of high Se under low S conditions. Both S and N are significantly correlated to Zn, and Zn uptake can be increased even from the soil with adequate Zn supplies in combination with S and N. The Se uptake may depend on the plant available Se and therefore successful vegetative loading and remobilisation to the grain will depend on the additional and correct supply of Se, which could improve Se density in human diets via the food chain.

The findings reported here indicate that S nutrition could play a role in improving nutrient uptake and have important implications for soil fertility management and fertiliser application in acidic, low N, S and Zn soils of Zambia in improving yield and nutrition quality of the grain. In maize, yield responses to N are more frequent than those of S fertilisation, but the central role of S and N in protein synthesis and efficient utilisation of N are cardinal in the improvement of quality of the grain and yield. The nutrient loading in the vegetative tissue is essentially the basis for the successful remobilisation in addition to adequate nutrient supply from the soil.

# CHAPTER 5

# Sulphur uptake and interaction with N, Se, Zn and yield responses in wheat

## 5.1 Introduction

This chapter will focus on S and Se as in the previous experiment S rate appeared to be an important influence on the uptake of Zn and Se. However, that experiment had effectively three treatments and there was a clear difference between the lack of additional S in the control and basal treatments and the two S treatments in terms of S concentration and there were interactions between the concentrations for S and the two micronutrients. Therefore, it was decided to use more rates of S to examine the response to S in more detail at two levels of Se while N and Zn were applied as basal nutrients in this study on wheat.

The survey of grain from Zambia established the prevalence of low concentrations of S in maize and wheat grain grown in Zambia. This suggests that S deficiency could be limiting productivity as well as Se and Zn uptake, causing suboptimal S amino acid status in the population. However, comprehensive agronomic practices, including fertiliser application strategies aimed at enhancing nutrient concentration for human consumption have yet to be pursued in Zambia even though wheat production in the country is mainly grown by large commercial farms.

Fertiliser applications aimed at improving grain nutrient concentration have also been reported to improve crop establishment when seed is sown in nutrient deficient soils (Ascher et al. 1994; Rengel and Graham 1995). In Zambia, most major soils are deficient in nutrients and S deficient areas are increasing (Tsuji et al. 2005). This trend is mostly as a result of an intensification of agriculture with greater use of improved, high yielding cultivars, a focus on N fertiliser as a means to increase yields and a greater use of high analysis fertilisers which are low in S. Consequently, nutrient exhaustion resulting in nutrient imbalances in the crop has occurred. Nitrogen fertiliser is applied regularly to crops in Zambia, but its efficiency could be hampered by lack of adequate S (Fazli et al. 2008). Therefore adequate S nutrition becomes necessary to achieve maximum efficiency of applied nitrogenous fertilisers. Sulphur nutrition in Zambian agriculture has not been considered a major problem and the main emphasis is on N, P and K. This is despite strong relationships between S and N for crop growth and yield established in some studies (Jamal et al. 2010; McGrath and Zhao 1996). However, the previous grain survey suggests that S should be included with the other macronutrients in fertiliser.

Wheat yield increased linearly with N fertiliser rate in an S and N interaction study by Reneau et al. (1986). The low S and N levels in the maize and wheat grain in Zambia suggests that responses to S application are likely in the farming systems. The application of N fertiliser in these farming systems is still low. Indeed, the combined application of S and N was reported to have had the largest effect on the uptake and concentration of S and N and protein of grains and yield (Aulakh et al. 1980) and Randall et al (1981) observed that S application increased wheat S concentration more with low N treatment, but had only small effects on N concentration in the grain. Strong correlations between S and Zn and S and Se, linked to uptake mechanisms involving Zn and Se, have also been reported (McDonald and Mousavvi-Nik 2009; Mikkelsen and Wan 1990). Therefore, S nutrition may enhance not only yield but also S, Zn and Se concentration in the grain given that protein is a sink for Zn and Se. This is likely to improve the delivery of these nutrients to the population. However, given the deficient S situation in Zambian soils it may be necessary to manage S and Se effectively. This can be achieved by applying additional Se fertilisers as the need to apply S to overcome S deficiency may interfere with Se uptake.

The results reported here will further help to understand the nutritional behaviour of S in relation to N, Se and Zn and inform balanced fertiliser recommendations in order to enhance yield and nutrient concentration in grain produced and consumed in Zambia.

## 5.2 Materials and methods

The experiment was conducted in the growth room with growing conditions the same as those described in Chapter 4. A different soil type was used in this experiment because of the high levels of Zn in the Echunga soil. Mt Compass soil was used in this experiment as preliminary experiments indicated it gave a greater response to Zn. The aim of the work was to assess the effects of S supply on the uptake of Zn and Se applied as a basal fertiliser. Mt Compass sand has been used extensively in studies in Zn deficiency (Genc et al. 2006). The soil is acidic and has inherently low fertility like most soils in Zambia. The chemical analysis of Mt. Compass sandy soil compared to a Zambian soil as outlined in Chapter 4 is presented in Table 5.1

The experiment was a factorial combination of 4 levels of S x 2 levels of Se. The experiment was set up as a completely randomised design with four replications. The total amounts of S applied as $CaSO_4$ were at the following levels: 0, 56 mg S/pot, 111 mg S/pot and 222 mg S /pot (equivalent to rates of 0, 20, 40 and 80kg S/ha) and the two Se levels included a low and high treatment at 2.4 μg Se/pot and 12 μg Se/pot (3 and 15g Se/ha). A basal treatment comprised of 177.6 mg nitrogen as $KNO_3$ (equivalent to 100 kg N/ha), 70.4 mg phosphorus in the form of $Ca_2 (H_2PO_4).H_2O$, (15 kg P/ha), 80.7 mg $MgCl_2.6H_20$ (25 kg Mg/ha), 84.5 mg $KNO_3$ (40 kg K/ha) was applied to all the treatments. Basal micronutrients were also applied to the pots, which consisted of 3.3 mg $MnSO_4.4H_2O$ (1kg Mn/ha), 3.2 mg $CuSO_4.5H_2O$ (1 kg Cu/ha), 4.6 mg $H_3BO_3$ (1 kg B/ha), 0.14 mg $Na_2MoO_4.H_2O$ (0.1kg Mo/ha), 3.3 mg $Fe_2SO_4.7H_20$ (2kg Fe/ha) and 2.5 mg $ZnSO_4.7H_2O$ (2.5 kg Zn/ha). The fertiliser rates used in this experiment represent normal commercial rates likely to be used in wheat production, whereas the low Se level represent an ideal level for grain agronomic biofortification and a high dose for comparison purposes based on the analysis in Lyons et al (2004). Before planting these nutrient solutions were homogeneously mixed through 3kg soil and then placed on pots lined with a plastic bag.

**Table 5.1. Characteristics of the soil used in the experiment compared with a soil from Zambia commonly used for wheat production.**

| Soil Property | Mt. Compass[A] | Typical soil (Zambia) |
|---|---|---|
| Texture | Sandy | Fine-loam |
| Ammonium Nitrogen (mg/Kg) | 1 | 47.0 |
| Nitrate Nitrogen mg/Kg | 14 | 38.0 |
| Phosphorus Colwell mg/kg | 5 | 29.8 |
| Potassium Colwell mg/Kg | 26 | 3.0 |
| Sulphur mg /Kg | 2.7 | - |
| DPTA Copper | 0.21 | |
| DTPA Zn mg/kg | 0.4 | 0.4 |
| DTPA Iron (mg/kg) | 35 | |
| DTPA Manganese (mg/kg) | 1.4 | 1.2 |
| pH ($H_2O$), | 5.8 | 6.6 |
| pH ($CaCl_2$) | 5.0 | 5.5 |
| Organic carbon (%) | 0.5 | 1.6 |
| Electrical conductivity (dS/m) | 0.04 | - |

[A] Source (Todd 2009)

Five seeds of a very early-maturing bread wheat (*Triticum aestivum* L, variety Axe) were planted and thinned to 3 seedlings per pot a week after emergence. The plants were watered daily with deionised (18MOhms resistivity) water to maintain field capacity. The plants were harvested at maturity, 8 weeks after sowing and grain was threshed from the ears, counted and weighed to determine grain yield and its components, grain number and mean kernel weight. Samples were ground for analysis for nutrient concentration as outlined in Chapter 3 after being dried in an oven for 48 hours. The nutrient analyses were done by Waite Analytical Services of the University of Adelaide according to the methods outlined in Wheal et al (2011). Analyses of S and other macro and micronutrients were performed using ICP-AES, N was analysed using the Dumas Combustion Technique using an Elementar total N analyser. Selenium was analysed using ICP-MS. Budgetary limitations meant that analysis of N and the other macro and micronutrients were conducted on composite samples to give two replicates for the ICP-AES and Elementar analyses. At each rate of S replicates 1 and 2 were combined as were replicates 3 and 4 to form the two replicate samples for analysis. In each case a 250 mg sample was weighed from each replicate and thoroughly mixed to form a 500 mg sample for each of the two composited replicates. The two replicates were further combined to give eight composite samples (4 S x 2 Se) on which the Se concentrations were measured. The nutrient measurements were checked by using certified standard reference materials. The results are presented in mg/kg or percentage units. The nutrient contents were obtained by multiplying the nutrient concentrations with the average values of the bulked grain yields.

Data were analysed using standard ANOVA procedures and their significances were evaluated based on $p<0.05$ level using the LSD test in GenStat 14th edition.

## 5.3 Results

### 5.3.1 Grain yield and yield components

There was a significant effect of S on growth (Appendix Table 5.1). Sulphur application significantly increased shoot biomass, number of ears, number of seeds and grain yield (Table 5.2). There was no significant S x Se interaction for any of the measurements. Growth and yield increased up to 20 kg S/ha with no significant increase at higher rates of S. The control treatment had fewer, smaller ears and produced a smaller number of grains.

**Table 5.2 Effects of S application on shoot biomass, grain yield and yield components. Values are means of four pot replicates each containing three plants, NS =not significant.**

| S level (kg/ha) | Shoot biomass (g/pot) | Number of ears (no./pot) | Grain yield (g/pot) | Grain no./pot | Kernel wt (mg) |
|---|---|---|---|---|---|
| 0 | 7.9 | 5.4 | 2.9 | 84.6 | 34.3 |
| 20 | 10.3 | 7.3 | 4.4 | 138.6 | 32.0 |
| 40 | 10.4 | 6.8 | 4.3 | 124.4 | 34.2 |
| 80 | 10.9 | 6.4 | 4.8 | 145.9 | 33.1 |
| LSD (P< 0.05) | 1.4 | 1.5 | 1.4 | 40.0 | NS |

At the 40 and 80 kg/ha S application, maturity was delayed by around 10 days. Although grain yield was significantly increased by S application, kernel weight was not and kernel weight tended to increase with lower grain numbers.

