POTENTIAL FOR SAFE AND EFFICIENT BIOFORTIFICATION OF MAIZE CROPS WITH SELENIUM IN MALAWI

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ABSTRACT

Selenium (Se) is an essential element for humans, which is derived primarily from dietary sources. Habitual suboptimal dietary Se intake is associated with reduced Se status and adverse health outcomes including cardiovascular disorders, impaired immune functions and some cancers. The global extent of suboptimal dietary Se intake is difficult to estimate, but is likely to be widespread where food choices are narrow, for example, in subsistence agricultural contexts. This study aimed to: (1) characterise the likely contribution of maize grain to dietary Se intake in rural Malawi; (2) test the dependency of maize grain Se concentrations on soil factors; and (3) identify agronomic methods to improve Se concentration in maize grain. 88 field sites across Malawi were sampled across Malawi in 2009 and 2010 before determining maize grain, total soil and KH_2PO_4-extractable soil Se concentrations by inductively coupled plasma-mass spectrometry (ICP-MS). Dietary Se intakes from other food sources were estimated from the literature. The median maize grain Se concentration in Malawi was 0.019 mg Se kg^{-1} (range 0.005-0.533), representing a median intake of 6.7 µg Se $person^{-1}$ d^{-1} from maize. Suboptimal (<30 µg d^{-1}) dietary Se intake is therefore likely to affect most of the rural population in Malawi. Maize grain Se concentration was *c*. 10-fold higher in crops grown on high pH (>6.5) soils (Vertisols), probably because the dominant species of Se at high soil pH Se(VI) is more available to crops than Se(IV), as evidenced by the KH_2PO_4-extractable soil concentrations recorded. Total soil Se concentration ranged between 0.0521 and 0.6195 mg kg^{-1} but provided a poor index of Se availability. The results showed that KH_2PO_4-extractable Se concentrations >0.01 mg kg^{-1} and soil pH values >6.5 produced grain Se concentrations exceeding 0.15 mg Se kg^{-1}, a value above which rural populations in Malawi would attain adequate Se intake. Field experiments in which three Se application methods ($Na_2SeO_{4\ (aq)}$, granular compound (NPK+Se) and granular calcium ammonium nitrate (CAN+Se) were applied were conducted at up to six sites in 2008/09 and 2009/10. Application of Se significantly increased grain and stover Se concentrations and the response was approximately linear for all sites and application methods in both years (R^2 >0.90). The results showed that application of Se at 5 g Se ha^{-1} to maize would deliver adequate intakes for much of the population in Malawi. As total plant recovery of Se ranged from 3-45%, further work is required to identify and address the sources of this variation. In more detailed experiments, the fate of applied Se was investigated at two sites using the stable ^{74}Se isotope. Recovery of applied Se was 0.65 and 1.08 g Se ha^{-1} at the Chitedze and Mbawa, sites respectively,

representing 6.5 and 10.8% of the applied 10 g Se ha^{-1} by the maize crop; 0.2 g Se ha^{-1} of native soil Se was also absorbed, leaving 9.35 and 8.92 g Se ha^{-1} unaccounted. Of the total soil and applied fertiliser Se, fertiliser-derived Se (^{74}Se-labelled) comprised 71 and 82% of plant-Se recovery at Chitedze and Mbawa, respectively. The residual effects of Se application on grain Se in maize crops grown in the subsequent cropping season were 0.3025 and 0.5858 µg kg^{-1} g^{-1} applied Se at Chitedze and Mbawa respectively. Residual Se detected as KH_2PO_4-extractable Se ranged from 0.0029 to 0.106 µg kg^{-1} g^{-1} applied Se between sites. Further studies are required to quantify the amount of Se immobilised in the soil pool or lost due to leaching or volatilisation. A further experiment examined how traditional processing procedures for maize grain affected Se concentration in maize flour. At Se fertilisation levels which would increase dietary Se intake to appropriate levels, there was no evidence that traditional milling produced any significant loss of Se from maize flour. Assessment of the contribution of maize to the dietary supply of other nutrients showed that calcium concentration, and hence intake from maize, were very low. Maize grain was low also in K, Cu and Zn but provided a good source of Fe, Mg, Mn and Mo. There is a need to monitor the concentrations of trace metals such as Cd, Co, Ni and Cr as these might exceed the daily allowance and pose a risk to human health.

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ACRONYMNS AND ABBREVIATIONS

ADD	Agricultural Development Division
AIDS	Acquired Immuno-Deficiency Disease Syndrome
AISP	Agricultural Input Subsidy Program
CAOBISCO	Association of the Chocolate, Biscuits and Confectionary Industries of the European Union.
DAP	Diammonium Phosphate
DRI	Dietary Reference Intake
DW	Dry weight
Eh	Redox potential gives a measure of the apparent electron activity (e^-) in solution (volts)
EPA	Extension Planning Area
FAO	Food and Agriculture Organisation of the United Nations
FISP	Farm Input Subsidy Program
GPx	Glutathione peroxidase activity
HAST	High Affinity Sulphate Transporters
HIV	Human Immuno-Suppression Virus
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
Mha	Million hectares
MPC	Maximum permissible concentration
MOAFS	Ministry of Agriculture and Food Security
NES	National Extension Systems
NIST	National Institute of Standards and Technology
pe or pE	Negative logarithm of electron activities
pH	Negative logarithm of hydrogen ion activities
RDA	Required Daily Allowance
Redox	Reduction-Oxidation
RNI	Required Nutrient Intake
SSA	Sub-Saharan Africa
WHO	World Health Organisation

CHAPTER 1: General Introduction

1.1 Background information

Malawi is a long, narrow, land locked country situated between latitudes 9° 22' and 17° 8' south and longitudes 33° 40' and 35° 55' east. Its total area is 11.85 Mha, of which 9.42 Mha is land and 2.43 Mha is covered by water (Anonymous, 2009b). Malawi has a population of 13.1 m and over 85% of the population derives its livelihood from agriculture (Anonymous, 2008). Approximately 6.2 Mha of land is divided amongst 2.4 m smallholder households under customary land tenure and an additional 1.2 Mha is controlled by estates. Of this, 1.1 Mha belongs to approximately 30,000 farmers under leasehold tenure. A further 34,000 ha belongs to large estates mainly producing tea. Finally, there is about 1.8 million ha of public lands (World Bank, 2007). Agriculture contributes over 40% to Gross Domestic Product (GDP), of which over 70% is generated by the smallholder sector. Agriculture generates 90% of export earnings and 65% of raw materials for the manufacturing sector, provides employment for over 85% of the country's population, and is the source of income for over 60% of the rural poor (World Bank, 2007). Malawian agriculture is characterised by a dual structure consisting of smallholder farms and estates. The estate sector produces mainly tobacco, tea, sugar and coffee for export. Although smallholder agriculture is mainly subsistence-oriented, dominated by maize and other food crops, smallholders are now contributing significantly to cash crops for export, particularly air cured tobacco, Burley tobacco (*Nicotiana tabacum* L.), which accounts for about 80% of total production. The other cash crops produced are cotton (*Gossypium hirsutum* L.), paprika (*Capsicum annuum* L.), groundnut (*Arachis hypogaea* L.*)* and chillies (*Capsicum annum* L.). The contribution of the estate sector to GDP has been 8% over the past decade, while the smallholder sub-sector has increased from <20% in 1994 to 20% by 2005 (World Bank, 2007). Agriculture and maize are critically important to the Malawian economy and the livelihoods of the majority of the population but

the combination of low agricultural productivity and the predominance of maize production leads to a high incidence of poverty and national/household food insecurity (Dorward and Chirwa, 2011). The food crops grown in Malawi are shown in Table 1.1 (Anonymous, 2009a).

1.1.1 Soil fertility in Malawi

The soils in Malawi are divided into two groups, namely upland soils and alluvial soils (Lowole, 1995). Luvisols represent one important type of upland soil, are well-drained and reddish in colour, and occur on flat to gently undulating land. The topsoil is a dark-brown or dark reddish brown sandy clay loam, while the subsoil is dark-red or red sandy clay. Luvisols have good or moderately good structure with soil pH ranging from acidic to almost neutral (pH 5.3 – 6.7). The organic matter level is normally medium but ranges from low to high (0.5–4.5%) (Brown and Young, 1965, Brown, 1966, Stobbs, 1971, Lowole, 1995).

One of the most commonly occurring soil types in Malawi is Ferralsols, which are also deep and well-drained. The topsoil is normally dark-brown sandy clay loam and the subsoil is yellowish-red strongly acid to slightly acid sandy clay (pH 4.5–6.3). Soil organic matter content ranges from 1.0–3.6% and the soils have rather low natural fertility but generally respond well to fertilisation (Brown, 1966, Lowole, 1995). Alluvial soils such as Vertisols normally occur in low lying areas and are characterised by very firm, plastic and dark clays. The high clay content is uniform through the whole soil profile, resulting in the formation of numerous wide and deep cracks during the dry season (Lockwood Survey Corporation Limited, 1970, Lowole, 1984). Vertisols have well-developed self-mulching properties and their pH is alkaline to neutral (pH 6.6-8.2); their soil organic matter content is medium (1.6-3.0%).

Eutric Fluvisols occur on depositional sites and are characterised by stratification of different textures and colours which alternate with increasing depth. These soils occur in active flood plains and their organic

matter is medium to high (1.7-5.0%). They have a high natural fertility and the major constraint to crop production is frequent flooding of the soil resulting from their poor drainage characteristics. Eutric Cambisols are chemically similar to Fluvisols but occur on old alluvial plains and have a high natural fertility and productivity (Lowole, 1995, Lowole, 1984). The soils of Malawi are shown in Table 1.2. The recommended fertiliser types in the early 1970s was 20:20:0 (20% N, 20% P_2O_5 and 0% K_2O) as a basal dressing and sulphate of ammonia applied as a top dressing to supply 92 kg N and 40 kg P_2O_5 ha^{-1}. High analysis Di-ammonium phosphate (DAP) fertiliser as a basal dressing fertiliser and urea as a top dressing were then introduced in the late 1980s (Anonymous, 1989).

The newly introduced recommendation is 23:10:5+3S+1Zn as this was superior to 23:21:0+4S in increasing maize yields. Current fertiliser recommendations are 92 kg N ha^{-1}, 20 kg P_2O_5 kg ha^{-1}, 10 kg K_2O kg ha^{-1}, 6 kg S ha^{-1} and 2 kg Zn ha^{-1} (Chilimba et al., 2006) and liming is now recommended for smallholder farmers to boost crop production as soil acidity is one of the major constraints the country is facing. The soil acidity status is shown in Figure 1.1.

The productive capacity of Malawi's soil resources has declined as a result of erosion and adverse changes in the hydrological, biological, chemical and physical properties of the soils (Chilimba, 2001). Continuous cultivation has resulted in mining of most of soil nutrients, which has been aggravated by burning of crop residues, particularly in central and northern Malawi. This has led to a decline in soil organic matter and a consequent reduction or depletion of the soil micro-organisms which are essential for recycling of nutrients in cropping systems (Chilimba, 2002). Increased use of organic and inorganic fertilisers might therefore improve soil productivity.

1.1.2 Fertiliser use in Malawi

The use of fertiliser in Malawi has varied over the past 20 years. The annual mean growth in fertiliser use was estimated to be 8.8% between 1964 and

the 1990s, but fertiliser consumption has increased more slowly during the past 15 years at a rate well below potential demand (MoAFS, 2003). Fertiliser sales averaged 186,000 t yr^{-1} between 1996 and 2003, whereas potential fertiliser applications for rain fed crops in Malawi was over 547,000 t yr^{-1}, based on the cropping area and application rates recommended in the guide to agricultural production published by the Ministry of Agriculture and Food Security (MoAFS, 2003). This value was calculated by multiplying the recommended application rate by the mean total land area planted with each crop during a specified period. The Food and Agriculture Organization has stated that increasing productivity on existing cropped land using more fertiliser remains the most likely path farmers will take to increase production (FAO, 2008).

Table 1.1: Food crop production by smallholders on 6.2 Mha of land in Malawi between 2004 and 2008 (source: Anonymous, 2009).

Crop	**Yearly production (metric tonnes)**				
	2004	**2005**	**2006**	**2007**	**2008**
Maize	1,608,349	1,225,234	2,611,486	3,444,655	2,777,438
Rice	49,693	41,270	91,450	113,166	114,905
Groundnut	153,414	141,078	203,071	273,757	260,573
Wheat	1,668	1,730	2,000	4,605	2,491
Sorghum	40,905	18,175	54,309	63,698	61,999
Millet	17,349	15,970	27,037	32,251	31,869
Common bean	76,964	85,759	117,808	132,689	129,948
Pigeonpea	93,084	63,883	130,987	159,365	149,873
Cowpea	15,048	8,584	19,737	27,721	29,058
Field pea	2,087	87	1,600	2,064	2,188
Grams	1,723	661	849	1,042	868
Soybean	33,758	40,396	55,248	71,295	64,489
Dolichus bean	3,030	1,429	2,327	2,923	2,590
Velvet Bean	7,650	4,382	6,583	7,142	6,694
Ground bean	7,300	4,178	8,480	10,347	10,375
Cassava	2,532,079	2,197,640	2,832,141	3,285,127	3,539,660
Sweet potato	1,762,034	1,081,463	1,781,595	2,307,354	2,362,425
European potato	420,490	404,420	527,831	594,003	673,344

Table 1.2: Soils of Malawi and their estimated areas based on the soil map of Malawi (source: (Lowole, 1995).

Soil Name	**Land area (km^2)**	**% of total land area**
Ferric luvisols	6636.3	7.0
Lithosols	23138.7	24.5
Ferric luvisols with lithosols	6551.7	6.9
Lithosols with some luvisols	1207.3	1.3
Orthic ferrasols	3175.0	3.4
Orthic ferrasols, chromic luvisols	4990.7	5.3
Xanthic ferrasols	14849.0	15.5
Orthic ferrasols and Xanthicferralsols	3868.0	4.1
Xanthic ferrasols over massive Laterite	3729.6	4.0
Xanthic ferralsols and lithosols	4214.0	4.5
Humic ferralsols	1222.7	1.3
Humic ferralsols with lithosols	861.3	0.9
Dystric nitosols	615.2	0.7
Dystric nitosols with some lithosols	699.8	0.7
Dystric nitosols and lithosols	1399.5	1.5
Pellic vertisols	1945.5	2.1
Chromic vertisols	144.4	0.1
Calcic phaeozems	201.0	0.2
Orthic solonetz	1222.7	1.3
Eutric regosols	307.6	0.3
Eutric fluvisols, Eutric cambisols	8797.2	9.3
Eutric gleysols	3875.7	4.1
Eutric fluvisols and Eutric gleysols	599.8	0.6
Total	94253.2	99.8

In the mid-1970s, government policies in Malawi favoured a "green revolution" approach which supported a universal fertiliser subsidy, subsidised smallholder credit and controlled the price of maize (Gladwin, 1992, Dorward and Chirwa, 2011). However, this package collapsed by

1992/93 cropping season due to the removal of government subsidies and economic liberalisation (Gladwin, 1992). Concomitantly, maize production decreased because most of the people were unable to access farm inputs because of their high cost and a consequent decline in the native fertility of the soils (Dorward and Chirwa, 2011).