There were no significant interactions between S and Se treatments on the grain yield and its components and the only significant effect of Se application was on numbers of ears per pot which significantly increased at the high Se treatment (Table 5.3).

**Table 5.3 Effect of Se treatments, NS =not significant.**

| Se level (g/ha) | Shoot biomass (g) | Number of ears/pot | Grain yield (g) | Grain no. | Kernel wt (mg) |
|---|---|---|---|---|---|
| 3 | 9.8 | 6.1 | 4.3 | 118.1 | 36.3 |
| 15 | 9.9 | 6.8 | 4.5 | 128.6 | 35.8 |
| LSD (P<0.05) | NS | 0.7 | NS | NS | NS |

### 5.3.2. Grain nutrient concentrations

There was no significant interaction of S and Se treatments on grain nutrient concentrations (Appendix Table 5.2), but the S and N concentrations were significantly affected by the S application, while Zn concentration was not significantly affected (5.4). The Zn concentration in the grain was very high. This indicated that the Zn rate used in the experiment was more than adequate for plant growth. Increasing S application caused a decline in grain N concentration.

**Table 5.4. The effects of S treatments on S, N, Zn concentration and N:S ratio in wheat grain. Values are means of the bulked replications, NS not significant.**

| S level (kg/ha) | Grain nutrient concentrations | | | |
|---|---|---|---|---|
| | S<br>mg/kg | N<br>% | Zn<br>mg/kg | N S<br>ratio |
| 0 | 1118 | 3.0 | 65 | 26.9 |
| 20 | 1368 | 2.4 | 53 | 17.7 |
| 40 | 1630 | 2.5 | 62 | 15.4 |
| 80 | 1518 | 2.3 | 55 | 14.8 |
| LSD (P<0.05) | 244 | 0.4 | NS | 1.8 |
| Critical concentration | 1200 | 2.0 | 23-30 | 16 |

[A] Based on Reuter and Robinson 1997

The concentrations of S, N and Zn were within adequate ranges except for S at S (0). The N:S ratio was significantly affected by S treatment and a decrease in the N:S as S rate increased (Table 5.4) was apparent. The N:S ratio, which is a reliable indicator of S deficiency, can be used to understand the S status of the crops (Stewart and Whitefield 1965). The N:S ratios indicated S deficiency in the control (S0) and moderate-low level in the S20 treatment and close to the critical concentration in the other two treatments (S40 and S80).

The effects of the Se treatments on the concentration of S, N and Zn are indicated in Table 5.5.and show that the Se treatment had no significant effect on their concentrations.

**Table 5.5. Effect of Se application on S, N and Zn concentrations in wheat grain. Values are means of bulked replicates NS = non-significant.**

| Se level (g/ha) | Nutrient concentration | | |
|---|---|---|---|
| | S<br>mg/kg | N<br>% | Zn<br>mg/kg |
| 3 | 1420 | 2.6 | 58 |
| 15 | 1396 | 2.5 | 59 |
| LSD (P<0.05) | NS | NS | NS |

### 5.3.3 Mineral content

The effects of application of S on grain S, N and Zn contents are given in Table 5.6. Adding S significantly increased the S uptake but N uptake appears to be depressed with addition of 20 kg S/ha from the S deficient treatment (S0). The Zn uptake was not significantly affected by S application.

**Table 5.6. Effect of S treatment on the grain S, N and Zn content. All the values are means of the bulked pot replicates, NS =not significant.**

| S level (kg/ha) | Grain nutrient content (ng/seed) | | |
|---|---|---|---|
| | S | N | Zn |
| 0 | 38.3 | 1026 | 2.2 |
| 20 | 43.9 | 777 | 1.7 |
| 40 | 55.8 | 862 | 2.1 |
| 80 | 50.2 | 707 | 1.8 |
| LSD (P<0.05) | 10.1 | 173.8 | NS |

The effect of S on Se concentration is shown in Table 5.7. The Se concentration was based on a bulked sample that formed only one replicate. The analysis showed a reduction in Se concentration with increasing S rate at both rates of Se. Though we added 5X the concentration of Se to the soil, the grain Se concentration increased by approximately 10X at the higher S treatments (40 and 80 kg S/ha). Relationships between nutrients were explored through correlation analysis (Table 5.8). A highly significant correlation between N and Zn concentration was observed whereas other associations were not significant.

**Table 5.7. The effect of S on Se concentration at low and high Se supply to soil. Values are for composite samples from four replicates.**

| S level (kg/ha) | Grain Se concentration µg/kg | |
|---|---|---|
| | 3 g Se/ha | 15 g Se/ha |
| 0 | 890 | 5000 |
| 20 | 580 | 3900 |
| 40 | 360 | 3100 |
| 80 | 250 | 2300 |

**Table 5.8. Linear correlations between the concentrations of S with N and Zn in the wheat grain grown under different S levels. * - P< 0.05; NS= not-significant.**

| Wheat | S (n=16) | Zn (n=16) | Se (n=8) |
|---|---|---|---|
| N | NS | 0.79* | NS |
| S | | NS | NS |

### 5.4 Discussion

With the exception of the nutrients of interest presented in this study, a summary of the other nutrient concentrations, shown in Appendix Table 5.3, generally did not show deficient concentrations when compared to the critical concentrations in the grain (Reuter and Robinson 1997).

The results obtained in the study reported here demonstrate that S fertiliser can have marked effects on grain yield and grain nutrient concentrations as well as nutritional quality of wheat. Sulphur deficiency resulted in decreased S amino acids content in grain and accumulation of non-protein compounds such as amides (Faete et al. 2005; Mortensen and Eriksen 1994). The positive effect of S fertiliser on grain yield was essentially as a result of higher numbers of kernels per spike (Table 5.2) which could have been due to initiation of more spikelets and /or of a reduction in mortality of florets (Archer 1974). Large increases in seed yield from 2.9 g/pot to 4.4 g/pot, or a 51 % increment, were obtained in response to S application of 20 kg S/ ha. Yield benefits of such scale indicate that S nutrition could play a role in increasing grain yield and protein quality due to higher S amino acids, (methionine and cysteine) and could be highly cost effective in S deficient areas if such response could be demonstrated in the field. A deficiency in S supply to crops lowers the utilisation of available soil N, consequently increasing nitrate leaching (Lakkineni and Abrol 1994) especially on sandy soil texture. Sulphur also increased green leaf retention and it might reduce grain N by keeping the N in the leaves for longer.

The concentrations of S in wheat grain increased with increasing S rate up to 40 kg S/ha but there was no further increase in the concentration with further application. The grain N was depressed compared to the control with addition of S, which appears to be due to other factors other than dilution effect as yield increased with addition of S, but N was reduced. A number of studies indicate a synergistic effect of combined application of S and N on the uptake of N and S in a number of crops (Jamal et al. 2010). Rabufeti and Kamprath (1977) reported that S and N fertilisation increased the percent total S in corn grain, but the same authors also indicate that S addition however significantly increased the percent N in the grain at S rate of 112 kg/ha or above, but slightly depressed the N content when applied at 50 kg N/ha. This could suggest that S application increased the wheat grain S concentration more with low N treatment, but had only small effects on N concentration in the grain. The present data suggest that S reduced N in all the S treatments and had the greatest influence on the N:S ratio. The reduction in N: S with addition of S also is in good agreement with earlier findings that S addition decreased the N:S in wheat at maturity (De Reuiter and Martin 2001).

The N:S ratio is often preferred over S concentration as a diagnostic criterion for S deficiency and a ratio of 16 as a criterion in the grain is reported (Stewart and Whitefield 1965). Thus, a ratio of above 16 indicates S supply is low relative to N or N supply is high relative to S, but the ratio alone does not tell whether supply of either nutrient is adequate, hence the use of both the ratio and concentration is preferred. The higher N content in the control could probably be attributed to S deficiency indicated by the N:S (Table 5.2). According to Zhao et al (1999), wheat grown in England rarely has N: S ratios exceeding 16 and there is an indication that N:S imbalance occurs at ratios greater than 16 and leads to yield reductions. This is consistent with the present results where S responses occurred when N:S ratio was greater than 16. In other studies, Janzen and Batteny (1984) while working with rapeseed found that maximum yields were obtained when both N and S were applied and the N:S ratio was in approximate balance. The ratio of total N content to total S content and protein S determine the degree of availability or deficiency of S in the protein (Jamal et al. 2010). An accumulation of N and S in the grain was reported by Lerner et al, (2006). These authors suggested that the amino acid composition of storage proteins changes with grain S concentration, under high S availability the synthesis and accumulation of S rich storage proteins is favoured at the expense of S-poor proteins. Consequently, compared to the grain S concentration of the samples from Zambia, similar nutrient concentrations in wheat could indicate that yield and quality can be improved with S application to production systems.

In the case of Zn uptake, there are two genetic mechanisms governing Zn movement from the soil to grain, namely Zn uptake from the soil commonly termed Zn uptake efficiency and the other is mobilisation of this Zn to the grain. Although there is no genetic relationship between Zn efficiency and Zn grain concentration (Kalayci et al. 1999), previous analysis of grain mineral concentrations in which S concentration has been correlated with Zn concentration has inferred a link between S and Zn transport (McDonald and Mousavvi-Nik 2009). Such correlations did not occur in the current experiment (Table 5.8) and could be due to high Zn concentration in the grain. However, N and Zn were significantly positively associated. The correlations between grain N and S concentration was not significant and this is confirmed by wide variation in the N:S ratio in the grain ranging from 14 to 26. The absence of interactive effect between N and S observed indicated that both N and S contribute effectively in improving grain yield and quality, irrespective of the level of application. A similar finding also was reported by Ercoli et al. (2012). The correlations of S treatments on the concentrations of S, Se and Zn could indicate a positive influence on uptake and /or transport for both macro and micronutrient and increased density in the grain (Rengel et al. 1999). This observation is very important and suggests that S application could also enhance the uptake and remobilisation of other macronutrients and micronutrients that were also deficient in the Zambian grain.

Sulphur application reduced Se concentration in the wheat grain (Table 5.7). Therefore, application of gypsum and high S fertilisers at commercial rates is likely to reduce Se concentrations in the grain of wheat and it may be necessary to add Se to fertiliser to avoid this when S is applied (Lyons et al. 2004). The uptake of Se by plants and subsequently in the food chain is governed by many soil factors including the presence of ions such as $SO_4^{2-}$ and $PO_4^{2-}$. The presence of abundant $SO^{2-}{}_4$ in the root zone typically reduces the Se concentration in the plants due to either antagonistic interaction between $Se^{6+}$ and $SO_4^{2-}$ for plant uptake (Gissel-Nielsen 1979; Lyons et al. 2005; Wan et al. 1988), or in the case where S is added to deficient soils, may reflect a dilution of plant Se due to increased plant growth (Mikkelsen et al. 1989). However, there was no correlation between S and Se concentration (Table 5.8). In the vegetative loading in maize and wheat grain biofortification experiments, reported in Chapter 4 and here respectively, both reductions and synergistic effects have been observed.

From treatments in this experiment reported here, we were able to increase the grain concentration of Se to above the target of around 300 µg/kg (more than adequate) (Lyons et al. 2005) with the low Se treatment of 0.02 mg Se per kg. This indicates that enhancing the concentration of organic Se in the grain can increase the Se intake of animals and humans. An intake of up to 450µg Se/day (the Upper Tolerable Intake Level) is regarded as safe and is unlikely to be achieved through the dietary sources only (McKenzie et al. 1998). Only small amounts of fertilisation were required to raise the grain Se concentrations of wheat to acceptable concentrations which could be an easy, efficient and cost-effective method for increasing Se intakes. Indeed, field trials in neighbouring Malawi show approximately 300 µg/kg increase in maize with addition of approximately 15 g Se/ha (Chilimba et al. 2011).

## 5.5 Conclusion

Our results showed that S application may play a role in increasing yield of wheat. The increase in yield and concentration of S was consistently high, whereas N was significantly correlated to Zn, indicating that high yields and adequate nutrient concentrations may be achieved for agronomic biofortification. It is hoped that these results from the pot experiment can be used to produce crops of improved yields and nutrient concentrations on major soils that are deficient in S, Se and Zn in Zambia. Increased concentrations of Se and Zn are likely to have health benefits by increasing the consumption of Se and Zn in the general population. Further, these results may lead to improved efficiency of N fertilisers and make farming more profitable and attractive and build health farming communities and the general population.

# CHAPTER 6

## General discussion and conclusion

The major aims and objectives of the work described in this thesis were to establish the current status of N, S, Se and Zn concentrations in maize and wheat grain in Zambia as a means of identifying possible nutritional limiting factors to yield and enhanced grain nutrient concentrations. The other objectives were to investigate the influence of N and S nutrition on uptake of Se and Zn to achieve agronomic biofortification in a situation where deficiencies in S and Zn and low Se levels of Se are apparent.

The surveys covering three zones and comprising grain samples showed that maize and wheat grain grown and consumed in Zambia generally is low to deficient in N, S, Se and Zn which infers there are large areas of Zambia deficient in plant-available N, S, Se and Zn. The high N:S ratio found in the grain suggests that N and S are out of balance possibly because of the focus on N nutrition in the past which may limit the effectiveness of N application. However, the N concentrations may still be improved. There is evidence from the experiments reported here and, in the literature, to suggest that improving S supply and application of small quantities of Se and Zn can lead to improvements in yield and in Se and Zn concentration in maize and wheat (Cakmak 2008; Haug et al. 2008). Nitrogen fertiliser use efficiency may be improved by including S fertiliser in the fertiliser programme (Fernando et al 2009), while Se and Zn concentration may need to be intentionally added if both yield and nutrient concentrations are targeted for improvement. A response to S, Se and Zn application is expected given that N fertiliser use is already on the increase in the production of crops in Zambia. In addition to improving Zn concentration and intakes for the population, higher seed Zn content improves seedling vigour and early growth, especially where plant-available Zn in the soil is limited (Rengel and Graham 1995).

In the past, yield and nutritional improvements of maize and wheat in Zambia have focused on the increased use of N fertiliser, and to a lesser extent P and K (Lungu and Dynoodt 2008). The use of N fertiliser as the major way of improving yield could lead to imbalances in nutrient uptake which over time could lead to low production (Lungu and Dynoodt 2008). Most major soils in Zambia are reportedly deficient in plant-available Zn and seed with enhanced Zn content would be of great benefit. The same authors also indicated that sowing seed with high Zn content can help to overcome problems of insufficient Zn fertilisation, unreliable supplies of Zn fertiliser as well as spatial and temporal variability in Zn availability and have measurable benefits of better grain yield. The positive correlations between N and Zn, and N and S reported in the current work in improving yields and concentration would infer that these nutrients may need to be applied in appropriate balanced combinations. This should therefore be addressed in the grain production in Zambia.

The ability to manage both S and Se becomes important, especially since both were identified as low in the survey and there is a commonly described negative interaction between S and Se in which high S can reduce Se uptake. However, both synergistic and antagonistic interactions have been reported between S and Se. Some work has suggested that S and Se enter plants through multiple transport pathways with contrasting selenate/sulphate selectivity, the activities of which vary between plants of contrasting nutritional status (White et al. 2004) and this could explain species and probably varietal differences in tolerance to Se toxicity. Wheat is reported to be more tolerant to Se phytotoxicity than many other crops (Lyons et al. 2004; Mikkelsen and Wan 1990). It is important therefore to elucidate the interactions between S and Se in order to ensure efficient delivery in nutrition programmes. The results reported here suggest that the interaction between S and Se can be complex with both negative and positive effects being evident depending on the Se and S concentration in the soil. Selenium concentrations were directly related to the Se rate applied. The addition of S where soil Se levels are low could have exacerbated low Se concentration in the grain, but a high Se concentration improved the uptake and concentration of S. White et al (2004) also reported that selenate promoted the uptake of sulphate a finding also observed in the results in Chapter 4, which suggest a synergistic relationship where Se increased S uptake at low S rates. Therefore, S and Se could be managed successfully to improve growth, crop yields and nutrient concentrations in crops in Zambia. However, additional research is required in the field to understand further the interactions between S and Se fertiliser regimes before a complete explanation of the synergistic and antagonistic phenomenon can be given, across soils of variable fertility levels and to evaluate further the rates of S and Se required for maize grown to maturity.

The N and Zn, S and Zn as well as the S and Se interactions are important in the agronomic biofortification of maize and wheat with Zn and Se. From both experiments described in Chapters 4 and 5 it is clear that S and N are important factors in the uptake of Zn and Se and could be important in subsequent remobilisation to the grain. The supply of Zn as $ZnSO_4$ ensured that S was supplied at the same time as Zn and could contribute to S concentration in the grain, but the amount of S supplied was low and only comprised a small proportion of the total S uptake. The positive effect of S fertiliser on grain nutritional quality may suggest the application of S fertiliser can be beneficial to grain N and Zn even in regions not deficient of soil S (Ercoli et al. 2012). Despite, relatively high soil fertility in the experiment with maize (Chapter 4), responses in growth and in nutrient concentration to added nutrients were measured. Photosynthesis and vegetative growth responded to additional N and S and even in soil where the background level of Zn was relatively high, increases in Zn concentration were obtained, depending on the supply of N and S. The results illustrate the importance of maintain a balanced nutrient programme to maximise the benefits of improvements in soil fertility and increase the effectiveness of agronomic biofortification. For example, increasing soil available Zn to overcome chronic Zn deficiency will be most effective if available N and S are also high. However, the results from the two pot experiments in the present study indicate that nutrient interactions are complex and can vary depending on the species and/or whether vegetative or yield responses are examined. For example, additional Zn+Se increased S uptake in vegetative tissue in maize but additional Se did not increase S in the seed of wheat (Chapter 5). This result could have been due to use of both different crop species and soils in the experiments. However, the other factors governing mobility of nutrients from the vegetative tissue to the grain may also influence the grain concentrations. Selenium added as selenate is easily translocated in the plant and into the grain whereas the mobility of S in the plant varies with N supply and may dependent on the constant uptake from the soil in addition to remobilisation, so a continuous supply of S is needed from emergence to crop maturity as S deficiency at any stage of growth can lead to reduced yields (Zhao et al. 2008). To enhance the effectiveness of biofortification programmes further studies will be required to characterise the nature of these interactions and to assess whether the types of responses observed under controlled conditions are reproduced in the field.

Two experiments were conducted using different species and examined different aspects of growth and consequently the results are not directly comparable. Nevertheless, it was decided to examine responses in maize and wheat to complement the results from the field survey and to investigate whether there were common responses under different conditions. It would be beneficial to grow maize to maturity to examine nutrient loading into the grain, while using a short season wheat variety allowed grain nutrient responses to be examined. The two soils used in the experiments also meant that results could have been influenced by different nutrient availabilities to plants in the soil. This could have contributed to the noticeable Zn effect on S uptake observed in the vegetative loading experiment but was not apparent in the wheat grain experiment. The Zn concentrations measured in the seed were high and above those normally measured in commercial crops. Despite this there was still a significant positive correlation between grain N and Zn concentrations, suggesting that interactions between Zn and N can occur over quite a wide range of Zn supplies. While there was variation in experimental conditions between the two experiments, there were some consistent results as well in the work reported here which shows positive interactions between N and Zn, S and Zn concentrations in both experiments and so it can be argued that adding N and S may enhance Zn uptake.

For Zambia in particular and SSA in general, there are a number of useful outcomes resulting from this study. There is an urgent need to address micronutrient deficiencies given that they affect a large segment of the population and levels of infectious diseases are high. Sulphur deficiency needs to be addressed as S can limit the success of agronomic biofortification of staple crops with Se and Zn. The results obtained in the vegetative loading of nutrients in maize (Chapter 4) with the control and basal treatments provides evidence to the effect that S deficient plants were low in Zn and less responsive to Zn, even though the soil test suggested that there was adequate Zn. It would appear that low S can therefore restrict ability to utilise soil Zn. Sulphur is one of the essential nutrients for plant growth with crop requirements similar to phosphorus. This element has received little attention for many years because fertilisers and atmospheric inputs (in mining and industrial areas) supplied the soil with adequate amounts of S. Now areas of S deficiency are becoming widespread throughout the world (Ercoli et al. 2012) and SSA is not an exception. In many western countries it has been associated with overcoming air pollution, but in SSA it is more related to decline in soil fertility. This is mainly caused by the use of high analysis-low S fertiliser, high yielding varieties and intensive agriculture and declining use of S containing fungicides (Scherer 2001).

An insufficient S supply can affect yield and quality of the crops, caused by the S requirement for protein and enzyme synthesis as well as it being a constituent of the amino acids; methionine, cystine and cysteine. Low yields and poor protein quality are expected to impact negatively on the health of the general population. Generally agronomic biofortification programmes (mainly with Zn) have focussed on the use of N (Cakmak 2008; Kutman et al. 2011), but the current work suggests that S is also important and may have been overlooked as a limitation. A possible reason may be that many of the previous biofortification trials have been done in collaboration with participating research institutions on research stations where background levels of fertility would generally be higher than farmers' fields so S deficiency may not show up as a problem.