The Malawian government is currently implementing an Agricultural Input Subsidy Program (AISP) which has greatly increased access to fertilisers by smallholder farmers (Fig. 1.2), and the country has attained food security, as shown by increase in maize production (Table 1.1; Fig. 1.3). An independent review of the AISP recently concluded that it has "led to significant increases in national maize production and productivity" (Dorward and Chirwa, 2011).

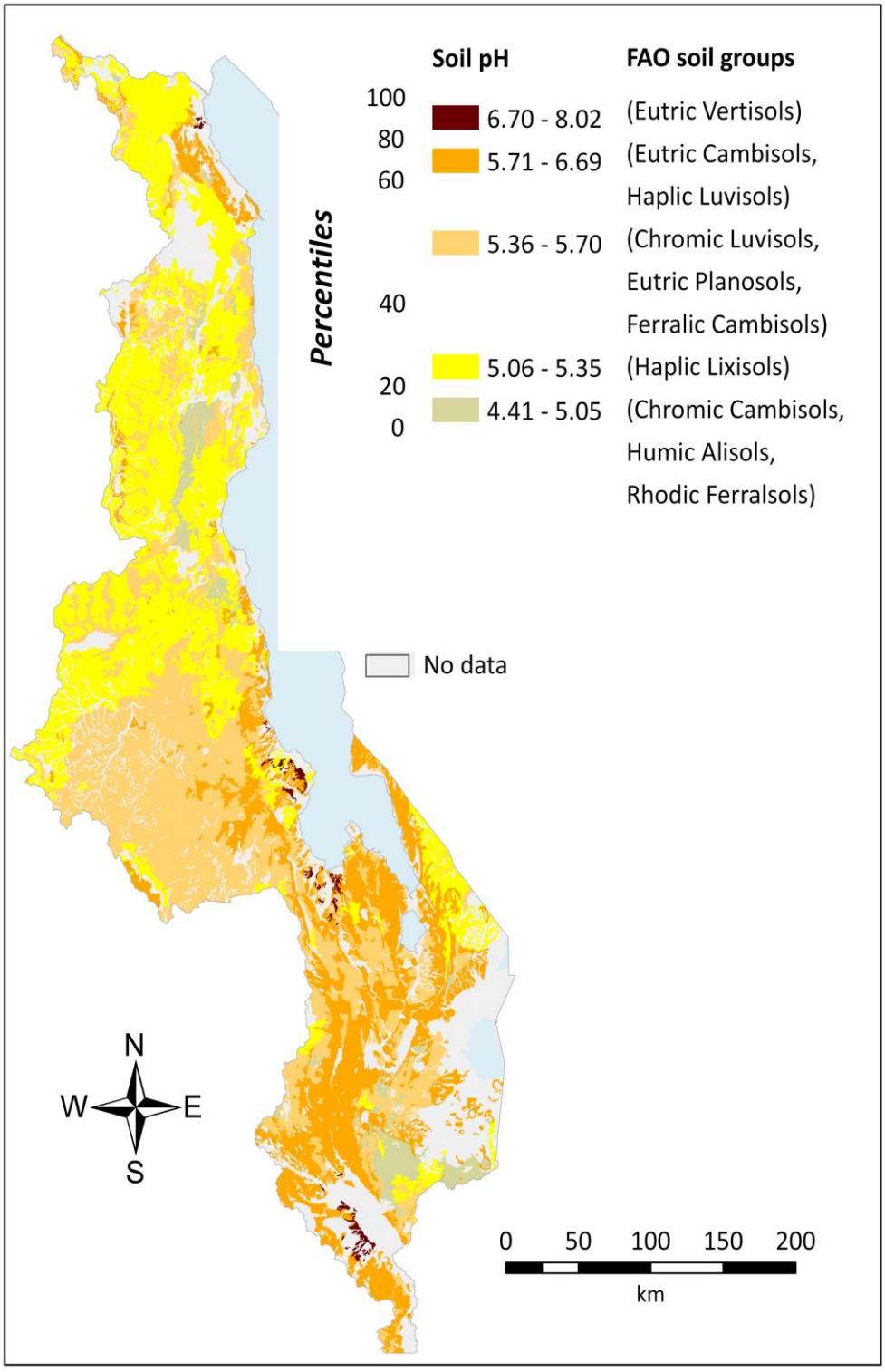

Figure 1.1: Map of Malawi showing areas of soil pH <5.5 and >5.5 (Green and Nanthambwe 1992). Figure produced using ArcGIS (v. 9.3, ESRI, Redlands, CA, USA).

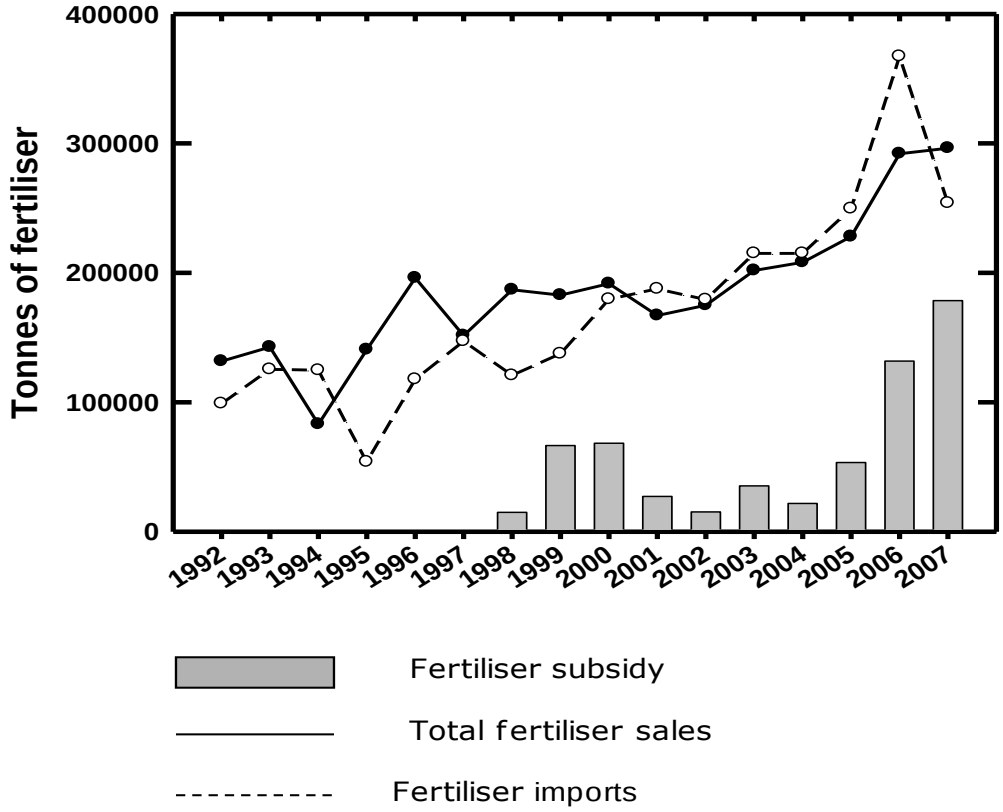

Figure. 1.2. Impact of fertiliser subsidy on fertiliser use in Malawi(Dorward and Chirwa, 2011).

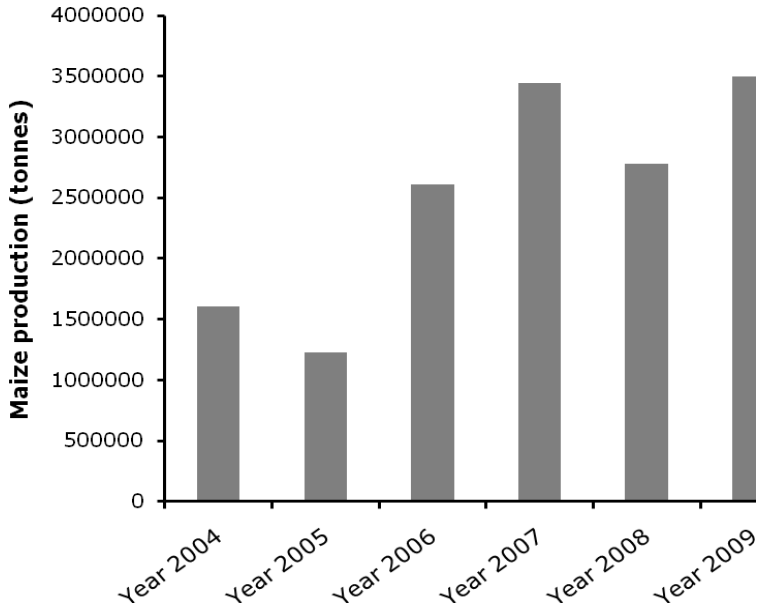

Figure 1.3. Effect of fertiliser subsidy on maize production in Malawi(Anonymous, 2009a).

1.2 Physical, chemical and biological properties of selenium

1.2.1 Physical properties

Selenium (Se) is a naturally occurring element within the oxygen group (Group V1A) (Fordyce, 2005), has an atomic mass of approximately 79 and has six natural isotopes, ^{74}Se, ^{76}Se, ^{77}Se, ^{78}Se, ^{80}Se and ^{82}Se. It is a chalcophile (sulphur-loving) element and replaces S in common sulphide minerals such as pyrite, chalcopyrite, pyrrhotite and sphalerite. Se has chemical and physical properties that are intermediate between those of metals and non-metallic elements (Johnson *et al.*, 2010) and also forms several rare minerals including crookesite (Cu, Ti, Ag) 2Se), berzelianite (CuSe) and tiemannite (HgSe) (Fordyce, 2005; Johnson *et al.,* 2010).

1.2.2 Chemical properties

Selenium was discovered in 1817 by Jan Jacob Benzelius and exists as elemental selenium (Se^0), selenide (Se^{2-}), selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}). The six natural stable isotopes of selenium are ^{74}Se (0.87%), ^{76}Se (9.02%), ^{77}Se (7.58%), ^{78}Se (23.52%), ^{80}Se (49.82%) and ^{82}Se (9.19%) (Mikkelsen *et al.*, 1989). Some of the commercially available forms of selenium are H_2Se, metallic selenides, SeO_2, H_2SeO_3, SeF_4, $SeCl_2$, selenic acid (H_2SeO_4), Na_2SeO_3, Na_2SeO_4 and various organic Se compounds (Terry and Zayed, 1994). Selenium in the +4-oxidation state can occur as selenium dioxide (SeO_2), SeO_3^{2-}, or selenious acid (H_2SeO_3), while in the +6-oxidation state, selenium is in the form of selenic acid (H_2SeO_4) or SeO_4^{2-} salts.

Selenite and selenate are thermodynamically stable under the pH and redox conditions found in most soils (Uden, 2005). Selenate is highly mobile under oxidising conditions, although its mobility declines with decreasing pH under reducing conditions (Gondi et al., 1992). Elemental Se or metal selenides form under conditions of low pH and redox potential (Eh) and the solution chemistry of Se is principally (oxy) anionic, comprising selenite (SeO_3^{-2}) (Elrashid et al., 1987) and selenate (SeO_4^{2-}). Elemental Se is also stable over a wide pH range under reducing conditions (Masscheleyn et al., 1990,

Masscheleyn and Patrick, 1993). Selenate is the major species in soil solution at high redox (pe + pH >15); in the medium redox range (pe+pH=7.5–15), selenite species predominate, while selenide species are stable only at low redox (pe + pH <7.5) (Elrashid et al., 1987). Selenite is more stable under lower redox conditions than in the high redox state, and selenate entering drainage systems is readily reduced to selenite if pe/Eh falls. At low pH levels, selenite is likely to be strongly absorbed by hydrous secondary iron oxides and, to a lesser extent, by clays and organic matter (Elrashid et al., 1987, Masscheleyn et al., 1990, Dhillon, 2009). In soils high in Ca and Mg, $CaSeO_4$ and $MgSeO_4$ both contribute to the total Se concentration, whereas KHSe, NH_4HSe and MnSe are the major contributors in acid soils (Elrashid et al., 1987).

1.2.3 Biological properties

Selenium is a biologically active element which can form direct selenium-carbon bonds to produce a range of organic compounds which include selenoamino acids and selenoproteins (Stadtman, 1983). Selenoproteins have essential functional roles in a wide array of prokaryotes, archaebacteria and eukaryotes (Nuttall, 1985, Driscoll and Copeland, 2006). Se is incorporated into selenoproteins such as selenomethionine and selenocystein (Sec), which is the twenty first amino acid and is encoded by an UGA codon in the selenoprotein mRNA.

1.3 Selenium inputs to soils

Soil Se originates from the weathering of Se-containing rocks, volcanic activity and dust arising from coal combustion (Combs and Combs, 1986). Soil Se content reflects, to some extent, that of the parent material from which the soil formed. Thus, in arid and semi-arid areas, soils with a high Se content have been derived from sedimentary rocks, usually shales and chalks (Fordyce, 2005). The selenate is easily leached from the surface soil and re-deposited in the sub-surface soil, where it is still available to plants (Olson *et al.*, 1942). Soil Se varies as a function of the parent material,

organic matter and humus content (Fordyce, 2005). Selenium has many pathways for distribution among geological, biological, atmospheric, aquatic and human activities. There are "sinks" which Se may enter, including complexation of selenides with ferric hydroxides or other metals to form metal selenides which may be recycled very slowly (Fordyce, 2005). There is a biological selenium cycle consisting of several oxidation-reduction states (Shrift, 1973). The other source of Se in soil results from atmospheric deposition (Deckers and Steinnes, 2004). Selenium generally occurs naturally in a number of inorganic forms, including selenide, selenate and selenite, has a biological role, and is found in organic compounds such as dimethyl selenide, selenomethionine, selenocystein and methyl selenocysteine. The missing link in the oxidative side of the biological selenium cycle was found when elemental Se was oxidised to selenite and to some extent, traces of selenate by *Bacillus megaterium* (Sarathchandra and Watkinson, 1981).

Selenium is also produced from selenide in many sulphide-containing ores such as those of copper, silver and lead, and is obtained as a by-product of processing of these ores (Johnson *et al.,* 2010). Natural sources of selenium include soils which are rich in Se and Se that has been bioconcentrated by plants, while anthropogenic sources include coal burning and the mining and smelting of sulphide. Approximately 70% of the anthropogenic emissions of Se is from combustion of coal, oil and other types of fuel, and production of copper, zinc and lead (McGrath, 2009). The major transport processes for Se in the soil-plant system have been characterised as uptake by plants, volatilisation to the atmosphere, accumulation of Se in soil and leaching to ground water (Lin, 2009).

1.4 Factors affecting Se availability for plant uptake

Geology exerts a fundamental control over Se concentrations in the soils on which crops and animals are raised to form the starting point in the human food chain (Fordyce, 2005; Johnson *et al.,* 2010). The Se status of populations, animals and crops varies markedly around the World as a

result of differing geological conditions (Fordyce, 2005, Johnson *et al.,* 2010). High Se concentrations are associated with some types of phosphate-rich rocks, organic rich black shales, coals and sulphide mineralisation, whereas most other rock types contain very low concentrations. Selenium-deficient environments are far more widespread than seleniferous ones (Davies, 1980). Soil Se also varies as a function of organic matter and soil texture, and it has been reported that Se concentrations are higher in organic soils than in mineral soils (Aubert and Pinta, 1977, Johnson *et al.,* 2010) and in soils with a high clay content than in coarse textured soils (Gardiner and Gorman, 1963). In addition, Se concentration increases with soil depth, particularly in horizons with alluvial clay accumulation (Wells, 1967). Although the underlying geology is the primary factor controlling the Se concentration of soils, the availability and uptake of Se into the plants is determined by several soil bio-physiochemical properties (Fordyce, 2005). These include the prevailing pH and redox conditions, the chemical form or speciation of Se, soil texture and mineralogy, organic matter and the presence of competitive ions. For example, the results for total soil Se concentration were contrary to expectation as the highest values in China were in areas where the incidence of Keshan disease was greatest; this disease is symptomatic of extreme Se deficiency (Fordyce, 2005). It was also noted that total soil Se closely reflected the quantity of soil organic matter; however, although soil Se content increased with soil organic matter, this did not increase Se concentration in the plant as Se in the soil was strongly adsorbed to organic matter (Fordyce, 2005).

In suboxic waters, the concentration of selenite increased while that of selenate decreased, reflecting changes in redox conditions in the environment (Takayanagi and Wong, 1985). The sharp increase in organic Se concentration in suboxic waters probably resulted from decomposition of organic matter and/or a diffusive flux from the underlying sediment (Takayanagi and Wong, 1985). When analyses were conducted to test for correlations between grain Se and various soil factors, the only positive correlation was with soil pH and the highest negative correlation was with soil Fe concentration (Johnson et al., 1996). A pH threshold of 7.6 was

noted below which grain Se concentration was low and grain Se concentration decreased with increasing organic matter (Johnson et al., 1996), and above which grain Se concentration was correlated with soil organic carbon in wheat, barley and oats, explaining up to 60% of the variation in Se concentration. When pH was below 6, Se uptake from added Se fertiliser was higher in soil with a high organic matter content, whereas when pH was above 6, Se uptake was higher in soil with a low organic matter content (Fordyce, 2005). Se availability in soil which was low in organic matter increased with increasing pH, but decreased in more highly organic soil (Eich-Greatorex et al., 2007). Soil pH, organic matter and clay content are the major factors affecting the transport and transformation of Se. These findings suggest that soil pH was the major factor influencing the availability of soil Se for plant uptake. Selenite sorption decreased with the increase in pH (Duc et al., 2006). The addition of phosphate fertiliser to soil leads to precipitation of phosphate and Se is fixed in the precipitate, so becoming unavailable for plant uptake. Conversely, phosphate may also lead to desorption of selenite ion bound by Fe and Al oxides because phosphate is more strongly adsorbed to these oxides than selenite (Liu et al., 2004, Nakamaru et al., 2006). Selenium concentration in plants may be further reduced by sulphate application due to competition between sulphate and selenate for transporters in plant roots (Lyons et al., 2003).

1.5 Geographical variation in soil selenium concentration

Se concentrations in most soils are within the range 0.01–2.0 mg Se kg^{-1} (Fordyce, 2005). In Australian soils, Se ranges between 0.11–0.41 mg kg^{-1} (Mikkelsen and Wan, 1990), while in Finland, a mean soil Se concentration of 0.21 mg kg^{-1} was found in the plough layer (Spencer and Siegel, 1978). In high precipitation areas in the west of Norway, soil Se concentration has risen from 0.2 to 1.4 mg kg^{-1} (Wu and Lag, 1988). Soil Se concentrations in the UK range from 0.1 to 4 mg Se kg^{-1}, with >95% of soils containing $\leq$1 mg Se kg^{-1} (Broadley et al., 2006). In Germany, mean values of 0.123 mg Se kg^{-1} for 195 agricultural soils and 0.158 mg Se kg^{-1} for 304 grassland soils have been reported (Hartfiel and Bahners, 1988). Selenium

concentrations of 0.24-0.55 mg kg^{-1} have been reported in India (Yadav et al., 2005). The mean total Se content was 0.112 mg kg^{-1} (range 0.059-0.190 mg kg^{-1}) in the low-Se area of China where Keshan disease is endemic, whereas the corresponding values for high-Se areas in China ranged from 6.39 to 10.66 mg Se kg^{-1} (Sun et al., 1985).

1.6 Availability of soil selenium for plant uptake

Plants absorb Se from the soil solution primarily as selenate and to a much lesser extent as selinite. Selenate is more readily available to plants and is stable in higher pH soils while selenite, which is dominant in acidic soils, is bound to sesquioxides, decreasing its availability for uptake (Geering et al., 1968). Results from experiments with rye seedlings showed that Se uptake by plants was significantly correlated with phosphate-extractable Se, and that a 0.1 M solution of KH_2PO_4 was a suitable extractant to determine available soil Se (Zhao et al., 2005). Generally, accumulation of insoluble elemental and selenide forms of Se and organic forms could account for the low availability of Se in soils despite the relatively large quantities present. Selenites are the form of Se which is preferentially adsorbed by clay minerals, particularly montmorillonite, Fe oxides and organic matter (Christensen et al., 1989, Vuori et al., 1989, Su and Suarez, 2000, Peak and Sparks, 2002), and its adsorption by geothite is highly pH dependent (Goldberg et al., 2009, Lee et al., 2011).

Measurements of water-soluble and base-soluble Se have been found to provide reliable estimates of Se availability to plants, although values for the former were more closely correlated with uptake by plants (Olson *et al.,* 1942, Nye and Peterson, 1975). Although a good correlation was observed between plant Se concentrations and hot water-extractable Se under greenhouse conditions, the correlation was poor under field conditions (Eurola et al., 2003). Sharmasarkar and Vance, (1995) reported that ammonium bicarbonate diethylene triamine penta acetic acid (AB-DTPA)

extractable Se provided the best predictor of plant-available Se under field conditions. However, Stroud *et al.* (2010) concluded that plant-available Se can be estimated effectively by KH_2PO_4 extraction, which removes both soluble and adsorbed Se from soil. This finding was in agreement with what was reported that phosphate exchanges with inorganic Se species adsorbed by clay minerals and oxides (Sharmasarkar and Vance, 1995, Jackson and Miller, 2000). A recovery of 91% of the soil selenate in 0.016 M KH_2PO_4 extraction solution has been reported, while recoveries of selenite ranged from 18.5 to 50.5% (Stroud et al., 2010).

1.7 Selenium uptake and assimilation by plants

Selenium has no proven function in plant nutrition (Broyer et al., 1966, Ziebur and Shrift, 1971, Hartikainen, 2005) and selenium fertilisation has generally not been found to increase yield in most crops, although some researchers have reported yield increases (Hartikainen, 2005). However, Se application at appropriate levels delayed senescence, improved the quality of produce and increased tolerance to tuber browning disease of European potato, *Solanum tuberosum L.* (Turakainen et al., 2004). Selenate enters root cells though sulphate transporters in their plasma membranes (Terry et al., 2000, White et al., 2004, White and Broadley, 2009), while selenite is thought to be transported by phosphate transporters (White and Broadley, 2009). Uptake of selenite and selenate ions by roots follows dissimilar mechanisms as experiments using excised roots showed that uptake of selenate ions requires energy (active uptake), whereas uptake of selenite ions is energy-independent (passive uptake) (Shrift and Ulrich, 1969).

Selenite is rapidly converted to organoselenium compounds in the root, whereas selenate is delivered to the xylem and transported to the shoot, where it is assimilated into organoselenium compounds and redistributed within the plant in similar manner to S (Terry et al., 2000, Broadley et al., 2006, White and Broadley, 2009). Following uptake, selenate is likely to be transported to the plastids or may remain in the cytoplasm, where it is assimilated *via* the S assimilation pathway. Briefly, selenate is activated by

adenosine triphosphate sulhurylase (ATP sulhurylase) to form adenosine 5'-phosphoselenate, which is reduced to selenite in the presence of adenosine 5'-phosphosulphate reductase and subsequently to selenides *via* a non-enzymic step in the presence of glutathione (Lu et al., 1995, White and Broadley, 2009). Selenide is assimilated into selenocystein and further into selenomethionine before being incorporated into proteins (Lu et al., 1995, White and Broadley, 2009). Some of the seleno amino acids may also be methylated and methylated selenoamino acids are converted to methyl selenol and ultimately to dimethyl selenide before being *volatilised* (Lu et al., 1995, Ip et al., 2002, Ellis and Salt, 2003).

Sulphur (S) and Se are both naturally occurring Group VIA elements and have similar chemical properties (Broadley *et al.,* 2006; White *et al.,* 2007). Sulphur is essential for plant growth, whereas Se is not known to be essential for plants but is essential for human nutrition and health. Competition for uptake between sulphate and selenate is known to occur and high concentrations of sulphate have been shown to inhibit selenate uptake by competing for the same binding site within cells, thus demonstrating that they follow the same transport path into roots (Epstein, 1955). Several other studies have also shown that sulphate and selenate follow similar uptake pathways being both taken up by sulphate transporters in the root plasma membrane (Terry et al., 2000). The sulphate transporters which catalyse the majority of the selenate and sulphate influx to plant cells are known as high affinity sulphate transporters (HAST) (Shinmachi et al., 2010). Sulphate competes with selenate for uptake by the sulphate transporter (Terry et al., 2000). However, crops such as rice and Indian mustard are able to absorb Se preferentially in the presence of high sulphate concentrations (Bell et al., 1992), whereas selenate uptake was inhibited by high sulphate concentrations in other crops such as alfalfa, wheat, ryegrass, barley and broccoli (Terry et al., 2000). Sulphate in the rhizosphere inhibits the uptake of selenate, whereas rhizospheric selenate promotes sulphate uptake and Se toxicity may occur because Se and S compete in biochemical processes for which S is vital (White et al., 2004).

1.8 The role of selenium in human health

Selenium (Se) is an essential element for humans and livestock. A total of 25 selenoproteins have been identified in humans, including iodothyronine deiodinases, thioredoxin reductases, glutathione peroxidases, and a range of other selenoproteins (e.g. SelP, SelM, SelT; (Brown and Arthur, 2001, Rayman, 2002, Fairweather-Tait et al., 2011). These proteins have critical roles in thyroid functioning, cell proliferation and survival through redox homeostasis, antioxidant defence and the immune response. When Se intakes are suboptimal, the selenoprotein status of humans decreases and there are increased risks of adverse health effects. At extremely low Se intake levels (i.e. where habitual intakes for adults are <20 μg Se d^{-1}), clinical deficiency disorders have been reported including Keshan disease (a cardiomyopathy) and Kashin-Beck disease (an osteoarthropathy). Where habitual intakes for adults are below the levels needed for maximal expression of glutathione peroxidase (typically ≥40 μg Se d^{-1}), there is an increased risk of health disorders, including cardiovascular disorders, impaired immune function and some cancers (Fairweather-Tait et al., 2011).

Fifteen selenoenzymes have been characterised for their biological function, including four glutathione peroxidases (GPx) which are antioxidant enzymes, three forms of thioredoxin reductases which have important roles in regenerating antioxidant systems and maintaining the intracellular redox state, and three forms of iodothyronine deiodinases that are involved in the production of active thyroid hormone (Brown and Arthur, 2001, Rayman, 2002). Selenium is a co-factor for the enzyme, glutathione peroxidase, which helps in the regeneration of glutathione, a major antioxidant responsible for protein stability, transcription of mRNA and other biochemical functions such as protection of cell membranes and prevention of free radical generation (Combs, 2005). Free radicals can destroy cells or impair cellular function, mutate DNA and initiate diseases associated with aging, cancer, heart and other blood diseases (Combs, 2005). Thioredoxin reductase (TR) is one of the selenoproteins involved in anti-cancer effects

(Ganther, 1999), and is also involved in the reduction of oxidised thioredoxin, recycling dehydroascorbate to ascorbate (*Vitamin* C). Se deficiency in humans has been linked to several physiological disorders (Rayman, 2000, Rayman, 2002, Jackson et al., 2004) and has a significant role in the treatment of severe bacterial infections, such as acute septicaemia and several other conditions which appear to be inversely correlated with soil Se concentrations, and the occurrence of disease such as endemic goitre, sudden infant death syndrome and multiple sclerosis (Foster, 1993).

There is substantial evidence that Se is a potent anti-carcinogen and studies continue to confirm that people with higher levels of Se in their blood enjoy lower rates of prostate and lung cancer (Reid et al., 2002, Vogt et al., 2003, Combs, 2005). It has been reported that prevention of cancer requires supra-nutritional levels of Se intake; for example, supplementation with 200 μg Se d^{-1} resulted in a 60% decrease in prostate cancer (Combs, 2001). An inverse relationship between serum Se level and carcinogenesis in various parts of the human body has been reported (Garlend et al., 1994). Recently, the largest ever prostate cancer prevention trial known as the Selenium and *Vitamin* E Cancer Prevention Trial (SELECT) showed that selenium or *Vitamin* E taken alone or in combination did not prevent prostate cancer in the population of relatively healthy men over an average period of five years (Lippman et al., 2009).

Selenium also has an important biological function in combating heart diseases. In China, where dietary intake is extremely low, Se deficiency is associated with health disorders such as Keshan disease, an endemic cardiomyopathy, and Kaschin Beck disease, a chronic and deforming form of arthritis (Fordyce, 2005). The most important biological function of Se is as an anti-oxidant and a protective agent against cancer and heart disease (Delmas-Beauvieux et al., 1996). The low soil Se status in parts of China where dietary Se intakes are extremely low is known to be involved in causing Keshan and Kashin Beck diseases (Fordyce, 2005, Johnson *et al.*, 2010). Low dietary Se intake has also been linked to pancreatitis, asthma and inflammatory response syndrome and impacts on immune system

functioning, response to viral infection, female and male fertility and thyroid functioning (Rayman, 2000, Rayman, 2002).