Efforts aimed at mapping S, Se and Zn deficiency in SSA can be much more effective when stakeholders from various backgrounds are involved, such as soil, plant, animal and human nutritional scientists, Ministries of Health and Agriculture, agriculture research institutes, and national and international NGOs that strive to alleviate malnutrition. Projects like the long-term Zn fertiliser project which is currently underway in neighbouring Malawi, with the main objectives of increasing yield and improving human health through increased consumption of Zn fortified crops (Zinc Nutrient Initiative 2012), could have important outcomes for the entire region as preliminary results reveal that after the first season of Zn fertilisation significant increases in maize yield and in Zn concentration occurred. In both Malawi and Zambia, the national governments are already supporting the farming communities with subsidised agricultural inputs that include seed and N, P, K fertilisers, and including Se and Zn in these programmes could have a positive impact on the health of the general population. In Zambia, initially the programme supported maize only but it has now been expanded to include other crops like sorghum, rice and millets normally produced for food security. Agronomic biofortification of cereal crops with Se and Zn could be an inexpensive and relatively easy way of improving yields and the nutritive value of food crops produced and consumed in the region.

Finally, the information presented in this thesis may have immediate application to the farming community and researchers seeking to improve the yields and nutrient concentration of staple food crops. However, further work is needed for detailed surveys to document the extent and degree of S deficiency in Zambia. Further, education on the importance of increasing Se and Zn concentrations and support in terms of the provision of mixed fertilisers that would include Se and Zn for the successful agronomic biofortification of staple food crops is required. That involves policy on the part of the national government and implies committing financial resources to this cause. Therefore, future work would include:

- Broadened and more systematic studies in order to draw definite conclusions on the status of micronutrients in the soils, staple crops and the general population in Zambia. This could include mapping areas of deficiency, further grain surveys and obtaining data on the Se and Zn status in the population.
- Addressing S deficiency and promoting Se and Zn fertiliser application into the farming systems as way of enhancing grain concentrations and combating micronutrient deficiencies in the population and improving yields of the staple crops. This could include other food crops

commonly consumed by the population so as to expand sources of micronutrients. It would be important also to do on-farm trials involving N and S and micronutrients to examine the interactions.

- Collaboration with other stakeholders as the problems of S, Se and Zn deficiency in the soil and food chain are regional in nature so that they are addressed holistically at regional level.

# CHAPTER 7

# REFERENCES

Allaway, WH 1986, Soil-plant-animal and human interactionships in trace element nutrition. In W.Mertz (ed.) Trace elements in human and animal nutrition. Academic Press, Orlando,FL. 465-488.

Allen, AY 1976, The effects of sulphur on maize yields in Western Kenya. *East African Agricultural Forum* 42, 313-322.

Alloway, BJ 2008, *Zinc in Soil and Plant Nutrition,*, International Zinc Association & International Fertilizer Association, Brussels and Paris,France.

Alloway, BJ 2009, Soil factors associated with zinc deficiency in crops and humans. *Environ Geochem Health* 31, 537-548.

Archer, MJ 1974, A sand culture experiment to compare the effects of sulphur on five wheat cultivars (*Triticum eastivum* L.). *Australian Joournal of Agricultural Research* 25, 369-380.

Arinola, OG, Nwozo, SO, Ajiboye, JA & Oniye, AH 2008, Evaluation of trace elements and total antioxidant status in Nigerian cassava processors. *Pakistani Journal Nutrition* 7(6), 770-772.

Ascher, JS, Graham, RD, Elliott, DE, Scott, JM & Jessop, RS 1994, Agronomic value of seed with high nutrient content. In proceedings of the CIMMYT Symposium on wheat in Tropical Environments, Bangladesh, Aug 1993.

Aulakh, MS, Pasricha, NS & Sahota, NS 1980, Yield, nutrient concentration and quality of mustard crops as influenced by nitrogen and sulphur fertilisers. *Agricultural Science Cambridge* 94, 545-549.

Baeten, JM, Mostad, SB, Hughes, MP, Overbaugh, J, Bankson, DD, Mandaliya, K, Ndinya-Achola, JO, Bwayo, JJ & Kreiss, JK 2001, Selenium deficiency is associated with shedding of HIV-1 infected cells in the female genital tract. *Acquired Immune Deficiency Syndrome* 26(4), 360-364.

Banda, DJ & Singh, BR 1989, Establishment of critical levels of zinc for maize in soills of the high rainfall areas of Zambia. *Norwegian Journal of Agricultural Sciences* 3, 221-227.

Banuelos, GS, Meek, DW & Hoffman, CJ 1990, The influence of selenium, salinity and boron on selenium uptake in wild mustard. *Plant and Soil* 127, 201-206.

Banzinger, M & Long, JK 2000, The potential for increasing the iron and zinc density in maize through plant breeding *Food and Nutrition Bulletin* 21, 397-400.

Barclay, MNI & MacPherson, A 1992, Selenium content of wheat for bread making in Scotland and the relationship between glutathione peroxidase levels in whole blood and bread consumption. *British Journal of Nutrition* 68(1), 261-270.

Baum, MK, Shor-Posner, G & Lais, S 1998, Micronutrient status in relationship to mortality in HIV-1disease. *Nutrition Research* 56 (1), S135-S139.

Beck, MA 2007, Selenium and Vitamin E Status: Impact on Viral Pathogenicity. *Nutrition* 137, 1338-1340.

Birringer, M, Pilawa, S & Flohe, L 2002, Trends in selenium biochemistry. *Nat Prod Rep* 19, 693-718.

Bisbjerg, B 1972, Studies on selenium in plants and soils , Riso Report, Copenhagen Denmark. *Danish Atomic Energy Commission Research Establishment Riso* 200.

Blaylock, MJ & James, BR 1994, Redox transformations and plan tuptake of selenium resulting from root-soil interactions. *Plant and Soil* 158, 1-12.

Bouis, HE & Welch, RM 2010, Biofortification- A Sustainable Agricultural Strategy for Reducing Micronutrient Malnutrition in the Global South. *Crop Science* 50, 20-32.

Branca, F & Ferrari, M 2002, Impact of micrnutrient deficiencies on growth:the stunting syndrome. *Annals of Nutrition and Metabolism* 46, 8-17.

Brennan, RF 1992, The relationship between critical concentration of DTPA extractable zinc from the soil for wheat production and properties of Southwestern Australian soils respponsive to applied zinc. *Communication Soil Science and Plant Analysis* 23(7-8), 747.

Brown, PH, Cakmak, I & Zhang, Q 1993, Forms and function of zinc in plants. In 'Zinc in soils and plants' (Ed. AD Robson). *Kluwer Academic Publishers:Dordrecht, The Netherlands*, 93-106.

Bugel, S, Sandstrom, B, Larsen, EH & Skibsted, LH 2002, Is selenium from animal sources bioavailable? 11th symposium on trace elements in man and animals, June 2-6, 2002, Berkeley, California.

Byerlee, D, Anandajayasekeram, P, Diallo, A, Gelaw, B, Heisey, PW, Lopez-pereira, M, Mwangi, W, Tripp, R & Waddington, S 1994, Maize research in sub-saharan Africa:an overview of the past impacts and future prospects.CIMMYT economics working paper.

Caballero, B 2002, Global patterns of child health:the role of nutrition. *Annals of Nutrition and Metabolism* 46, 3-7.

Cakmak, I 2008, Enrichment of cereal grains with zinc:agronomic or genetic biofortification? *plant soil* 302, 1-17.

Cakmak, I 2009, Enrichment of fertilisers with zinc :An excellent investment for humanity and crop production in India. *Trace Elements in Medicine and Biology* 23, 281-289.

Cakmak, I, Pfeiffer, WH & McClafferty, B 2010, Biofortification of Durum Wheat with Zinc and Iron. *Cereal Chemistry* 87, 10-20.

Campa, A, Shor-Posner, G & Indacochea, F 1999, Mortality risk in selenium deficient HIV positive children. *Acquired Immune Deficiency Syndrome* 20(4), 508-513.

Cary, EE & Allaway, WH 1969, The stability of different forms of selenium applied to low selenium soils. *Soil Science Society of America, Special Proc.* 33, 571.

Chandler, WH 1937, Zinc as a nutrient for plants. *Botanical Gazzette* 98, 625-646.

Chileshe, F, Nkonde, GK & Simukanga, S 2000, Zambian phosphate resources: Local benefits. *Phosphorus and Potassium* 226, 9-18.

Chilimba, ADC, Young, SD, Black, CR, Meacham, MC, Lammel, J & Broadley, MR 2011, Agronomic biofortification of maize with Selenium (Se) in Malawi. *Field Crops Research* 125, 118-128.

Chinene, VRN 1984, Generating field data for validation of crop models in Zambia. In proceeding of the XI international forum on soil taxonomy and agrotechnology transfer in Zambia. July 15-August 1 1985. *Government Report*, 181-186.

Chirwa, M & Yerokun, O 2012, The distribution of zinc fractions in surface samples of selected agricultural soils of Zambia. *International Journal of Soil Science* 7(2), 51-60.

Combs, GF 1998, Selenium in foods. In Chichester CO and Schweigher BS(eds) *Advances in Food Research.* San Diego: Academic Press., 85-113.

Combs, GF 2001, Selenium in global food systems. *British Journal of Nutrition* 85, 517-547.

Damaseke, MI, Sakala, GM & Munyinda, K 1993, Agronomic effectiveness of partially acidulated phosphate rock in Southern Zambia. In: The Zambia Fertiliser Technology Development Committee- ZFTDC. Proceeding of an international workshop: phospate rock-derivatives and their use. *Government Report*, 49-56.

De Reuiter, JM & Martin, RJ 2001, Management of nitrogen and sulphur fertiliser for improved bread wheat (*Triticum aestivum*) quality. *New Zealand Journal of Crop Horticultural Science* 29, 287-299.

Dev, G, Jaggi, RC & Aulakh, MS 1979, Study of nitrate-sulphate interaction on growth and nutrient uptake of maize using $^{35}$S. *Indian Society Soil Science* 27, 302-307.

Dhillon, KS & Dhillon, SK 2003, Distribution and management of seleniferous soils. *Advances in Agronomy* 79, 119-184.

Dow, AI & Roberts, S 1982, Proposal: Critical nutrient ranges for crop diagnosis. *Agronomy* 74, 401-403.

Ercoli, L, Arduini, I, Marliotti, M, Lulli, L & Masoni, A 2012, Management of sulphur fertiliser to improve durum wheat production and minimise S leaching. *European Journal of Agronomy* 38, 74-82.