The Human Immuno-suppression Virus (HIV) causes a depletion of body selenium which in turn induces the immune system failures manifested as Acquired Immuno-Deficiency Disease Syndrome (AIDS; Burbano *et al.*, 2002). Supplementation with 200 µg Se d^{-1} has been shown to forestall the progression of HIV infection to the development of AIDS, reduce the symptoms of AIDS, improve the lifespan of AIDS patients and reduce hospitalisation rates of HIV positive adults (Burbano et al., 2002). Deficiency of selenium, more than any other nutrient, has been documented to be correlated with the progression and mortality of HIV (Cirelli et al., 1991, Look et al., 1997). It is estimated that at least one billion people are Se-deficient (Combs, 2001). Several studies have confirmed that low Se status impacts on the functioning of the immune system and that Se deficiency in much of Sub-Saharan Africa is an important determinant of the rapid spread of HIV/AIDS (Foster, 2003). Thus, Senegal, which has similar cultural values to other African countries, has very low prevalence of HIV/AIDS of 1.77%, whereas the corresponding figure for other African countries is >12%. This difference has been attributed to the greater dietary intake of Se in Senegal (Foster, 2000). In Tanzania, it was reported that low plasma Se levels were significantly associated with an increased risk of mortality, and that Se status is important in determining clinical outcomes related to HIV disease in Sub-Saharan Africa (Kupka *et al.*, 2004), while in Malawi, low Se status and HIV load were associated with anaemia and pulmonary tuberculosis (van Lettow et al., 2005).

Low Se status is also an important risk factor for the development of myco-bacterial diseases such as tuberculosis in HIV positive individuals (Gupta et al., 2009, Eick et al., 2009, van Lettow et al., 2004). Marked Se deficiency may also lead to the development of rheumatoid arthritis, an inflammatory condition (Köse et al., 1996, Aaseth et al., 1998). An international study of asthma and allergies in childhood reported that Se-rich foods protected children from asthma and supplementation with 200 µg Se d^{-1} reduced the use of inhaled and systemic corticosteroids (Ellwood et al., 2001). Low Se

intake increases the risk of cardiovascular disease and it was reported in Niger that 40% of the patients with peripartum cardiomyopathy had plasma Se concentrations <45 ng mL^{-1}, which is regarded as a risk factor for the disease (Cénac et al., 1992). Low Se also increases the incidence of diseases such as endemic goitre, asthma, sudden infant death syndrome and multiple sclerosis (Foster, 1993).

The relationships between Se intake, Se status in terms of selenoprotein expression and health outcomes are still being resolved (Hurst et al., 2010, Lippman et al., 2009). These uncertainties are reflected in the wide range of Dietary Reference Intake (DRI) levels in different countries (Fairweather-Tait *et al.,* 2011). Some DRIs are set to reduce the risks associated with overt deficiency (i.e. recommending intakes of ~40 µg Se d^{-1}), although most countries have recommended intake levels of 50-70 µg Se d^{-1}. At high levels of habitual Se intake (>400-900 µg Se d^{-1}), Se is potentially toxic and so care must be taken in setting DRIs and recommending dietary supplementation. Reported Se intake values for different countries (Tahtat et al., 2003, Reilly, 2006) are shown in Table 1.3. However, the values presented are old and current intake might differ significantly as various countries have been working to address their low Se intake.

1.8.1 Selenium intake and daily required allowance

In early studies of Se in humans, blood and plasma levels were determined in attempts to assess Se status but there were no reference values to enable interpretation of the results (Burk et al., 1967). Functional measurements of Se became possible when Se was discovered to be an essential component of glutathione peroxidase (Rotruck et al., 1973). A measurement of GPx was therefore used as an accessible biomarker of Se status in humans (Yang et al., 1987) and Se intake was correlated with plasma Se concentration and the activity of the most abundant selenoprotein glutathione peroxidase (GPx).

Although there is no international recommended dietary Se intake because this varies with age, sex and source of dietary Se (Thomson, 2004), subjects with plasma/serum Se levels >70 μg Se L^{-1} (>0.89 μmol Se L^{-1}) showed no further glutathione peroxidase (GPx) response to Se supplementation (Neve, 1995). As a result, some countries have established recommended Se intake levels which are adequate to meet nutritional needs based on maximal GPx activities (Adams, 2008). Plasma Se level exceeding 120 μg L^{-1} may be a useful target value for minimising cancer risk (Combs, 2001). In the United States, the recommended dietary intake for men and women was set at 55 μg d^{-1}, with a recommended upper safe limit of 400 μg Se d^{-1}. According to the Recommended Daily Allowances in the United States, the average intake should be 50-200 μg Se d^{-1} (McConnell et al., 1981). Globally, Se intake ranges from 6-500 μg d^{-1}, and in Australia, Bangladesh, Canada, Finland, Greece, Russia, United Kingdom, USA, Venezuela and Germany intake ranges from 29-500 μg d^{-1} (Reilly, 1998). The mean intake in Finland increased from 30 to 113 μg Se d^{-1} between 1984 and 1986 due to the national supplementation programme (Eurola et al., 2003). Se intakes and status in New Zealand increased when Australian wheat containing higher levels of Se was imported (Thomson and Robinson, 1980, Watkinson, 1981, Thomson and Robinson, 1996). In the UK, the reference nutrient intake (RNI), a level considered to be sufficient or more than sufficient for most of the population, is set at 75 and 60 μg Se d^{-1} respectively for males and females (Stahl et al., 2002, Broadley et al., 2006). Selenium intake in humans declined from >60 μg d^{-1} in the 1970s to <40 μg d^{-1} in the 1990s with a concomitant decline in Se status. The change in Se status of people in UK was attributed to the use of locally produced wheat against imported wheat which was known to have a higher Se concentration. Other reasons advanced were reduced use of coal, depletion of soil Se as a result of intensive cropping, and dietary changes, particularly reduced consumption of offal (Rayman, 2000, Broadley et al., 2006, Fairweather-Tait et al., 2011).

The recommended Se intake in the US, Canada and Europe is 55 μg d^{-1} (Thomson, 2004), a value intended to achieve and maintain the maximum plasma GPx activity. However, as there is growing evidence that additional

beneficial effects, such as cancer prevention, may be provided when dietary Se intake exceeds the normal nutritional range, it may be inappropriate to rely solely on GPx activity to define optimal Se intake (Rayman, 2002). Plasma Se concentrations >120 μg L^{-1} may be a useful target to minimise the risk of cancer (Combs, 2001). To provide this level of plasma Se would require a dietary intake of at least 1.5 μg Se kg^{-1} body weight d^{-1}, equivalent to 90 and 120 μg d^{-1} respectively for people weighing 60 or 80 kg (Hawkesford and Zhao, 2007). Selenium intakes <11 μg d^{-1} are often associated with serious health effects and intakes <20 μg Se d^{-1} have been observed to induce deficiency symptoms (Fairweather-Tait *et al.,* 2011).

As plasma Se concentration and the activity of the most abundant selenoprotein, glutathionine peroxidase (GPx), are correlated with Se intake, some countries have established recommended intake levels which are sufficient to meet the nutritional needs of the majority of healthy people, based on maximal GPx activities (Adams, 2008). Table 1.4 shows that Se concentrations in serum or plasma in healthy adults vary between countries due to differences in Se intake.

1.8.2 Approaches for addressing low dietary Se intake

Several approaches have been suggested to address low Se intake, including dietary diversification, fortification of food with Se and introduction of mineral supplements containing Se. For example, in Finland, people began to take tablets containing Se when its importance for human health became known, but the government noted the danger that some individuals might still be subject to Se deficiency while others were at risk of an overdose (Eurola, 2005). The primary disadvantage of consuming inorganic Se is that a substantial proportion is excreted as sodium selenate, while sodium selenite is not incorporated into the body. Selenium present in biofortified grain is more readily assimilated by the body than inorganic forms of Se and thus offers improved bioavailability (Lyons et al., 2003, Xu and Hu, 2004). Selenium in biofortified grain is present in the form of selenomethionine, which can be incorporated into muscle tissue and the Se

is released when required to provide a significant increase in antioxidant activity (Xu and Hu, 2004). Selenium-enriched wheat was found to contain the most effective form of Se in reducing the incidence of the precursors of colon cancer (Finley, 2007).

The population of Norway, despite having a relatively modest total Se intake, has the highest mean serum Se level in Europe of 119 μg L^{-1}. The probable explanation is that the major source is relatively Se-rich North American wheat (Broadley et al., 2006). The Se contained in wheat grain is highly bioavailable; for example, when participants in a trial consumed Se-enriched bread providing a Se intake of 100, 200 or 300 μg d^{-1} for six weeks, their serum concentrations were increased by 20, 37 and 53 μg L^{-1}, respectively (Meltzer et al., 1993). By contrast, supplementation of livestock with Se did not increase human intake of this element (Thomson and Robinson, 1980, Watkinson, 1981, Thomson and Robinson, 1996). Similarly, in a study in Serbia, consumption of wheat grain enriched with Se increased plasma glutathione peroxidase activity in blood by 53%, reduced oxidative stress parameters and increased the levels of copper, iron and zinc in erythrocytes relative to individuals consuming low-Se wheat (Djujic et al., 2000).

Cereals and cereal products contribute *c*. 70% of the total dietary intake of Se in populations living in the low Se areas of China and 40-54% in low income populations in India (FAO/WHO, 2001). A survey of the Se status of people in 27 regions of Russia revealed a highly significant correlation between serum Se and Se concentration in wheat flour from different areas (Golubkina and Alfthan, 1999). These findings suggest that agronomic biofortification is the most suitable approach in addressing the problems associated with low Se intake.

1.8.3 Current Se intake and status in Malawi

The Se concentration of food grown in Zomba, Malawi is consistent with areas where soil Se is low (Donovan et al., 1991). A low intake of Se with a median intake of 44-46 μg d^{-1} and an interquartile range of 28-30 μg d^{-1} was also reported for Mangochi (Eick et al., 2009). Nevertheless, these values may be the highest in the country as Mangochi is situated beside Lake Malawi where Se-rich fish contribute a greater proportion of the local diet; crops grown in Mangochi may also contain higher Se concentrations than in other parts of Malawi due the higher soil pH. This suggestion is supported by results which show that Se concentrations in maize grain and pigeon pea were respectively 0.026 and 0.064 mg kg^{-1} for Zomba (Donovan et al., 1991) whereas the corresponding values for Mangochi were 0.065 and 0.155 mg kg^{-1} respectively (Eick et al., 2009). These results confirm that Se concentration in the edible yield component is dependent on bioavailable soil Se levels and soil properties, even in legumes.

Table 1.3. Estimated mean selenium intakes of adults in various countries (adapted from Reilly, 2006).

Country	**μg d^{-1}**	**Reference**
Algiers	130	Djamel Tahtat *et al.* , 2003
Australia	55-87	McOrist and Fardy, 1989
Bangladesh	63-122	Beeri and Ahmed, 1976
Belgium	30	Amiard *et al.* , 1993
Burundi	17	Benemariya *et al.* , 1993
Canada	98-224	Giessel-Nielsen, 1998
China (Keshan areas)	<11	Combs and Coms, 1986
China (Seleniferous	750-4990	Yang *et al.* , 1989
China (urban)	53-80	Zhang *et al.* , 1989
Finland (post-1984)	67-110	Korpela *et al.,* 1989
Finland (pre-1984)	25-60	Westermarck, 1977
France	29-43	Ducros *et al* ., 1997
Germany	38-47	Oster and Prellwitz, 1989
India	28-105	Dang *et al.* , 2001
Ireland	44	Murphy *et al.* , 2002
Japan	104-127	Yoshita *et al.* , 1998
Malawi, Mangochi	44-46	Eick *et al.* , 2009
Malawi, Zomba	15-21	Donovan *et al.* , 1992
Mexico	61-73	Valentine *et al.* , 1994
New Zealand	19-80	Thomson and Robinson, 1993
Poland	30-40	Wasowkz *et al.* , 2003
Russia	54-80	Aro and Alfthan, 1998
Serbia	30	Djujic *et al.* , 1995
Slovakia	27-43	Kadrabova *et al.* , 1998
Turkey	18-53	Aras *et al.* , 2001
United Kingdom	32-58	Food Standards Agency, 2009
USA	60-220	Longnecker *et al.* , 2001
Venezuela	200-350	Combs and Combs, 1986

In Zomba, Malawi, blood plasma Se was reported <0.89 μmol L^{-1}, the critical value below which deficiency occurs (Fairweather-Tait et al., 2011). The observation that 73-95% of the population of Malawi had blood plasma Se concentrations of 0.5-0.7 μmol L^{-1} demonstrates that Se deficiency is widespread (Van Lettow *et al.,* 2003, Van Lettow *et al.,* 2004).

Maize is one of the World's most important cereal crops and provides an estimated 15% of its protein and 20% of its calories. It is a dietary staple for over 200 million people and its importance is expected to grow as the

World's population approaches 8 billion by 2025 (Nuss and Tanumihardjo, 2010). In Malawi, the main food groups contributing to Se intake are cereals, fish, legumes, groundnut, fruit and vegetables (Donovan et al., 1991, Donovan et al., 1992). In Burundi, the majority of the rural population who could afford only staple food, including maize and vegetables, had a mean Se intake of 17 μg d^{-1} (Benemariya et al., 1993), while a study in Zomba, Malawi reported that *c*. 43% of 4-6 year old children had an Se intake of ≤20 μg d^{-1}. The four main dietary sources of Se were cereals (35-60%), fish (20-27%), legumes (10-28%) and fruit and vegetables (5-11%; Donovan *et al.*, 1992).

Maize grain is the dominant staple food in Malawi where *c*. 52% of the mean total dietary calorie intake of 2172 kcal $person^{-1}$ d^{-1},is derived from this single source (FAO, 2011, 2007 data); this equates to 0.354 kg maize grain person d^{-1}. Maize is therefore a critical factor in determining Se intake in the average Malawian diet despite being relatively low in terms of Se concentration according to local food composition tables. For example, whole-grain maize flour contained 25 μg Se kg^{-1} in Zomba District (Donovan *et al.,* 1991) and 49 μg Se kg^{-1} in Mangochi District (Eick, 2007). Se intake from maize would therefore be 8.9 and 17.4 μg d^{-1} respectively in Zomba and Mangochi .

Table 1.4. Selenium concentrations in serum or plasma of healthy adults in different various countries (µg Se L^{-1}) (Source: Reilly, 2006; Fairweather-Tait *et al.,* 2011).

Country	**Se concentration (µg L^{-1})**	**Reference**
Australia	67±24	Tiran *et al.,* 1992
Austria	91±12	McOrist and Fardy, 1989
Canada	146±27	Vezina *et al.,* 1996
China (urban) Keshan areas Seleniferous areas	80±10 21±6 494±140	Whanger *et al.,* 1994
England	88±21	Thulurath and Vath, 1992
Finland (Pre 1984) Finland (Post 1984)	66±11 110±8	Westermarck, 1977 Korpela *et al.,* 1989
France	83±4	Ducros *et al.,* 1997
Germany	86±13	Meissner, 1997
Ireland	94±14	Darling *et al.,* 1992
Italy	87±17	Casaril *et al.,* 1995
Japan	117±16	Matsuda *et al.,* 1997
The Netherlands	69±6	Vander Torre *et al.,* 1991
Malawi	**58±18**	**Van Lettow *et al.,* 2005**
New Zealand	53±6	Thomson and Robinson, 1993
Norway	119±16	Mertzer and Haurg, 1995
Spain	94±3	Ferrer *et al.,* 1999
Turkey	71±2	Ozata *et al.,* 1999
USA (Eastern) USA (Central)	113±15 133±15	Salvini *et al.,* 1995 Smith *et al.,* 2000
Zaire	82±3	Vanderpas *et al.,* 1993

1.9 Agronomic biofortification

1.9.1 Introduction

Biofortification is defined as increasing the bio-available concentrations of essential elements in the edible portions of crop plants (Graham et al., 2001, Bouis, 2003, Lyons et al., 2003). The potential for using Se-enriched fertilisers to increase crop Se concentrations and dietary intake in the United Kingdom has been proposed previously (Adams et al., 2002, Rayman, 2002, Arthur, 2003, Broadley et al., 2006) and the application of Se fertiliser to pastures and forages has been demonstrated (Gissel-Nielsen, 1998, Gupta and Gupta, 2002). The best example of biofortification of food crops with Se for human consumption using fertilisers comes from Finland. As a consequence of the nationwide low dietary Se intake and its potential health consequences, the Finnish Ministry of Agriculture and Forestry decided in 1983 that Se would be incorporated into all multi-nutrient fertilisers used for crop production from 1 July 1984 onwards (Ylaranta, 1984, Varo et al., 1988, Eurola et al., 1989, Eurola et al., 1991, Aro et al., 1995, Rayman, 2002, Eurola et al., 2004).

The primary aim of the Finnish policy was to achieve a 10-fold increase in Se concentration within cereal grain (Eurola, 2005). For grain production and horticulture, 16 mg Se kg^{-1} was added to multi-nutrient fertiliser formulations, whilst 6 mg Se kg^{-1} was added for fodder crops and hay production. Following initial applications of Se, a new directive came into force from 16 June 1990, when fertilisers containing 16 mg Se kg^{-1} were removed from the market and a single supplementation level of 6 mg Se kg^{-1} was adopted (Eurola, 2005). In 1998, Se supplementation was increased to 10 mg Se kg^{-1} fertiliser for all crops. The effect of adding Se to fertiliser for crops in Finland was to increase Se concentration in 125 indigenous food items; most notably, the Se concentration of wheat bread was increased 10-fold from 0.03 to 0.35 mg Se kg^{-1} DW (Eurola et al., 1991). Finland has now achieved sufficient Se levels and the addition of selenate to NPK fertilisers has proved an effective and safe method to increase the selenium

status of the entire population (Eurola, 2005, Aro et al., 1995). Other countries such as Norway applied 6.5 g Se ha^{-1} using calcium nitrate enriched with 25 mg Se kg^{-1} as top dressing for spring wheat, thereby increasing Se concentration in wheat grain to the desired level (Tveitnes et al., 1995). In Australia, applications ranging from 4 to 120 g Se ha^{-1} have been used to increase grain Se concentration progressively by up to 133-fold when sprayed onto soil at the time of sowing, and by up to 20-fold when applied after flowering (Lyons *et al.,* 2005). In the United Kingdom, application of Na_2SeO_4 solution as a single, high volume drench significantly increased Se concentration in wheat grain and straw for all four sites examined; in this study, Se concentration was increased by 0.0167 mg kg^{-1} DW for straw and 0.026 mg kg^{-1} DW for grain for each g Se ha^{-1} applied (Broadley et al., 2010). However, selenium fertilisation appears not to increase yield in many crops, although some researchers have reported increases (Hartikainen, 2005). As grain Se concentration is determined primarily by the concentration of bio-available Se in the soil and there is limited genotypic variation in grain Se concentration, breeding approaches may not be worthwhile in attempts to biofortify the edible portion of crop species (Lyons *et al.,* 2005).

Early studies demonstrated that selenate (Na_2SeO_4 or K_2SeO_4) is more available for immediate uptake by pasture crops than selenite (Gissel-Nielsen, 1998). However, in the years following Se application, selenite and/or less soluble forms of selenate ($BaSeO_4$) provided more persistent effects (Gissel-Nielsen, 1998, Gupta and Gupta, 2002, Broadley et al., 2006) and application of 3-5 g Se ha^{-1} yr^{-1} as slow release Selcote provided an adequate Se supply (Gupta and Gupta, 2002). Application of 5 or 10 g Se ha^{-1} yr^{-1} increased Se concentration in first cut of livestock pasture crops from 0.067 to 0.187 and 0.220 mg kg^{-1} respectively (Gupta and Gupta, 2002). Addition of 10 g Se ha^{-1} was necessary to ensure that the Se concentration of barley grain was adequate in a study carried out in Canada (Gupta, 1995). Selenium concentration in wheat grain consistently increased with increasing rates of Se-enrichment of calcium nitrate (CN) or NPK (Tveitnes et al., 1995), although the superiority of Se-enriched CN over NPK in increasing grain Se concentration depended on both location and

growth conditions (Tveitnes et al., 1995). At the same rate, both methods of Se-application were equally effective in raising the Se concentration of wheat grains. Calcium nitrate enriched with 25 mg Se kg^{-1} (6.45 g Se ha^{-1}) increased Se concentration in wheat grain to the desired level (Tveitnes et al., 1995), while application of Na_2SeO_4 solution as a single, high volume drench significantly increased Se concentration in wheat grain and straw for all four sites examined (Broadley et al., 2010).

Genotypic variation in grain Se concentration is limited as this trait is determined primarily by available soil Se, suggesting that breeding approaches may be ineffective (Lyons *et al.,* 2005). Agronomic biofortification is likely to be the most feasible method to increase Se status in most situations as it represents a food systems approach that can deliver increased selenium to whole populations safely, effectively, efficiently and in the most suitable chemical forms (Welch and Graham, 1999). Selenium is available in a range of widely used commercial fertilisers (Broadley et al., 2006), although these workers concluded that field experiments using different cropping systems and climatic conditions are vital to establish the optimum rate of Se fertilisation.

1.9.2 Recovery of applied selenium by plants

Several studies have shown that recovery of Se by field crops is <10% because the applied selenate is converted to selenite, which is easily adsorbed by iron oxides and hydroxides in acidic soils (Cary et al., 1967, Geering et al., 1968, Christensen et al., 1989, Balistrieri and Chao, 1990). Extractable Se has given high recovery of applied Se of >91% with recovery of selenite ranging from 18.5-46.1% (Stroud et al., 2010) but Se recovery by crops has remained low. The proportion of applied Se recovered in grain varied with Se application rate, timing of application, method of application and crop yield (Curtin et al., 2006). When Se was applied at top dressing Se recoveries of 20% was attained while Se applied as seed coat treatment, the Se recovery was less than 5% of applied Se (Curtin et al., 2008). Grain recovery of approximately 5% of the applied Se by dryland crops yielding 4-

5 t ha^{-1} has been reported, leaving the great majority of the applied Se in the soil or inedible plant components (Stephen et al., 1989). Uptake of selenate-Se by crops declines within weeks of application, even when large amounts are applied, and uptake by cereals in the second year was minimal (Gissel-Nielsen and Bisbjerg, 1970, Mikkelsen *et al.,* 1989). Laing et al. (2009) found that 80-95% and 94-98% of the Se applied annually was not taken up by grass and maize respectively, whereas a total recovery (grain and straw) of 20-35% was reported for wheat although a split application of Se was slightly less effective (Broadley et al., 2010).

Selenium uptake by plants differs between crop types, as cereals accumulate less than brassicas and legumes (Bisbjerg and Gissel-Nielsen, 1969), and varies with soil type, Se application rate or soil Se concentration and the method of application (Davies and Watkinson, 1966b, Bisbjerg and Gissel-Nielsen, 1969, Ylaranta, 1984). Grant, 1965 reported that Se recovery by plants applied as selenite was 1 to 2 % following application and 65% of the added selenite was adsorbed to soil colloids while the remaining 30% was unaccounted for (Davies and Watkinson, 1966). In Finland, <10% of the applied Se was taken up by the crop (Eurola, 2005), but other workers have reported recovery values of 18% (Lyons et al., 2004). The method of application apparently affects the efficiency of Se recovery, as some studies have shown that foliar application is several times more efficient than application in the form of conventional fertilisers (Aspila, 2005, Curtin et al., 2006). Lyons *et al.* (2004) reported that foliar applications were less efficient than application to the soil.

1.9.3 The fate of applied selenium in the soil-plant system

Chemical extraction cannot distinguish which soil Se pools is plant-available or determine whether plants can access non-labile soil Se fractions. Similarly, computation of Se recovery from field experiments involving fertiliser applications cannot accurately determine the proportion of Se within plants that came from applied fertilisers and that which was already present within the soil (Goodson et al., 2003). Stable isotopes provide a

powerful tool for determining nutrient uptake from various sources and elucidating the processes which influence the uptake efficiency of applied fertiliser and its fate (Zapata and Hera, 1995). The use of stable isotopes in research is preferred because the use of radioactive isotopes is often limited by their long biological half life or their high-energy emissions and the associated issues of radiation exposure (Janghorbani, 1981). Stable Se isotopes have been used in studies of human metabolism (Janghorbani et al., 1981, Young et al., 1982, Finley, 1995, González Iglesias et al., 2007). The use of stable isotopes to study nutrient uptake from different sources is possible because plants do not discriminate between applied and pre-existing soil nutrients during their uptake (Hera, 1995). The use of stable isotopes as a label offered the potential of safely studying the bioavailability of minerals (Weaver, 1985). As noted previously, agronomic biofortification of food crops with selenium has been adopted successfully in Finland, although the behaviour and cycling of added selenate in the soil-plant system, the efficiency of Se uptake by plants, and the proportion removed from soil are still unknown (Keskinen et al., 2009).

1.9.4 Effect of grain processing on Se concentration

A field study of wheat in the United Kingdom showed that the Se concentration of wheat flour ranged from 30 ng g^{-1} in white flour and 35 ng g^{-1} in wholemeal flour produced from grain from untreated plots to >1800 ng g^{-1} in white flour and >2200 ng g^{-1} in wholemeal flour from grain from plants receiving selenium at the highest application rate of 100 g ha^{-1} (Hart et al., 2011). Moreover, the relationship between the quantity of Se applied and the Se content of flour and bread was approximately linear, indicating minimal loss of Se during grain processing and bread production (Hart et al., 2011). It was reported that Se and S are more evenly distributed throughout the grain of wheat than other mineral nutrients and a smaller proportion is removed in the milling residue. Post-milling processing therefore did not affect the Se concentration of wheat products (Lyons et al., 2005a), and there was no evidence of significant Se loss after bread baking using either low or high Se flour (Garvin et al., 2011). These findings

could be of great benefit as contamination of food systems by mycotoxins is a major problem in many countries in Eastern and Southern Africa (Garvin et al., 2011). Infection of maize grain with mycotoxins is common under field conditions (Garvin et al., 2011) and processing was considered desirable to reduce their concentrations in grain, although there were fears that processing might lead to losses of nutrients (Hotz and Gibson, 2007). Samples of sorghum grain and malt, the traditional opaque sweet beverage (thobwa), and beer prepared from sorghum malt were all found to be contaminated with aflatoxins (Matumba et al., 2011). Processing of cereals can have a major impact on their HT-2 and T-2 toxin concentrations; for example, de-hulling reduced the mycotoxin content of oats by >90% (Edwards et al., 2009). Processing generally has significant effects on the levels of mycotoxins in the final products and the removal of bran in cereals consistently reduced deoxynivalenol levels (Garvin et al., 2011).

1.10 The need to address low Se intake in Malawi

As a developing country, Malawi needs to implement effective agricultural programmes to mitigate the impact of HIV/AIDS, cancer, asthma and other diseases. The prevalence of diseases such as HIV/AIDS throughout sub-Sharan Africa (SSA) adversely affects productivity and food security. Increases in the frequency and severity of illness and mortality and reduced productivity resulting from malnutrition have exacerbated poverty and continue to retard economic growth and development. Good nutrition is a precondition for human and economic development. The immediate cause of malnutrition in SSA is inadequate dietary intake resulting from a combination of underlying factors such as insecurity over household food supplies and deficiencies in the food produced locally for essential mineral elements including selenium (Bowie, 2006).

Selenium deficiency in much of Sub-Saharan Africa has been postulated to be an important factor influencing the rapid spread of HIV/AIDS (Foster, 2003). HIV-1 has spread more rapidly in SSA countries such as Uganda, Kenya, Tanzania and South Africa than in Senegal, where the infection rate

has remained at 2% or less. A contributory factor to this contrast is that soils in Senegal are rich in calcium phosphate derived from selenium-enriched phosphorite; they are therefore rich in selenium, with the result that the food chain provides a consistently elevated supply of selenium which is highly protective against both cancer and HIV-1 infection (Foster, 2003). The prevalence HIV-1 has reached 25.8% in Zimbabwe, 25.1% in Botswana, 19.1% in Zambia, 12.9% in South Africa, 10.1% in Côte d'Ivore, 9.4% in Tanzania, 9.3% in Ethiopia and 4.4% in the Democratic Republic of Congo, but is only 1.8% in Senegal (Foster, 2000). It was also reported that HIV-positive Se-deficient women are more likely to infect their sexual partners than HIV-positive females with high Se levels; inadequate dietary Se also accelerates the rate at which HIV-1 sero-positive individuals progress to AIDS (Baeten et al., 2001, Foster, 2003). It therefore seems likely that food chains which consistently provide a sufficient supply of Se may be highly protective against both cancer and HIV-1 (Foster, 2000).

The AIDS pandemic may become the greatest human health catastrophe in history unless a safe and effective vaccine is developed quickly or preventive strategies are widely applied (Foster, 2002). The HIV-1 virus depletes the host of selenium and spreads rapidly in highly Se-deficient populations, particularly in Sub-Saharan Africa (SSA) where soil Se concentrations are naturally low (Foster, 2003). Malawi has recently experienced an increase in diseases which were previously uncommon, including diabetes, asthma, heart disease and cancer. The severe impact of HIV and AIDS in Malawi is creating great challenges which require urgent mitigation programmes (National AIDS Commission, 2009). The national HIV prevalence rate among adults is about 11.8%, ranking Malawi as one of the most AIDS-affected countries in the World (World Bank, 2007). Although Se concentrations in soils and food are generally low, there is likely to be significant geographical variation in soil Se concentration and hence in the food crops grown in different areas. Such differences might lead to substantial variation in Se intake within a country where the diet is strongly maize-based and there is currently no adequate information concerning soil and maize grain Se concentrations and Se intake. This is a

major deficiency considering the importance of Se in human nutrition and its impact on the incidence of major human diseases.