Erdal, I, Yilmaz, A, Taban, S, Eker, S & Cakmak, I 2002, Phytic acid and phosphorus concentrations in seeds of wheat cultivars grown with and without zinc fertilisation. *Plant Nutr* 25, 113-127.

Erik, F 2007, Food intake of seleniumm and sulphur amino acids in tuberculosis patients and healthy adults in Malawi.MPhil Thesis, Faculty of Medicine, University of Oslo, Norway.

Eurola, M, Efholm, P, Ylinen, M, Koivistoinen, P & Varo, P 1990, Effects of selenium fertilisation on the selenium content of cereal grains, flour and bread produced in Finland. *Cereal Chemistry* 67, 334-337.

Faete, NES, Hollung, K, Ruud, L, Sogn, T, Faergestad, EM, Skarpeid, HJ, Magnus, EM & Uhlen, AK 2005, Combined nitrogen and sulphur fertilisation and its effect on wheat quality and protein composition measured by SE-FPLC and proteomics. *Cereal Science* 41, 357-369.

Famine Early Warning Systems Network 2006, ' Zambia food security update'.

FAO 1973, *Luangwa Valley Conservation and Development Project.report on project results ,conclusion and recommendations.FO:DP/ZAM/68/510 terminal report.*, Rome,Italy.

FAO. 2004, The state of food insecurity in the world. Annual Report by Economic and Social Department, Rome: FAO

FAO 2008, Food and agriculture organisation of the united nations statistical database. www.fao.org

FAO 2009, Food and agriculture organisation aof the united nations statistical database. www.fao.org.

Fazli, IS, Ahmad, A, Masoodi, M, Khan, JS & Abdin, MZ 2008, Interactive effect of sulphur and nitrogen on nitrogen accumulation and harvest in oilseed crops differing in nitrogen assimilation potential. *Plant Nutrition* 31, 1203-1220.

Geering, HR, Cary, EE & Allaway, WH 1968, Solubility and redox criteria for the possible forms of selenium in soils. *Soil Science Society of America, Special Proc.* 32, 35.

Genc, Y, McDonald, GK & Graham, RD 2006, Contribution of different mechanisms to Zinc efficiency in bread wheat during early vegetative stage. *Plant and Soil* 281, 353-367.

Gissel-Nielsen, G 1979, Uptake and translocation of selenium-75 in Zea mays. In *Isotopes and radiation in researh in soil plant relationships*, Vienna. *IAEA*, 427-436.

Gitau, R, Makasa, M, Kasonka, L, Sinkala, M & Chintu, C 2005, Maternal micronutrient status and decreased growth of zambian infants born after the maize prices increases resulting from southern african drought of 2001-2002. *Pub Health Nutr* 8, 837-843.

Golden, MHN 1991, The nature of nutritional deficiency in relation to growth failure and poverty. *Acta paediatrica scandinavica* 374, 95-110.

Graham, HL, Geoffrey, JJ, Ortiz-Monasterio, I, Yusuf, G, Stangoulis, JCR & Graham, RD 2005, Selenium in Australia:Selenium status and biofortification of wheat for better. *Trace Elements in Medicine and Biology* 19, 75-82.

Graham, RD, Welch, RM & Bouis, HE 2001, Addressing micronutrient malnutrition through enhancing the nutritional quality of staple foods:principles,perspectives and knowledge gaps. *Advances in Agronomy* 70, 77-527.

Grant, PM 1981, The fertilisation of sandy soils in peasant agriculture. *Zimbabwe Agricultural* 78, 169-175.

Grant, PM & Rowell, AWG 1976, Studies on sulphate fertilisers for Rhodesian crops.1. Effect of sulphur in fertiliser compounds on the yield and sulphur status of maize. *Rhodesian Journal of Agricultural Research* 16(1), 43-59.

Grantham-McGregor, SM & Ani, CC 1999, The role of micronutients in psychomotor and cognitive development. *British Medical Bulletin* 55, 511-527.

Greenwood, NN & Earshaw, A 1984, Chemistry of elements , 882-899. Oxford :Pergamon Press.

Haug, A, Graham, RD, Christopherson, OA & Lyons, GH 2008, How to use the world's scarce selenium resources effectivectly to increase the selenium concentration in food. *Microbial Ecology in Health and Disease*, 209-228.

Hays, VW & Swenson 1985, Minerals and bones.In:dukes'physiology of domestic animals,tenth edition. 449-466.

Hopper, JL & Parker, DR 1999, Plant availability of selenite and selenate as influenced by the competing ions phosphate and sulphate. *Plant and Soil* 210, 199-207.

Hossain, MD, Hanafi, MM, Saleh, G, Foroughi, M, Behmaram, R & Noori, Z 2012, Growth, photosynthesis and biomass allocation of different kenaf (*Hibiscus cannabinus* L). *Australian Journal of Crop Science* 6(3), 480-487.

Huang, C, Barker, SJ, Langridge, P, Smith, FW & Graham, RD 2000, Zinc defficiency up regulates expression of high affinity phosphate transporter genes in both phosphate sufficient and deficient barley roots. *Plant Physiology* 124, 415-422.

IZiNCG 2004, Assessment of the risk of zinc deficiency in populations and options for its control. *Food and Nutrition Bulletin*, S113.

Jamal, A, Yong-Sun, M & Malik, ZA 2010, Sulphur- a general overview and interactions with nitrogen. *Australian Journal of Crop Science* 4(7), 523-529.

Janzen, HH & Bettany, JR 1984, Suplphur nutrition of rapeseed. Influence of fertiliser nitrogen and sulphur rates. *Soil Science Society of America* 48.

Kaiser, BN, Grindley, KL, Tyerman, SD & Ngaire Bradey, J 2005, Molybdenum nutrition in plants. *Ann. Bot.*

Kalayci, M, Torun, B, Aydin, M, Oztuk, L & Cakmak, I 1999, Grain yield, zinc efficiency and zinc concentration of wheat genotypes grown in a zinc-deficient calcareoous soil in feild and greenhouse. *Field Crops Research* 63, 87-98.

Kalima, C & Veldkamp, WJ 1985, Land evaluation methodology in Zambia; in XI International Forum on Soil Taxonomy and Agrotechnology Transfer. *Government Report*, 148-157.

Kang, BT & Osiname, OA 1976, Sulphur response of maize in Western Nigeria. *Agronomy* 68, 333-336.

Kanwar, JS & Youngdahl, LJ 1985, Micronutrient needs of tropical food crops. *Fertiliser Research* 7, 43-63.

Kirk, GJD & Bajita, JB 1995, Root induced iron oxidation, pH changes and zinc solubilization in rhizoshere of lowland rice. *New Physiologist* 131, 129-137.

Koivistoinen, P & Huttunen, JK 1986, Selenium in food and nutrition in Finland. An overview of research and action. *Annual Clinical Research* 18, 13-17.

Kumwenda, JDT, Waddington, SR, Snapp, SS, Jones, RB & Blackie, MJ 1996, Soil fertility management research for the maize cropping systems of smallholders in southern africa:a review. *Nrg paper* 96-02.

Kutman, UB, Yildiz, B & Cakmak, I 2011, Improved nitrogen status enhances zinc and iron concentrations both in the whole and the endosperm fraction of wheat *Cereal Science* 53, 118-125.

Lakkineni, KC & Abrol, YP 1994, Sulphur requirements of crop plants:Physiological analysis. *Fertiliser News* 39, 11-18.

Lauchli, A 1993, Selenium in plants: uptake, functions and environmental toxicity. *Bot. Acta* 106, 455-468.

Lerner, SE, Molfese, ER, Ponzio, NR, Cogliatti, M & Rogers, WJ 2006, N and S fertiliser effects on grain composition, industrial quality and end-use in durum wheat. *Cereal Science* 44, 2-11.

Li, FL, Bao, WK & Wu, N 2009, Effects of water stress on growth, dry matter allocation and water use effeciency of a leguminous species, *sophora davidii*. *Agroforestry Systems* 77, 193-201.

Long, JK, Banzinger, M & Smith, ME 2004, Diallel analysis of grain iron and zinc density in Southern African-adapted maize inbreds. *Crop Science* 44, 2019-2026.

Lungu, OIM & Chinene, VRN 1993, *Cropping and soil management systems and their effects on on soil productivity in Zambia. A review*, The University of Norway, Aas.

Lungu, OIM & Dynoodt, RFP 2008, Acidification from long term use of urea and its effect on selected soil properties. *African Journal of Food Agriculture Nutrition and Development* 8 (1), 63-76.

Lyons, GH, Judson, GJ, Ortiz-Monasterio, I, Genc, Y, Stangoulis, JCR & Graham, RD 2005, Selenium in Australia: selenium status and biofortification of wheat for better health. *Trace Elements in Medicine and Biology* 19, 75-82.

Lyons, GH, Lewis, J, Lorimer, MF, Holloway, RE, Brace, DM, Stangoulis, JCR & Graham, RD 2004, High-selenium wheat: agronomic biofortification strategies to improve human nutrition. *Food, Agriculture and Environment* 2(1), 171-178.

Mafongoya, PL, Bationo, A, Kihara, J & Waswa, BS 2006, Appropriate technologies to replenish soil fertility in southern Africa. *Nutr Cycl Agroecosyst* 76, 137-151.

Malhotra, VK 1998, Biochemistry for students.Tenth Edition Jaypee brothers medical publishers(P)ltd,New Delhi,India.

Malik, MFA, Ashraf, M, Qureshi, AS & Ghafoor, A 2007, Assessment of genetic variability, correlation and path analysis for yield and its components in soyabean. *Pakistani Journal of Botany* 39(2), 405-413.

Mapelli, V, Hillestrom, PR, Patil, K, Larsen, EH & Olsson, L 2011, The interplay between sulphur and selenium metabolism influences the intracellular redox balance in sacchomyces cerevisiae. *FEMS Yeast Res* 12, 20-32.

Marschner, H 1995, Mineral nutrition of higher plants,2nd edn.London Academic Press.

Matula, J 2004, The effect of chloride and sulphate application to soil on the changes in nutrient content in barley shoot biomass at an early phase of growth. *Plant Soil Environment* 50, 295-302.

McDonald, GK, Genc, Y & Graham, RD 2008, A simple method to evaluate genetic variation in grain zinc concentration by correcting for differences in grain yeild. P*lant Soil* 306, 49-55.

McDonald, GK & Mousavvi-Nik, M 2009, Increasing the supply of sulphur increases the grain zinc concentration in bread and durum wheat. *eScholarship,University of California*.

McGrath, SP & Zhao, FJ 1996, Sulphur uptake, yield responses and interactions between nitrogen and sulphur in winteroilseed rape (*Brassica napus*). *Journal of Agricultural Science, Cambridge* 126, 53-62.