Agronomic biofortification of food crops with selenium, and potentially other vital trace elements, has been shown to be desirable (Adams et al., 2002, Rayman, 2002, Arthur, 2003) and has already resulted in Finland achieving safe and sufficient selenium levels in the human diet (Eurola, 2005). Biofortification has been described as an effective and safe method to increase the selenium status of the entire population of countries where rigorous programmes are implemented (Aro et al., 1995). Agronomic biofortification is likely to be the most feasible method for increasing selenium status in most circumstances as it provides a food systems approach that can deliver increased dietary selenium to the entire population safely, effectively, efficiently and in the most suitable chemical forms (Welch and Graham, 1999). As in Finland, an agronomic biofortification strategy is politically feasible in Malawi because the Malawian government implements a subsidised fertiliser distribution programme to benefit smallholder farmers. Maize was chosen as the focus for the present study as it is the main staple food crop in Malawi and many other countries in SSA and is vital in determining food security and dietary supplies of Se in such countries. Detailed studies were carried out to establish the extent of variation in Se concentrations in soil and maize grain throughout Malawi and the impact of fertilising maize crops with Se on dietary intake.

1.10.1 Aims and objectives

1.10.2 Aims

The aims were to explore the potential for safe and efficient biofortification of a staple Malawian food crop, maize, with selenium in order to increase Se intake.

1.10.3 Objectives

The specific objectives were to:

1. determine soil and maize grain Se concentrations across the country and local variation in dietary intake of Se
2. examine the effect of Se application on maize yield and Se concentration in maize grain and stover
3. determine the effect of different sources of selenium, application rates and times and split applications on maize yield and Se concentration in maize grain and stover
4. establish the efficiency of Se recovery from soil by maize
5. study the fate and residual effect of Se applied to maize crops using ^{74}Se and sodium selenate
6. determine the effect of processing on Se concentration in maize flour
7. assess the contribution of a maize-based diet to supplies of other nutrients (Ca, Mg, K, Mn, Fe Cu, Cr, Cd, Mo and Zn) to the Malawian population

CHAPTER 2: Materials and Methods

2.1 Introduction

This Chapter describes the experimental approaches and analytical methods used to (i) investigate the background Se concentrations in soils and maize in Malawi (Chapter 3), (ii) undertake Se biofortification studies and evaluate the effect of maize flour processing on Se concentration (Chapter 4), (iii) determine the fate of applied and residual applied Se (Chapter 5) and (iv) determine the concentrations of Ca, Mg, K, Fe, Zn, Cu, Mo, Cr, Co, Cd and Ni in maize grain (Chapter 6).

2.2 Assessment of soil and maize grain selenium concentration

2.2.1 Site selection

A non-structured sampling approach was adopted based on the National Extension Systems (NES) in Malawi to provide a representative spatial coverage of maize grain and soil from the major maize producing areas of the country. The NES comprises Agricultural Development Divisions (ADDs; n=8), Districts (n=28), Extension Planning Areas (EPAs; n=195) and Sections (n=~2,300). The first round of sampling was undertaken in May 2009 at 73 sites within 27 EPAs, representing seven of the eight ADDs. Six of these sites were under standard maize cultivation at research stations in the Blantyre, Lilongwe, Machinga, Mzuzu, Salima and Shire Valley ADDs. The remaining 67 sites were selected as being representative of farmers' fields. Based on the high maize grain Se concentrations from a single site in Shire Valley ADD (Mikalango EPA) sampled in 2009, a further 15 farms were sampled from the Shire Valley ADD in 2010 within three EPAs (Dolo, Mangoti and Mikalango). The locations of the sample sites are shown in Figure 2.1.

2.2.2 Sampling of soil and maize grain

For each of the selected 1-2 ha fields, eight maize cobs and the corresponding soil from the top of planting ridges were sampled and pooled to produce composite samples. A "W" transect was used across the field, with samples being collected at the two basal and two top corners and the four mid-points on transect. Whole cobs were harvested when ripe at approximately 20% moisture content and taken to Chitedze Research Station, where they were shelled and oven-dried at 66 °C to 13% moisture. 500 g samples of dried maize grain were ground using a Christy and Norris Lab Mill (Christy Turner Ltd, Ipswich, UK). Multi-elemental analysis was carried out to determine the concentrations of Se and Ca, Mg, K, Cu, Mn, Zn, Co, Fe, Mo, Cd, Cr and Ni. Soil samples were collected using an auger to a depth of 0.15 m from the 1-2 ha fields selected for study, again using a "W" transect to obtain eight samples from each field. The soil samples from individual fields were placed on clean plastic sheets, mixed thoroughly before taking a 500 g sub-sample of the composite sample. The composite samples from each field were placed in plastic bags and transported to Chitedze Research Station where they were air-dried and sieved to 2 mm before being stored in carefully labelled paper bags. A 20 g sub-sample of each sample was packed in a small plastic bottle (30 mL) and dispatched to the University of Nottingham for analysis. Multi-elemental analysis was carried out to determine the concentrations of Se and Ca, Mg, K, Cu, Mn, Zn, Co, Fe, Mo, Cd, Cr and Ni (Section 2.1.3). Other soil parameters such as soil reaction in water (pH), organic matter content and soil texture were also determined at Chitedze Research Station (Section 2.1.5).

2.2.3 Selenium analysis

2.2.3.1 Plant Analysis

For Se analysis, 400 mg of milled grain was digested under microwave heating for 45 min, at a controlled pressure of 2.0 MPa, in 3.0 mL of 70% trace analysis grade (TAG) HNO_3, 2.0 mL H_2O_2 and 3.0 mL milli-Q water (Fisher Scientific UK Ltd, Loughborough, Leicestershire, UK). Digested samples were diluted to 20 mL with milli-Q water (18.2 MΩ cm) and stored

at room temperature pending elemental analysis. Immediately prior to analysis, samples were diluted 1-in-10 with milli-Q water. Selenium analysis (as ^{78}Se) was undertaken using inductively coupled plasma-mass spectrometry (ICP-MS; X-Series^{II}, Thermo Fisher Scientific Inc., Waltham, MA, USA).

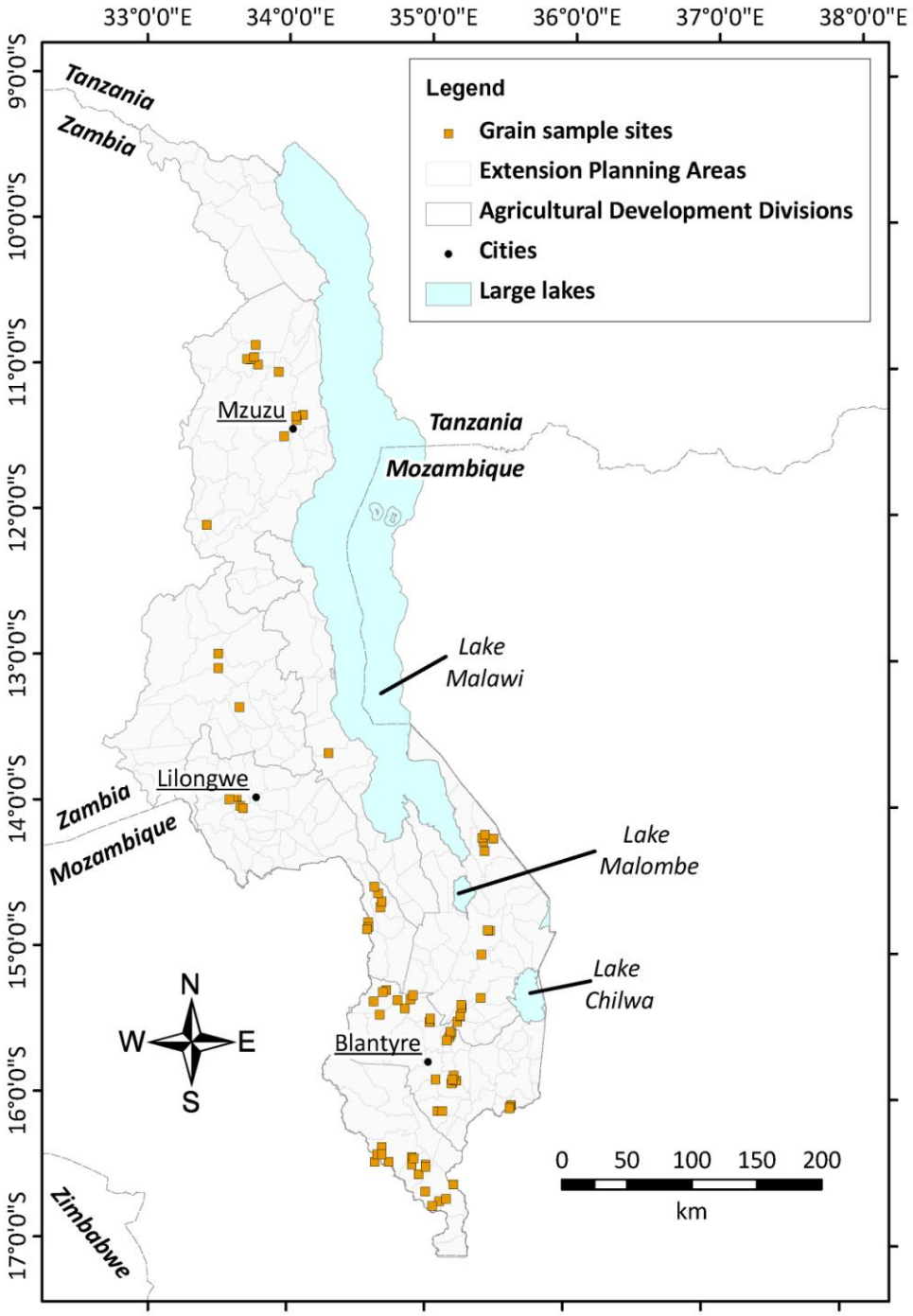

Figure 2.1. Location of sampling sites in farmers' fields in Malawi (Produced using ArcGIS v. 9.3).

The instrument was used in 'collision-reaction cell with kinetic energy discrimination (CCT-KED)' mode using a hydrogen reaction cell to remove polyatomic interference and enhance ^{78}Se transfer to the analytical quadrupole. Samples were introduced from a covered auto sampler (Cetac ASX-520 with 4 x 60-place sample racks) through a concentric glass venturi nebuliser (Thermo-Fisher Scientific; 1 mL min^{-1}) and Peltier-cooled (3 ^{o}C) glass mixing chamber. Internal standards introduced to the sample stream *via* a T-piece included Sc (100 µg L^{-1}), Ge (50 µg L^{-1}), Rh (20 µg L^{-1}) and Ir (10 µg L^{-1}) in 2% TAG HNO_3 and 4 % methanol; typically only Ge and Rh were used, in combination, to correct Se signals. Methanol increases sensitivity to Se, possibly by a charge-transfer reaction in which C^+ ions in the plasma enhance ionisation of the Se atoms. A single element Se stock solution (Claritas-PPT grade 1000 ppm Se, Certiprep/Fisher) was used to make external Se calibration standards, typically 0, 5.0 and 10.0 µg L^{-1}.

The ICP-MS was also used in CCT-KED mode for all other elemental analyses, but with a 7% H_2 in He gas mix in the hexapole collision cell. The internal standard solution was as described for Se determination but all four elements (Sc, Ge, Rh and Ir) were used selectively for drift correction and methanol was omitted from the solution matrix. Multi-element calibration standards (Claritas-PPT grade CLMS-2, Certiprep/Fisher) included Al, As, Ba, Bi, Ca, Cd, Co, Cr, Cs, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Rb, Se, Sr, U, V and Zn, all in the preferred range of 0-100 µg L^{-1}. A bespoke standard solution (Claritas-PPT grade CLMS-2, Certiprep/Fisher) containing 1000 ppm Ca, Mg, K and Na was used to produce a second set of calibration standards for the major cations with concentrations of 10, 20 and 30 mg L^{-1}.

All sample processing was undertaken using Plasmalab software (version 2.5.4; Thermo-Fisher Scientific) employing internal cross-calibration where required to convert 'analogue' detector responses to equivalent 'pulse-counting' values. For each digestion batch (48 samples) data were corrected using two blank digestions and quality control was assessed using reference material from the National Institute of Standards and Technology. Quality control was confirmed using a series of certified reference materials, principally NIST 1567a wheat flour (NIST, Gaithersburg, MD, USA).

All elemental concentrations were converted to mg kg^{-1} dry weight (DW) using Equation 2.1.

$$C_{plant} = \frac{(C_{sol} - C_{blank}) \times Vol}{W_{plant}} \quad \text{(Eq. 2.1)}$$

where C_{plant} is the elemental concentration (mg kg^{-1}) in the plant, C_{sol} and C_{blank} are the concentrations (µg L^{-1}) in the plant and blank digests, corrected for dilution, Vol is the digest volume (20 mL) and W_{plant} is the mass of plant digested (400 mg).

2.2.3.2 Total soil Se analysis

All soil samples were air-dried and sieved to <2 mm. Approximately 200 mg of soil was digested in PFA digestion vessels with 70% hydrofluoric acid, nitric acid and perchloric acid (Trace Element Grade (TEG); Fisher Scientific, UK) using a 48-place teflon-coated graphite Block Digester (Analysco, UK). A 2 mL aliquot of nitric acid (TAG) and 1 mL perchloric acid were added to 200 mg soil sample and heated 80 °C for 8 h and 100 °C for 2 h in the block digester overnight. A 2.5 mL aliquot of hydrofluoric acid was then added before heating at 120 °C for 1 h, 140 °C for 3 h and 160 °C for 4 h until a dry residue formed. Finally 2.5 mL nitric acid and 2.5 mL mill-Q water were added and the sample was left at 50 °C for 1 h to re-dissolve the digested residue. The solution was cooled and the digested samples were diluted to 50 mL using milli-Q water (18.2 MΩ cm), without filtration and stored un-refrigerated in 'universal' sample bottles (5% HNO_3) pending elemental analysis. All digests were diluted by 1-in-10 with milli-Q water immediately prior to analysis. Multi-element analysis of diluted soil digestions was undertaken by ICPMS (Thermo-Fisher Scientific X-Series^{II}) in CCT-KED mode with 7% H_2 in He as the collision-reaction cell gas, as described for plant analysis. The data for each digestion batch (48 samples) were corrected using two blank digestions and quality control was assessed using two samples of a reference material from the National Institute of Standards and Technology (NIST 1646a, estuarine sediment).

All elemental concentrations were converted to mg kg^{-1} DW using Equation 2.2.

$$C_{soil} = \frac{(C_{sol} - C_{blank}) \times Vol}{W_{soil}} \quad \text{(Eq. 2.2)}$$

where C_{soil} is the elemental concentration (mg kg^{-1}) in the soil, C_{sol} and C_{blank} are the concentrations (µg L^{-1}) in the soil and blank digests, corrected for dilution, Vol is the digest volume (50 mL) and W_{soil} is the mass of soil digested (*c*. 200 mg).

2.2.3.3 Extractable soil selenium

The phosphate extraction method proposed by Zhao and McGrath (1994) for S and subsequently adopted for Se was used to determine 'extractable' or 'available' Se (Stroud et al., 2010). A 30 mL aliquot of KH_2PO_4 (0.016 M, pH 4.8) was added to 10 g of dry soil, shaken for 1 h, centrifuged for 20 min at 2200 rpm and filtered through 0.22 µm syringe into ICP tubes. The samples were diluted 1:10 prior to analysis by ICP-MS as described previously. Data were corrected using two blank solutions; currently no certified reference materials exist for phosphate-extractable Se in soil.

2.2.4 Quality control

Quality of analysis was assured by running ICP-MS performance checks during each analytical run, determining methodological limits of detection, using replicate analyses, generating procedural blanks and analysing certified reference materials, mainly from the National Institute of Standards and Technology (NIST 1646a, estuarine sediments for soil and NIST 1567a, wheat flour for plant material).

Table 2.1. Measured and certified Se concentrations in soil reference material (estuarine sediments; NIST 1646a).

Soil reference material codes	Measured Se concentration (mg kg^{-1})	Certified Se concentration (mg kg^{-1})
NIST 1646a	0.2073	0.193
NIST 1646a	0.1890	0.193
NIST 1646a	0.1891	0.193
NIST 1646a	0.1953	0.193

All data of multi-element analyses were within 20% of the certified values and half the elements listed were within 15% of certified values (Table 2.2) and this is acceptable considering the range of elements being determined simultaneously and the low concentrations being presented to the instrument in the case of the trace metals. NFA, 2009 guidelines indicate that beyond ±20% is potentially unacceptable in pesticide analysis (http://ec,europa.eu/food/plant/protection/resources/qualcontrol_en.pdf and Nordval protocol No. 2, 2010 gave recovery % of 40–120 with concentration of 0.001 mg kg^{-1} to be acceptable (http://www.nmkl.org/Nordval/Nordval%20protocolNo2.pdf).

Table 2.2. Measured and certified elemental concentrations in wheat flour reference material (wheat flour; NIST 1567a).

Nutrient Element	Mg	K	Ca	Mn	Fe	Co	Cu	Zn	Mo	Cd
Measured concentration (mg kg^{-1})	327	1163	173	7.75	11.25	0.006	1.72	10.73	0.43	0.02
Certified concentration (mg kg^{-1})	400	1330	191	9.40	14.10	0.006	2.10	11.60	0.48	0.03
Recovery %	82	87	91	82	80	100	82	84	90	77

Table 2.3. Wheat flour reference material codes and measured and accredited Se concentrations.

Sample batch number	Wheat flour reference material used during both seasons	Measured Se concentration (mg kg^{-1})	Certified Se concentration (mg kg^{-1})
1	07/13943F	0.029	0.032
2	NIST 1567a	1.074	1.100
3	07/13941F	0.030	0.027
4	07/13946F	0.318	0.316
5	07/13948F	1.794	1.625
6	07/13948F	1.778	1.625
7	07/13948F	1.622	1.625
8	07/13943F	0.030	0.032
9	07/13943F	0.029	0.032
10	NIST 1567a	1.057	1.100
11	NIST 1567a	1.110	1.100
12	07/13946F	0.320	0.316
13	07/13946F	0.368	0.316
14	07/13946F	0.299	0.316
15	07/13946F	0.285	0.316

The measured and certified Se concentrations were closely correlated (R^2=0.9976; Fig. 2.2).

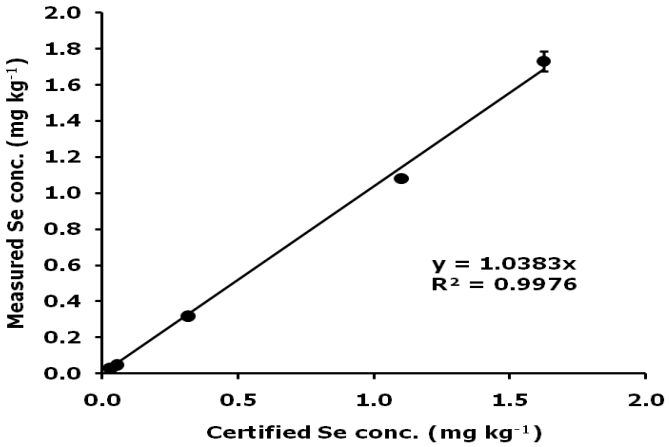

Figure 2.2. Relationship between measured and certified Se concentrations for several certified materials.

The effect of storage of digested maize flour at room temperature on measured Se concentrations was evaluated by re-analysing samples originally digested and analysed in 2009 during the following year, 2010

(Fig. 2.3). The slope of the relationship and R^2 value were 0.98 and 1.00 respectively, suggesting that storage of digested samples in sealed universal tubes at room temperature produces minimal changes in sample composition.

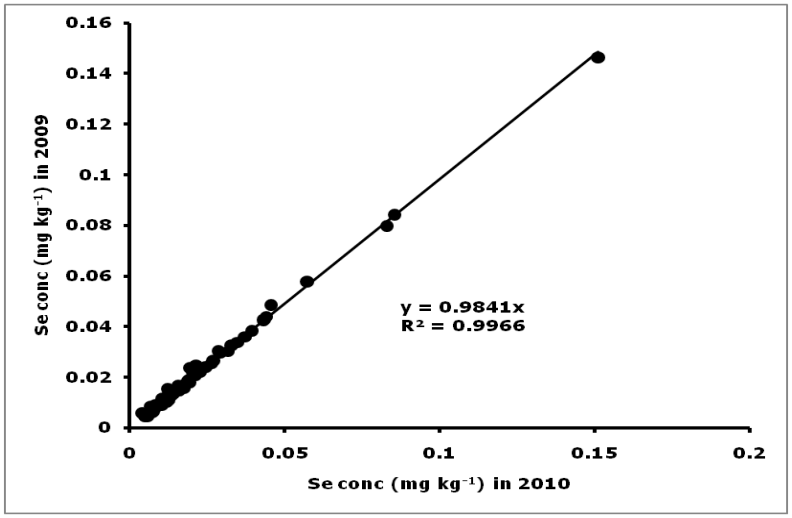

Figure 2.3. The effect of storage of digested samples on measured Se concentration.

Blank digest assays were aggregated across all individual analytical runs for determination of Se in grain flour samples; the 'figures of merit' are shown in Table 2.4.

Table 2.4. Figures of merit for Se analysis by ICP-MS, determined from aggregated blank digest samples and assuming digestion of 0.4 g flour, uptake in 15 mL solution and a further 1-in-10 dilution prior to analysis.

METHOD DETECTION LIMITS	mg kg^{-1}
Standard deviation of method blank concentration	0.00122
Limit of detection (LOD)	0.00384
Limit of quantification (LOQ)	0.01223
Practical quantification limit (PQL)	0.01920

LOD = 3.14 x σ_{blank}

LOQ = 10 x σ_{blank}

PQL = c. 5 x LOD (MDL)

2.2.5 Analyses of other soil properties

2.2.5.1 Soil mechanical analysis (soil texture)

Mechanical analysis of soils was undertaken using the method proposed by Gee and Bauder (1986). Samples (50 g) of air-dry <2 mm sieved soil were saturated with distilled water and 10 mL of 10% Calgon solution (sodium hexametaphosphate) in 400 mL plastic beakers. Suspensions were allowed to stand for 10 min, mixed for 2 min with a high speed stirrer and quantitatively transferred, with rinsing, into graduated cylinders. A hydrometer was inserted into the suspension and water added to provide a volume of 1130 mL; the initial hygrometer reading was then determined. After removing the hydrometer, the cylinders were sealed with tight-fitting rubber bungs and mixed by inverting them carefully ten times. Amyl alcohol (2-3 drops) was added to suppress frothing and, after 20 s, the hydrometer was gently replaced into the suspension. Temperature and suspension density were measured after 40 s and 2 h to determine sand and clay content respectively; silt content was determined by difference (Gee and Bauder, 1986).

2.2.5.2 Soil pH

Soil pH was determined in suspensions of 20 g of soil in 50 mL distilled water after shaking for 1 min on a mechanical shaker; this process was repeated three times during a 30 min period. The pH meter was calibrated against buffer solutions with pH values of 4.0, 7.0 and 9.0 pH values were recorded to the nearest 0.5 unit as soon as the reading was stable.

2.2.5.3 Total soil organic carbon

Soil organic carbon (SOC) was determined using the Walkley-Black Method (Walkley and Black, 1934). Finely ground soil samples (*c*. 1.0 g) were suspended in 10 mL of 1 N potassium dichromate solution and 15 mL concentrated sulphuric acid, shaken for 1 min and allowed to stand for 30 min. The suspensions were diluted with 150 mL distilled water and 5 mL concentrated phosphoric acid and allowed to cool. After addition of 1 mL diphenylamine indicator, the suspensions were immediately titrated against 0.5 N ferrous ammonium sulphate to determine unreacted dichromate, signified by a colour change from deep blue to dark green.

2.3 Agronomic biofortification of maize with selenium

2.3.1 Overview

Three sets of field experiments were conducted in Malawi in both 2008/09 and 2009/10 to determine the response of maize to three different forms of selenate-Se containing fertiliser. These were were (1) a liquid drench of $Na_2SeO_{4(aq)}$ (41.8% Se, Sigma-Aldrich Company Ltd, Dorset, UK), (2) compound granular fertiliser containing NPK+Se, representing a 25:5:5+Na product marketed under the trade name Top Stock® (Yara UK, Immingham, UK) which contains 0.0015% Se (w/w) in the form of Na_2SeO_4, and (3) calcium ammonium nitrate (CAN+Se; Yara) containing 0.005% Se (w/w), also in the form of Na_2SeO_4.

2.3.2 Site and crop selection, cultivation and experimental design

In both years, fields were selected at research stations of the Malawi Ministry of Agriculture and Food Security (MoAFS) at Bvumbwe, Chitala, Chitedze, Makoka, Mbawa and Ngabu (Table 2.5). All sites were rain-fed. However, as crop failure occurred at Ngabu in 2009/10 due to lack of rain, a late-sown replacement site under irrigation was selected at nearby Kasinthula, within the same Shire Valley Agricultural Development Division (ADD). Soils at all sites were Luvisols except for the Shire Valley ADD sites, which were Eutric Vertisols. Experiments with $Na_2SeO_{4(aq)}$ and NPK+Se were conducted at six sites in 2008/09 and 2009/10 using *Zea mays* L. var. SC627 (a local hybrid). Experiments with CAN+Se were conducted at a subset of three sites in each year (Bvumbwe, Chitedze and Ngabu/Kasinthula) using two varieties; *Z. mays* L. var. SC627, and ZM623 (an open pollinated variety).

At each site, the soil was ploughed to 30 cm depth and subsequently harrowed. Ridges 30 cm in height were prepared at 75 cm spacing. Shortly after first rainfall two maize seeds were sown on the top of each ridge with 25 cm spacing. Each experimental plot comprised four ridges 5 m in length.

The two outer ridges and three terminal maize plants in each ridge were regarded as guard rows, giving a net plot size of two ridges by 4 m in length. After approximately two weeks, plants were thinned to leave one plant at each planting station and basal NPK fertiliser was applied. All plots were weeded twice during the growing season and the crops were harvested after the grain had ripened and dried in the field; the plants separated into cobs and stover.

A randomised block design was adopted in which each site, Se fertiliser type and year was considered as a discrete experimental unit. For experiments involving $Na_2SeO_{4(aq)}$ and NPK+Se, there were four replicates per treatment, except at Bvumbwe in 2009/10 where three replicates per treatment were used due to space constraints. For experiments involving CAN+Se, there were three replicates per treatment at all sites. All data analyses were conducted in GenStat (V.13.3.0.5165, VSN International, Hemel Hempstead, UK).

2.3.3 Fertiliser applications

In the $Na_2SeO_{4(aq)}$ experiment, eight treatment levels of Se (0, 5, 10, 15, 25, 50, 75, 100 g ha^{-1}) were applied at six sites in each of two years, representing 376 plots in total. The $Na_2SeO_{4(aq)}$ was applied at early stem extension stage (~'knee high'; Table 2.6). To ensure even application to the crop, the $Na_2SeO_{4(aq)}$ was applied as a high-volume drench using a knapsack sprayer, with the operator wearing personal protective equipment of overalls, boots, face-shield and nitrile gloves (Broadley *et al.,* 2010). A 16 L Berthoud Vermorel 2000Pro knapsack tank (Exel GSA, Villefanche-sur-Saône, France) was connected to a 1 m boom, housing three Lurmark 110 °, flat-fan spray nozzles (Hypro EU Ltd, Longstanton, Cambridge, UK), spaced equally, with a spray-swath of 1.5 m. A coarse nozzle type "08 white" was used (1180 mL $nozzle^{-1}$ min^{-1}; British Crop Protection Council, 2001) to minimise potential aerosol drift. Ergonomically acceptable drench rates were calibrated to treat four replicate plots from a single tank at appropriate walking speed with two passes (833 L water ha^{-1}).

Table 2.5. Experimental sites characteristics.

Trial site	Location	Soil type[b]	Soil textural class[c]	Soil pH[d]	OM[e]	Total soil selenium	Topography	Rainfall
	(Lat., Long.)				%	(mg kg^{-1})[a]		(mm)
Bvumbwe (Dwale[f])	-15.92, 35.07	Chromic Luvisols	Sl	5.2	1.08	0.288	Medium altitude (Shire Highlands)	800-1500
Chitala (Chinguluwe)	-13.68, 34.28	Chromic Luvisols	Scl	5.6	2.38	0.362	Lakeshore plain (Salima Lakeshore)	500-1200
Chitedze (Chitsime)	-13.98, 33.63	Chromic Luvisols	Scl	5.9	2.03	0.300	Medium altitude (Lilongwe Plain)	800-1000
Makoka (Thondwe)	-15.52, 35.22	Chromic Luvisols	Scl	5.4	1.87	0.272	Medium altitude	800-1200
Mbawa (Mbawa)	-12.12, 33.42	Haplic Luvisols	Ls	5.7	1.86	0.124	Medium altitude (Southwest Mzimba Plain)	700-900
Ngabu (Mikalango)[g]	-16.60, 34.35	Vertisols	C	7.9	2.64	0.217	Low altitude(Shire Valley)	500-700
Kasinthula (Mitole)[h]	-16.05, 34.81	Vertisols	Sl	7.4	2.95	0.197	Low altitude(Shire Valley)	500-700

[a]total soil Se; [b]FAO classification (Green and Nanthambwe, (1992); [c]sl = sandy loam, scl = sandy clay loam, c = clay

[d]water; [e]organic matter; [f]Extension Planning Area (EPA) in parentheses; [g]2008/09 only; [h]2009/10 only (irrigated site).