McKenzie, RC, Rafferty, TS & Beckett, GJ 1998, Selenium: An essential element for immune function. *Immunology Today* 19, 342-345.

McPhilips, JK 1986, Sulphur in maize nutrition in Zambia, soils and crops advisory officer's report to Mount Makulu Research Statiion. Department of Agriculture, Zambia. *Government Report*.

Melse-Boonstra, A, Hogenkamp, P & Lungu, OIM 2007, Mittigating HIV/AIDS in Sub-Saharan Africa through selenium in food. *Farmer Publications, Golden Valley Agricultural Trust (GART)*, 43-65.

Meltzer, HM, Norheim, G & Holm, H 1992, Supplementation with wheat selenium induces a dose response-dependent response in serum and urine of a selenium-replete population. *British Journal of Nutrition* 67.

Mikkelsen, RL, Page, AL & Bingham, FT 1989, Factors affecting selenium accumulation by agricultural crops. In:*Selenium in agriculture and the environment. Soil Science Society of America, Special Publication* 23, 65-94.

Mikkelsen, RL & Wan, HF 1990, The effects of sulphur uptake in barley and rice. *plant soil* 121, 151-153.

Mortensen, J & Eriksen, J 1994, Effects of sulphur deficiency on amino acid composition. *Norwegian Journal of Agricultural Sciences* 15, 135-142.

Murray, RK, Granner, DK, Mayes, PA & Rodwell, VW 2000, Harper's biochemistry.

Nestel, P, Bouis, HE, Meenakshi, JV & Pfieffer, W 2006, Biofortification of staple food crops. *Nutrition Research* 136, 1064-1067.

Oldfield, JE 1981, The selenium story:a second chapter. In Spallholz J, Martin J, Ganther H (eds). *Selenium in Biology and Medicine*, Westport: AVI. 1-9.

Orman, S & Ok, H 2012, Effects of sulphur and zinc applications on growth and nutrition of bread wheat in calcareous clay loam soil. *African Journal of Biotechnology* 11(13), 3080-3086.

Ortiz-Monasterio, JI, Palacios-Rojas, N, Meng, E, Pixley, K, Trethowan, R & Pena, RJ 2007, Enhancing the mineral and vitamin content of wheat and maize through plant breeding. *Cereal Science* 46, 293-307.

Pangani & Echeveria 2011, Performance of sulfur diagnostic methods for corn. *Agronomy* 103, 413-421.

Pearson, DP, Hansen, TH, Laursen, KH, Schjoerring, JK & Husted, S 2009, Simultaneous iron, zinc, sulphur and phosphorus speciation analysis of barley grain tissues using SEC-ICP-MS and IP-ICP-MS. *Metallomics* 1, 418-426.

Pollack, M & Leeuwenburgh, C 1999, Molecular mechanisms of oxidative stress in aging:free radicals, antioxidant and disease. In Handbook of oxidants and antioxidants in exercise (Sen CK,Parker,L,Hinninen,O. *Elsevier Science,BV*, 881-923.

Pratley, JE & McFarlene, JD 1974, The effect of sulphate on the selenium content of pasture plants. *Australian Journal of Experimental Animal Husbandry* 14, 533-538.

Rabufetti, A & Kamprath, EJ 1977, Yield, N and S content of corn as affected by N and S fertilisation on coastal plain soils. *Agronomy* 69, 785-788.

Ramakrishnan, U, Manjrekar, R, Rivera, J, Gonzales-Cossio, T & Martorell, R 1999, Micronutrients and pregnancy outcome:a review of the literature. *Nutrition Research* 19, 103-159.

Randall, PJ, Spencer, K & Freney, JR 1981, Sulphur and nitrogen fertiliser on wheat I. Concentration sulphur and nitrogen to sulphur ratio in grain, in relation to yield response. *Australian Journal of Agricultural Research* 32, 203-212.

Reddy, KR & Matcha, SK 2010, Quantifying nitrogen effects on castor bean (*Ricinus communis* L) development, growth and photosynthesis. *Indian Crops Production* 13, 185-191.

Reneau, RBJ, Bran, DE & Donohue, SJ 1986, Effect of sulphur on winter wheat grown in the coastal plain of Virginia. *Communication Soil Science Analysis* 17, 149-158.

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

Rengel, Z & Graham, RD 1995, Importance of seed Zn content for wheat on Zn-deficient soil *I. Vegetative Growth*. *Plant and Soil* 173, 259-266.

Reuter, DJ & Robinson, JB (Eds) 1997, Plant analysis an interpretation manual second edition, CSIRO Publishing Melbourne 118.

Riley, MM, Gartrell, RF, Brennan, RF, Hamblin, J & Coates, P 1992, Zinc deficiency in wheat and lupins in Western australia is affected by the source of phoshate fertiliser. *Australian Journal of Exprimental Agricultural* 32, 455-463.

Rosegrant, MW, Cai, X, Cline, SA 1996, World water and food to 2025:Dealing with sacarcity. International Food Policy Research Institute, Washington DC.

Saasa, OS 2003, Agricultural intensification in Zambia :the role of policies and policy processes(Macro study). *Government Report*, 6-14.

Salvagiotti, F & Miralles, DM 2007, Wheat development as affected by nitrogen and sulphur nutrition. *Australian Journal of Agricultural Research* 58, 39-45.

Sanchezi, PA 2002, Soil fertility and hunger in Africa. *Science* 295, 2019-2020.

Sanchezi, PA & Swaminathan 2005, Hunger in Africa:the link between unhealth people and healthy soils. *Lancet* 365, 442-444.

Saurez, DL, Grieve, CM & Poss, JA 2003, Irrigation method affects selenium accumulation in forage *Brassica* species. *Plant Nutrition* 26, 191-201.

Scherer, HW 2001, Sulphur in crop production-invited paper. *European Journal of Agronomy* 14, 81-111.

Schnug, E 1998, Diagnosis of Sulphur nutrition. In Sulphur in agroecological systems, part of the series 'nutrients in the ecosystems'.

. *Kluwer Academic Publishers:Dordrecht* 2, 1-38.

Schwarz, K & Foltz, CM 1957, Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. *American Chemistry Society* 79, 3292-3293.

Serlemitsos, J & Fusco, J 2001, Vitamin a fortification of sugar in Zambia 1998-2001.

Silesi, G, Akinnifesi, FK, Debusho, LK, Beedy, T, Ajayi, OC & Mong'ombo, S 2010, Variarion in Maize yield gaps with plant nutrient inputs,soil type and climate across sub-saharan Africa. *Field Crops Research* 116, 1-13.

Sinyinda, L & Mwala, M 2010, Characterisation of micronutrient (zinc and iron) dense tropical maize hybrids grown in two different environments in Zambia.Second RUFORUM Biennial Meeting 20-24 September 2010,Entebbe,Uganda. 593-596.

Soeten, KO, Olaiya, CO & Oyewole, OE 2010, The importance of mineral elements for humans and domestic animals and plants:a review. *African Journal of Food Science* 4(5), 200-222.

St.Clair, BS & Lynch, JP 2010, The opening of pandora's box:climate change impacts on sol fertility and crop nutrition in developing countries. *Plant and Soil* 335, 101-115.

Stein, AJ 2010, Global impacts of human mineral malnutrition. *plant soil* 335, 133-154.

Stewart, BA & Whitefield, CJ 1965, Effect of crop residues, soil temp and sulfur on growth of winter wheat. *Soil Science Society of America, Special Proc.* 29, 752-755.

Swaine, DJ 1962, Technical communication: The trace element content of fertilisers. *Commonwealth Agricultural Buraux* 52.

Szabo, G, Chavan, S, Mandrekar, P & Catalato, D 1999, Acute alcoholic consumption attenuates IL-8 and MCP-1 induction in response to ex vivo stimmulation *Clin.Immunol.* 19, 67-76.

Terry, N, Zayed, AM, de Souza, MP & Tarun, AS 2000, Selenim in higher plants. *Ann Rev plant Mol Biol* 51, 401-432.

Thorne, W 1957, Zinc deficiency and its control. *Advances in Agronomy* 9, 31-65.

Todd, CA 2009, Rhizoctonia diesease on potatoes: The effects of anastomosis groups, fungicides and zinc on disease. *PHD Thesis*, 40.

Tsuji, T, Mambo, A, Phiri, LK, Msoni, R, Sokotela, SB & Yerokun, O 2005, Studies on nutrient distribution in some Zambia soils with refernce to sulphur using GIS(Geographical Information Systems) II. evaluation of pplant available sulphur and its distribution in major Zambian soils *Soil Science Plant Nutrition* 51(7), 943-952.

Uauy, C, Distelfeld, A, Fatima, T, Blechl, A & Dubcovski, J 2006, A NAC gene regulating senescence improves grain protein, Zinc and iron content in wheat. *Science* 314, 1298-1301.

Ulrich, A & Hills, FJ 1967, Principles and practices of plant analysis. In 'Soil Tests and Plant Analysis'. Part II. *Soil Science Society of America, Special Prob.* No.2, 11-12.

Van Campen, DR & Glahn, RP 1999, Micronutrient bioavailability techniquees: accuracy, problems and limitations. *Field Crops Research* 60, 93-113.

Wan, HF, Mikkelsen, RL & Page, AL 1988, Selenium uptake by some agricultural crops from central California soils. *Environmental Quality* 17, 269-272.

Weil, RR & Mughogho, SK 2000, Sulphur nutrition of maize in four regions of Malawi. *Agronomy* 92, 649-656.

Welch, RM 1999, Importance of seed mineral nutrient reserves in crop growth and development. *Experimental Botany* 55, 353-364.

Welch, RM & Graham, RD 2004, Breeding for micronutrients in staple food crops from a human nutrition perspective. *Experimental Botany* 55, 353-364.

Wendt, JM, Jones, RB & Itimu, OA 1994, *An integrated approach to soil fertility improvement in malawi,including agroforestry.In E.T Craswell and J.Simpson(eds.),Soil Fertility and Climatic Constraints in dry land Agriculture.ACIAR proceedings No.54,Canberra,Austraalian Council for International Agricultural Research(ACIAR)*, pp. 74-79.

Wheal, SM, Fowles, T & Palmer, L 2011, A cost-effective acid digestion method using closed polypropylene tubes for inductively coupled plasms optical emmission spetrometry (ICPOES) and analysis of plant essential elements. *Royal Society of Chemistry* 3, 2854.

White, CL, Robson, AD, Fischer, HM 1987, Variation in nitrogen, sulphur, selenium, cobalt, manganese, copper and zinc contents of grain from wheat and two lupin species grown in a range of mediterranean environments. Australian Journal of Agricultural research. 32, 47-59

White, P, J. & Broadley, MR 2009, Biofortification of crops with seven mineral elements often lacking in human diets – iron, zinc, copper, calcium, magnesium, selenium and iodine. *New Phytologist* 182, 49-84.

White, PJ, Bowen, HC, Parmaguru, P, Frirtz, M, Spracklen, WP, Spiby, RE, Meacham, MR, Mead, A, Harriman, M, Trueman, LM, Smith, BM, Thomas, B & Broadley, MR 2004, Interactions between selennium and sulphur nutrition in *Arabidopsis thaliana*.Sulphur metabolism in plants special issue. *Experimental Botany* 55, 1927-1937.

White, PJ & Broadley, MR 2005, Biofortifying crops with essential mineral elements. *Trends in Plant Science* 10, 1360-1385.

White, PJ & Johnson, LA 2003, Corn Chemistry and Technology. *American Assocition of Cereal Chemists*, 571-547.

WHO 2002, The world health report 2002.reducing risks, promoting healthy life,Geneva,Switzerland,World health organisation 1-168.

Wilhelm, NS, Bramford, TA, Riggs, JL, Allen, JL & Auhl, L 1993, Critical levels for zinc deficiency of field grown wheat. In 'Proceedings of the 7th Australian agronomy conference'. Adelaide. (Eds GK McDonald, WD Bellotti). *Australian Society of Agronomy: Parkville, Vic.*, 119-121.

Wissuwa, M, Ismail, AM & Graham, RD 2008, Rice grain concentrations as affected by genotype, native soil zinc availability and zinc fertilisation. *Plant and Soil* 306, 37-48.

Yilmaz, A, Ekiz, H, Gultekin, I, Torun, B, Barut, H, Karanlik, S & Cakmak, I 1998, Effect of seed zinc content on grain yield and zinc concentration of wheat grown in zinc defficient calcareous soils. *Plant Nutr* 21, 2257-2264.

Ylaranta, T 1990, Effects of liming and addition of sulphate and phosphate on the selenium content of Italian rye grass. *Ann. Agric. Fenn* 29, 141-149.

Zeng, L, Cheng, Z, Jiang, X, Bei, X, Zheng, Y, Glahn, RP, Welch, RM, Miller, DD, Lei, XG & shou, H 2010, Nicotianamine, a Novel Enhancer of Rice Iron Bioavalaibility to Humans. *PloS ONE* 5(4).

Zhao, FJ, Salmon, SE, Witters, PJA, Evans, EJ, Monagham, JM, Shewry, PR & McGrath, SP 1999, Responses of bread making quality to sulphur in three wheat varieties. *Science Food Agriculture* 79, 1865-1874.

Zhao, FJ, Tausz, M & Dekok, LJ 2008, Role of sulphur for plant production in agricultural and natural ecosystems. In IRHea (eds) (ed.), *Sulphur Metabolism in Phototropic Organisms*, pp. 417-435.

# APPENDICES

Appendix Table 3.1: Sample of the questionnaire used in the survey of Zambian maize and wheat

THE UNIVERSITY OF ADELAIDE

SCHOOL OF AGRICULTURE, FOOD AND WINE

CROP SURVEY QUESTIONNAIRE

1. Basic information

Location of farm……………….

Total farm area

Crop…………………..

Cultivar…………….

Season……………………..

2. Management practice

Previous crop

Date planted……………..............

Fertiliser type………………………………

Fertiliser rate.........................................

When applied…………………………………

How applied (drilled, broadcast,)....................

Other (specify)...........................................

Sowing rate...............................................

Sowing method............................................

Has lime been applied?...............................

If yes Rate and when last applied.....................

Use of crop residues:

Use of legume trees/shrubs (e.g. Gliricidia, pigeon pea)?...............................

Use of intercropping (e.g. maize + beans, cowpea)?.........................

3. Soil Type……………………………………

Texture………………………………………

pH……………………………………………..

Rating of soil structure: 1. very good; 2. Good;   3. Poor;   4. Very bad

4. Climatic data

Rainfall received over past season/year……………………………

Temperature………………………………..

5. Yield data

Ha planted………………………………….

No. of bags harvested (50kg)……………………..

6. Farmer perceptions

How does the farmer rate his maize yields

Above average;   2. Average;   3. Below average

Over the past 5 years have the yields of the farm's maize crops

Improved;      2. Stayed the same;    3. Declined

What does the farmer consider the main limiting factors to crop yields? Rank these from 1 (most important) to 7 (least important)

Poor seasons(rainfall flood or drought)

Poor soil

Poor varieties

Weeds

Disease

Insect pests

Availability of fertiliser

Information contained in this questionnaire is for academic purposes only. Thank you for your time.

Appendix Table 3.2a. The mineral concentration of Iron (Fe) Manganese (Mn), copper (Cu), Calcium (Ca) Magnesium (Mg), Potassium (K) and Phosphorus (P) in maize samples from the surveyed areas in the agro ecological zones of Zambia.

| Zone | Crop | Fe | Mn | Cu | Ca | Mg | K | P |
|---|---|---|---|---|---|---|---|---|
| | | mg/kg | mg/kg | mg/kg | mg/kg | mg/kg | mg/kg | mg/kg |
| 2 | Maize | 20 | 8.5 | 2.1 | 46 | 1290 | 3800 | 3500 |
| 2 | Maize | 15 | 4.2 | 1.5 | 32 | 860 | 3100 | 2000 |
| 2 | Maize | 14 | 3.4 | 1.2 | 30 | 970 | 3300 | 2400 |
| 3 | Maize | 24 | 4.6 | 1.2 | 24 | 970 | 3100 | 2600 |
| 2 | Maize | 13 | 3.8 | 1.1 | 32 | 940 | 3200 | 2500 |
| 2 | Maize | 13 | 7.1 | 1.7 | 47 | 920 | 3600 | 2500 |
| 2 | Maize | 18 | 5.8 | 1.5 | 43 | 1070 | 3700 | 3200 |
| 2 | Maize | 16 | 4.9 | 2.0 | 48 | 900 | 2800 | 2500 |
| 3 | Maize | 15 | 5.2 | 1.1 | 22 | 860 | 2800 | 2200 |
| 3 | Maize | 16 | 3.0 | 1.2 | 20 | 900 | 3200 | 2600 |
| 3 | Maize | 15 | 5.5 | 1.5 | 22 | 980 | 3200 | 2600 |
| 3 | Maize | 15 | 4.5 | 1.2 | 18 | 920 | 3500 | 2500 |
| 2 | Maize | 16 | 5.8 | 1.6 | 33 | 1260 | 3900 | 3700 |
| 2 | Maize | 16 | 6.8 | 1.3 | 58 | 1390 | 4100 | 3800 |
| 2 | Maize | 15 | 7.5 | 1.0 | 43 | 1200 | 3600 | 3400 |
| 1 | Maize | 20 | 4.2 | 2.2 | 26 | 1200 | 4300 | 3400 |
| 1 | Maize | 15 | 5.0 | 2.0 | 43 | 840 | 3200 | 2100 |
| 1 | Maize | 18 | 5.0 | 1.4 | 51 | 930 | 3100 | 2400 |
| 1 | Maize | 20 | 5.3 | 2.2 | 33 | 930 | 3100 | 2100 |
| 1 | Maize | 15 | 6.3 | 1.8 | 45 | 890 | 2400 | 1920 |
| 2 | Maize | 16 | 5.2 | 1.4 | 40 | 1080 | 3000 | 2900 |
| 2 | Maize | 14 | 4.8 | 2.0 | 42 | 910 | 2700 | 2100 |
| 2 | Maize | 15 | 5.6 | 1.5 | 31 | 1090 | 3100 | 2800 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 2 | Maize | 13 | 3.6 | 1.6 | 30 | 890 | 3500 | 2200 |
| 2 | Maize | 16 | 3.4 | 2.2 | 29 | 930 | 3600 | 2800 |
| 2 | Maize | 17 | 5.7 | 2.2 | 51 | 1010 | 3600 | 2300 |
| 2 | Maize | 17 | 4.2 | 2.6 | 34 | 870 | 3100 | 2000 |
| 2 | Maize | 16 | 8.0 | 2.5 | 37 | 1140 | 3200 | 3200 |
| 2 | Maize | 15 | 4.0 | 1.2 | 30 | 850 | 2900 | 2300 |
| 2 | Maize | 19 | 4.1 | 1.6 | 26 | 1000 | 3200 | 2500 |
| 2 | Maize | 15 | 6.3 | 1.2 | 30 | 1050 | 3200 | 2900 |
| 2 | Maize | 14 | 5.0 | 1.6 | 43 | 1060 | 3100 | 2700 |
| 2 | Maize | 16 | 3.6 | 2.3 | 29 | 960 | 3000 | 2600 |
| 2 | Maize | 18 | 4.1 | 1.9 | 43 | 920 | 3100 | 2400 |
| 2 | Maize | 16 | 4.6 | 2.0 | 47 | 1230 | 3800 | 2700 |
| 2 | Maize | 13 | 2.7 | 0.9 | 37 | 1010 | 3800 | 2500 |
| 2 | Maize | 23 | 5.8 | 1.6 | 28 | 1130 | 3500 | 3100 |
| 2 | Maize | 20 | 5.7 | 1.7 | 52 | 1020 | 3100 | 2600 |
| 2 | Maize | 17 | 5.1 | 1.8 | 33 | 970 | 3300 | 2800 |
| 2 | Maize | 23 | 5.1 | 2.0 | 49 | 910 | 2800 | 1870 |
| 2 | Maize | 19 | 5.6 | 1.6 | 46 | 1030 | 3100 | 2600 |
| 2 | Maize | 20 | 5.2 | 2.0 | 45 | 920 | 3100 | 2300 |
| 2 | Maize | 16 | 5.1 | 1.4 | 34 | 1010 | 3600 | 3100 |
| 2 | Maize | 19 | 4.7 | 1.6 | 72 | 1140 | 3300 | 2900 |
| 2 | Maize | 18 | 5.3 | 1.4 | 76 | 1170 | 3600 | 3300 |
| 2 | Maize | 16 | 3.9 | 1.7 | 41 | 890 | 3400 | 2500 |
| 2 | Maize | 17 | 5.3 | 1.7 | 56 | 940 | 3300 | 2300 |
| 2 | Maize | 14 | 5.0 | 1.7 | 38 | 950 | 3000 | 2400 |
| 2 | Maize | 20 | 7.9 | 2.0 | 50 | 950 | 2700 | 2400 |
| 2 | Maize | 18 | 6.2 | 1.7 | 42 | 1010 | 3100 | 2900 |
| 2 | Maize | 20 | 6.0 | 1.8 | 62 | 1060 | 3500 | 2900 |
| 2 | Maize | 19 | 6.7 | 1.7 | 40 | 1030 | 3800 | 2800 |
| 2 | Maize | 18 | 5.2 | 1.8 | 44 | 1080 | 3500 | 3000 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 2 | Maize | 11 | 4.2 | 1.7 | 55 | 1050 | 3200 | 2500 |
| 2 | Maize | 17 | 5.7 | 2.3 | 58 | 950 | 3200 | 2200 |
| 1 | Maize | 21 | 7.3 | 2.1 | 49 | 1260 | 3700 | 3200 |
| 1 | Maize | 16 | 4.7 | 1.8 | 43 | 870 | 2800 | 2100 |
| 1 | Maize | 20 | 6.4 | 1.9 | 45 | 1150 | 3500 | 3000 |
| 1 | Maize | 18 | 5.7 | 1.8 | 46 | 1190 | 3600 | 3300 |
| 2 | Maize | 20 | 5.4 | 1.6 | 43 | 920 | 3200 | 2400 |
| 3 | Maize | 20 | 5.5 | 1.5 | 50 | 910 | 3100 | 2300 |
| 3 | Maize | 17 | 5.8 | 1.7 | 63 | 1020 | 3200 | 2500 |
| 2 | Maize | 18 | 4.5 | 1.9 | 44 | 910 | 3500 | 2600 |
| 2 | Maize | 21 | 5.2 | 1.9 | 38 | 870 | 2800 | 1810 |
| 1 | Maize | 18 | 3.5 | 1.7 | 71 | 980 | 3200 | 2500 |
| 1 | Maize | 15 | 4.8 | 1.9 | 38 | 850 | 3100 | 2300 |
| 2 | Maize | 18 | 5.2 | 2.1 | 75 | 1010 | 2900 | 2400 |
| Critical conc. | | - | 5.0 | - | - | - | 3600 | 2900 |

Appendix Table 3.2b. The mineral concentration of Iron (Fe) Manganese (Mn), copper (Cu), Calcium (Ca) Magnesium (Mg), Potassium (K) and Phosphorus (P) in the wheat samples from the surveyed areas in the agro ecological zones of Zambia.

| zone | | Fe | Mn | Cu | Ca | Mg | K | P |
|---|---|---|---|---|---|---|---|---|
| | Crop | Mg/kg | Mg/kg | Mg/kg | Mg/kg | Mg/kg | Mg/kg | Mg/kg |
| 2 | Wheat | 29 | 28 | 3.2 | 390 | 1480 | 4400 | 3900 |
| 2 | Wheat | 28 | 36 | 3.0 | 390 | 1440 | 4400 | 3800 |
| 2 | Wheat | 51 | 53 | 3.5 | 440 | 1330 | 4300 | 3400 |
| 2 | Wheat | 35 | 61 | 2.8 | 500 | 1260 | 4100 | 3400 |
| 2 | Wheat | 26 | 30.000 | 3.8 | 520 | 1400 | 4400 | 4000 |
| 2 | Wheat | 30 | 34.000 | 3.5 | 490 | 1340 | 3700 | 3000 |
| Critical conc. | | - | 19.4 | 1.2 | - | - | 4700 | 3700 |

Appendix Table 4.1. Summary of analysis of variance photosynthesis rate, transpiration, stomatal conductance and transpiration efficiency.

| Source of variation | d.f | Mean squares | | | |
|---|---|---|---|---|---|
| | | Photosynthesis rate | Transpiration rate | Cond (X $10^4$) | TE |
| Fertiliser | 2 | 54.32*** | 0.744** | 14.31* | 0.945* |
| Fertiliser.N | 1 | 150.98*** | 1.541** | 32.83** | 2.000 |
| Fertiliser.S | 1 | 8.70 | 0.068 | 2.57 | 0.001 |
| Fertiliser.Zn+Se | 1 | 0.10 | 0.649* | 12.72** | 2.375 |
| Fertiliser.N.S | 1 | 13.91 | 0.290 | 6.41 | 0.812 |
| Fertiliser.N.Zn+Se | 1 | 3.35 | 0.276 | 8.52† | 2.680 |
| Fertiliser.S.Zn+Se | 1 | 7.90 | 0.250 | 5.92 | 0.278 |
| Fertiliser.N.S.Zn+Se | 1 | 15.93 | 0.434 | 9.65 | 0.026 |
| Residual | 43 | 4.27 | 0.139 | 2.993 | 0.893 |
| CV (%) | | 16.3 | 19.4 | 20.6 | 14.4 |

Appendix Table 4.2 Summary of the analysis of variance for water use, water use efficiency, plant height and shoot dry mater.

| Source of variation | d.f | Mean squares | | | |
|---|---|---|---|---|---|
| | | Total water use (X10$^{-2}$) | WUE | Plant height | Shoot dry matter |
| Fertiliser | 2 | 75.88** | 3.269 | 0.159 | 9.169*** |
| Fertiliser.N | 1 | 4.72 | 4.060 | 0.23 | 2.12 |
| Fertiliser.S | 1 | 8.70 | 0.016 | 0.003 | 0.469 |
| Fertiliser.Zn+Se | 1 | 3.56 | 0.420 | 4.815 | 0.025 |
| Fertiliser.N.S | 1 | 14.74 | 0.282 | 3.190 | 0.047 |
| Fertiliser.N.Zn+Se | 1 | 4.95 | 5.122† | 4.378 | 3.111* |
| Fertiliser.S.Zn+Se | 1 | 22.68 | 2.769 | 9.690* | 0.144 |
| Fertiliser.N.S.Zn+Se | 1 | 38.41 | 8.239* | 0.211 | 1.771 |
| Residual | 20 | 12.85 | 1.605 | 1.965 | 0.567 |
| CV (%) | | 11.7 | 18.5 | 7.3 | 11.6 |

Appendix Table 4.3. Average concentrations of nutrients in whole shoots of maize grown for 28 days and the corresponding critical values and the coefficient of variation for the mean. Values for concentrations are shown as the mean±SEM.

| Nutrient | Mean | Critical value | Coefficient of variation (%) |
|---|---|---|---|
| Calcium (mg/kg) | 3625±371 | 2500 | 10.2 |
| Magnesium (mg/kg) | 3285±461 | 1500 | 14.1 |
| Phosphorus (mg/kg) | 3525±401 | 2900 | 11.4 |
| Potassium (%) | 3.1±0.82 | 2.18 | 26.4 |
| Copper (mg/kg) | 3.1±0.43 | 5-20 | 14.1 |
| Manganese (mg/kg) | 21.3±1.48 | 8-9 | 6.9 |

Appendix Table 4.4 Summary of the analyses of variance of the concentrations of N, S, N:S ratio, Zn and Se concentration in whole shoot of maize.

| Source of variation | d.f | Mean square | | | | |
|---|---|---|---|---|---|---|
| | | Nitrogen ($X10^2$) | Sulphur ($X10^{-5}$) | N:S ratio | Zinc | Selenium |
| Fertiliser | 2 | 11.87 | 13.36*** | 52.22*** | 29.32 | 554.7*** |
| Fertiliser.N | 1 | 1.54 | 0.02 | 0.23 | 5.33 | 47.82* |
| Fertiliser.S | 1 | 19.55 | 2.33 | 0.13 | 39.57 | 113.8** |
| Fertiliser.Zn+Se | 1 | 3.65 | 47.88*** | 81.05*** | 11.44 | 4280.50*** |
| Fertiliser.N.S | 1 | 3.07 | 1.52 | 1.63 | 0.11 | 132.10** |
| Fertiliser.N.Zn+Se | 1 | 46.74* | 0.56 | 5.70 | 12.20 | 76.53* |
| Fertiliser.S.Zn+Se | 1 | 2.00 | 0.04 | 0.29 | 0.01 | 77.57* |
| Fertiliser.N.S.Zn+Se | 1 | 13.07 | 0.07 | 3.86 | 8.39 | 91.95* |
| Residual | 10 | 6.76 | 1.14 | 3.14 | 13.27 | 13.16 |
| CV (%) | | 15.9 | 18.2 | 17.9 | 19.9 | 24.2 |

Appendix Table 5.1 Summary of the analysis of variance of yield and yield components of wheat.

| Source of variance | d.f | Mean squares | | | | |
|---|---|---|---|---|---|---|
| | | Shoot biomass(g) | No.of ears/pot | No.of seeds/pot | Grain yield(g) | Kernel weight |
| Sulphur | 3 | 7.3* | 2.5* | 2988.5* | 2617.5* | 4.8 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Selenium | 1 | 0.1 | 2.3 | 441.0 | 62.5 | 2.2 |
| Sulphur. Selenium | 3 | 0.6 | 0.5 | 272.1 | 100.8 | 2.2 |
| Residual | 8 | 1.3 | 0.8 | 599.0 | 755 | 4.9 |
| CV (%) | | 11.4 | 14.3 | 19.8 | 21.3 | 6.6 |

Appendix Table 5.2 Summary of the analysis of variances for the grain S, N, N:S ratio and Zn concentration of wheat grain.

| Sourec of variation | d.f | Mean square | | | |
|---|---|---|---|---|---|
| | | S ($10^{-5}$) | N ($10^{2}$) | Zn | N: S ratio |
| Sulphur | 3 | 1.96* | 0.4* | 124.7 | 126.0*** |
| Selenium | 1 | 0.02 | 0.01 | 3.6 | 0.13 |
| Sulphur. Selenium | 3 | 0.13 | 0.02 | 23.0 | 0.13 |
| Residuals | 8 | 0.22 | 0.06 | 87.5 | 1.18 |
| CV (%) | | 10.6 | 9.9 | 16.0 | 5.8 |

Appendix Table 5.3. Average concentrations of nutrients in wheat grain and the corresponding critical values and the coefficient of variation for the mean. Values for concentrations are shown as the mean±SEM.

| Nutrient | Mean | Critical value | Coefficient of variation |
|---|---|---|---|

| | | | (%) |
|---|---|---|---|
| Iron (mg/kg) | 35±3.6 | - | 11.9 |
| Manganese (mg/kg) | 65±5.2 | 19.4 | 6.9 |
| Copper (mg/kg) | 2±0.3 | 1.0 | 15.4 |
| Molybdenum (mg/kg) | 6±3.1 | <0.1 | 28.7 |
| Calcium (mg/kg) | 277±37.0 | - | 7.2 |
| Magnesium (mg/kg) | 1496±76.5 | - | 5.1 |
| Potassium (mg/kg) | 5238±885.3 | 4100 | 6.0 |
| Phosphorus (mg/kg) | 4544±340.5 | 2200 | 2.8 |