Plots were treated in ascending order of target Se application rates. A basal application of N, P_2O_5 and K_2O (46, 20 and 10 kg ha^{-1} in total, respectively) was made to all plots using a 23:10:5 fertiliser (Yara UK) and a top dressing of urea (46 kg N ha^{-1}) was subsequently applied. Fertiliser granules were applied *via* calibrated cups to the base of individual plants using a hand-placement method.

For the NPK+Se experiment, five treatment levels of Se were used (0, 1.5, 3.0, 4.5 and 6.0 g Se ha^{-1}) at six sites in each of two years. A split Se treatment was included as an experimental sub-factor, giving nine NPK+Se treatments, and representing 423 plots in total. Splits represented basal:top applications of Se as follows: 0 g Se ha^{-1} (0:0), 1.5 g Se ha^{-1} (100:0, 0:100), 3 g Se ha^{-1} (100:0, 50:50, 0:100), 4.5 g Se ha^{-1} (25:75, 75:25) and 6 g Se ha^{-1} (50:50). Fertiliser granules were applied using the hand-placement method described previously. To ensure that the 50:50 basal:top split applications of NPK were identical for all plots, applications were balanced using Super Grass® (25:5:5; Yara UK), i.e. only the Se applications were split. In total, each plot received the equivalent of 100, 20 and 20 kg ha^{-1} of N, P_2O_5 and K_2O, respectively.

For the CAN+Se experiment, five treatment levels of Se were used (0, 5, 10, 15 and 20 g Se ha^{-1}) at three sites in each of two years using two varieties of maize, representing 180 plots in total. A basal application of N, P_2O_5 and K_2O (46, 20 and 10 kg ha^{-1} in total, respectively) was made to all plots using a 23:10:5+3S fertiliser (Yara UK). CAN+Se fertiliser was applied as a top dressing using the hand-placement method described previously. Nitrogen was balanced using calcium ammonium nitrate (CAN) without Se. In total, each plot received the equivalent of 130, 45, and 23 kg ha^{-1} of N, P_2O_5 and K_2O, respectively.

Table 2.6. Experimental timelines.

Trial Site	2008/09				2009/10			
	Sowing	N (basal)	N (top)	Harvest	Sowing	N (basal)	N (top)	harvest
Bvumbwe	11 Dec.	23 Dec.	14 Jan.	30 Apr.	25 Dec.	15 Jan.	15 Feb.	3 May.
Chitala	10 Dec.	27 Dec.	16 Jan.	28 Apr.	23 Dec.	3 Jan.	23 Jan.	26 Apr.
Chitedze	9 Dec.	30 Dec.	22 Jan.	4 May.	15 Dec.	1 Jan.	19 Jan.	27 Apr.
Makoka	9 Dec.	22 Dec.	15 Jan.	14 Apr.	15 Dec.	22 Dec.	23 Jan.	27 Apr.
Mbawa	10 Dec.	29 Dec.	27 Jan.	6 May.	21 Dec.	4 Jan.	21 Jan.	29 Apr.
Ngabu	11 Dec.	24 Dec.	14 Jan.	15 Apr.	Na	Na	Na	Na
Kasinthula	Na	na	na	Na	16 Feb.	25 Feb.	18 Mar.	6 Jun.

Ergonomically acceptable drench rates were calibrated to treat four replicate plots from a single tank at appropriate walking speed with two passes (833 L water ha^{-1}). Plots were treated in ascending order of target Se application rates. A basal application of N, P_2O_5 and K_2O (46, 20 and 10 kg ha^{-1} in total, respectively) was made to all plots using a 23:10:5+3S fertiliser (Yara UK) and a top dressing of urea at 46 kg N ha^{-1} was subsequently applied. Fertiliser granules were applied *via* calibrated cups to the base of individual plants, using a hand-placement method (Fig. 2.5).

Figure 2.4. Liquid Se application of sodium selenate as a source of Se at Mbawa in 2009.

Figure 2.5. Application of a basal dressing of granular selenium-enriched fertiliser NPK+Se (Topstock) at Makoka in 2009.

2.3.4 Maize grain Se analysis

Milled grain (*c*. 0.4 g dry weight, DW) was digested under microwave heating for 45 min at a controlled pressure of 2.0 MPa in 3.0 mL of 70% trace analysis grade (TAG) HNO_3, 2.0 mL H_2O_2 and 3.0 mL milli-Q water (Fisher Scientific UK Ltd, Loughborough, Leicestershire, UK). The microwave system comprised a Multiwave 3000 platform with a 48-vessel 48MF50 rotor (Anton Paar GmbH, Graz, Austria). Samples were digested in vessels comprising perfluoroalkoxy (PFA) liner material and polyethylethylketone (PEEK) pressure jackets (Anton Paar GmbH). Digested samples were diluted to 15 mL (30% HNO_3) with milli-Q water (18.2 MΩ cm) and stored at room temperature pending elemental analysis. Immediately prior to analysis, samples were diluted 1-in-10 with milli-Q water. Selenium (^{78}Se) analysis was undertaken using ICP-MS (X-Series^{II}, Thermo Fisher Scientific Inc., Waltham, MA, USA) using a hydrogen reaction cell. Samples were introduced from a covered autosampler (Cetac ASX-520, Omaha, NE, USA) with 4 x 60-place sample racks, at 1 mL min^{-1} through a concentric glass venturi nebuliser and Peltier-cooled (3 °C) spray chamber (Thermo Fisher Scientific Inc.). Internal standards were introduced to the sample stream *via* a T-piece and included Sc (50 ng mL^{-1}), Rh (10 ng mL^{-1}) and Ir (5 ng mL^{-1}) in 2% TAG HNO_3. An external wheat flour standard (NIST 1567a; National Institute of Standards and Technology, Gaithersburg, MD, USA)

was used as reference material. Each digestion batch (n=48) included two blank digestions; final Se concentrations were converted to mg kg^{-1} DW.

2.3.5 Soil analyses (total Se, KH_2PO_4-extractable Se and other soil properties)

For total Se analyses, sieved soil (~0.2 g DW) was fully digested in 70% HF, 70% HNO_3 and 60% $HClO_4$ (TAG; Fisher Scientific UK Ltd), using PFA digestion vessels and a 48-place teflon-coated teflon-coated graphite block digestor (Model A3, Analysco Ltd, Chipping Norton, UK). Digested samples were diluted to 50 mL using milli-Q water and stored in 5% HNO_3 at room temperature in universal sample bottles pending elemental analysis. Most samples were analysed in triplicate and estuarine sediment (NIST 1646a, NIST) was used as an external standard reference material. For extractable Se analyses, the method of Zhao and McGrath (1994) was used. Triplicate samples of air-dried sieved soil (10 g) were shaken with 30 mL 0.016 M KH_2PO_4 (pH 4.8) in polycarbonate centrifuge tubes for 1 h. The soil suspensions were then centrifuged for 20 min at the speed of 2200 rpm, filtered to <0.22 µm using a Millex syringe driven filter unit (Millipore, Cork, Ireland) and stored at room temperature prior to analysis for Se by ICP-MS, as described previously. Other soil properties measured included soil pH in water, texture using the hydrometer method (Gee and Bauder, 1986), and organic carbon using an adapted chromic acid titration method (Walkley and Black, 1934).

2.3.6 Soil sampling

Soils from the 0-15 and 15-30 cm horizons at all field sites were sampled at harvest using an auger. At each site, eight soil samples were collected from each replicate using "W" transects, and a composite sample was obtained using the quartering process whereby the soil samples were placed on clean plastic sheet, mixed thoroughly and reduced in size by removing one quarter of each sample after mixing until 500 g composite sample was obtained. The samples were placed in plastic bags and transported to

Chitedze Research Station where they were air-dried at room temperature. The air-dried soils were ground to pass a 2 mm sieve pending Se analysis.

2.3.7 Maize harvesting

The maize cobs in the two central ridges in each plot were harvested, shelled and weighed in the field to allow moisture content to be measured and 500 g samples were placed in paper bags. The stover was weighed and five plants from each plot were cut into small pieces and placed in paper bags. The samples were transported to Chitedze Research Station where they were oven-dried at 66 ^{o}C before being milled and taken to University of Nottingham for analysis.

2.4 Determining the fate of applied Se using ^{74}Se

Full details of the preparation of ^{74}Se and specific experimental approaches are given in Chapter 5. The experiment was conducted at Chitedze and Mbawa. The field experiment was laid out as randomised complete block design with four replicates. ^{74}Se isotope was applied at a single application rate of 10 g Se ha^{-1}. Plot size was 2 x 2 m, providing a net plot area of 1 x 1 m from which samples were collected and yield data recorded.

2.5 Effects of traditional maize processing on flour Se concentration

Samples of maize grain from the Liquid experiment described in Section 2.2.1 and six levels of Se application (0, 5, 10, 15, 25 and 50 g Se ha^{-1}) were selected. Approximately 4 kg of dry maize (13% moisture content) was placed in a de-huller before adding 500 mL of water and de-hulled for 30-60 min. Winnowing was done using flat baskets to remove the husk (bran) and obtain de-hulled grain. Three traditional maize processing methods were then applied to assess their effect on the Se content of the processed maize flour. These included: a) milled whole grain brown flour

known as Mgaiwa in Malawi; b) dehulled grain was milled to produce flour known locally as gramil and; c) dehulled grain was soaked in water for three days at room temperature, washed and air-dried to produce flour known locally as Ufa woyera.

2.6 Statistical Analysis

Genstat Release 12.1 (VSN International Limited, Hertfordshire, United Kingdom) was used to analyse the data. The results for grain and stover yield and Se concentration were analysed after checking the assumption of constant variance and normality using the model checking plots. None of the data required transformation or any other remedial measure prior to analysis.

CHAPTER 3: Nationwide survey of selenium concentrations in soil and maize grain in rural Malawi

3.1 Introduction

Selenium (Se) is an essential element for humans and livestock. A total of 25 selenoproteins have been identified in humans, including iodothyronine deiodinases, thioredoxin reductases, glutathione peroxidases, and a range of other selenoproteins (e.g. SelP, SelM, SelT; Fairweather-Tait *et al*., 2011). These proteins have critical roles in thyroid functioning, cell proliferation and survival through redox homeostasis, antioxidant defence and the immune response. When Se intake is suboptimal, the selenoprotein status of people decreases and there are increased risks of adverse health effects. At extremely low Se intake levels (where habitual intakes for adults are <20 μg Se d^{-1}), clinical deficiency disorders have been reported including Keshan disease (a cardiomyopathy) and Kashin-Beck disease (an osteoarthropathy). Where habitual intakes for adults are less than the levels needed for maximal expression of glutathione peroxidase, typically at least 40 μg Se d^{-1}, there is an increased risk of health disorders, including cardiovascular disorders, impaired immune functions, and some cancers (Fairweather-Tait et al., 2011). The relationships between Se intake, Se status in terms of selenoprotein expression and health outcomes have still to be fully resolved (Hurst et al., 2010, Goldson et al., 2011). These uncertainties are reflected in the wide range of Dietary Reference Intake (DRI) levels in different countries (Fairweather-Tait et al., 2011). Some DRIs are set to reduce risks of overt deficiency (i.e. recommending intakes of *c*. 40 μg Se d^{-1}), although most countries have recommended intake levels of 50-70 μg Se d^{-1}. As high habitual levels of Se intake are potentially toxic (>400-900 μg Se d^{-1}), care must be taken in setting DRIs and recommending dietary supplementation.

Selenium intake in human populations is derived primarily from dietary sources and can be determined from direct dietary analyses or surveys and

food composition tables. Reported Se intakes range from 3 to 7000 µg Se d^{-1} globally due to differing dietary preferences and the levels of plant-available Se in the soil on which crops are grown for consumption (Rayman, 2002, Fordyce, 2005, Rayman, 2008, Johnson et al., 2010, Fairweather-Tait et al., 2011). Populations in many European countries and elsewhere have intakes <50 µg Se d^{-1}, which are likely to be suboptimal in terms of selenoprotein expression (Hurst et al., 2010). Higher dietary Se intake levels (>150 µg Se d^{-1}) occur in Se-rich (seleniferous) environments (e.g. parts of China, India, North America and Venezuela) and where seafood-based diets containing high concentrations of Se are prevalent (e.g. notably in parts of Greenland and Japan). Selenium intake from water and air is usually insignificant, except where environmental Se concentrations are high due to natural or anthropogenic factors (Fordyce, 2005).

The extent of Se deficiency in human populations is unclear, although it is likely to be widespread in global terms and especially where food choices are narrow. For example, surveys of Se concentrations in rice grain show that Se intake is likely to be suboptimal in many populations reliant on a staple diet of rice (Williams et al., 2009). In Sub-Saharan Africa (SSA), Se intake levels are often very low in rural populations where fish consumption is low. Thus, in rural Burundi, intakes of 17 µg Se d^{-1} have been reported in adults (Benemariya et al., 1993). In southern Malawi, intakes of 15-21 µg Se d^{-1} have been reported among children living in rural areas of Zomba District (Donovan et al., 1992), consistent with low blood plasma Se concentrations (<55 µg L^{-1}) among adults in the same area (van Lettow et al., 2004, van Lettow et al., 2005). A substantial proportion of dietary Se intake in SSA has been attributed to fish consumption (Donovan et al., 1991, Donovan et al., 1992). Indeed, higher Se intakes (44-46 µg Se d^{-1}) have been reported in Mangochi District, adjacent to the southern end of Lake Malawi, where fish consumption is high (Eick et al., 2009). In Burundi, higher Se intakes have also been reported in middle-class men (82 µg Se d^{-1}) and mothers (38 µg Se d^{-1}) which have been linked to variation in fish consumption between groups (Benemariya et al., 1993).

In rural SSA, maize grain is the dominant staple food. In Malawi (mean energy intake 2172 kcal person^{-1} d^{-1}) and neighbouring Zambia (1873 kcal person^{-1} d^{-1}), *c*. 52% of total dietary calorie intake was derived from maize in 2007 (FAO, 2011). This equates to 0.354 and 0.315 kg person d^{-1} in Malawi and Zambia, respectively. Consumption of animal products from all sources (meat, offal, fats, milk and eggs) is typically low, accounting for 64 and 97 kcal person^{-1} d^{-1} in Malawi and Zambia, respectively, of which fish accounts for 9 and 11 kcal person^{-1} d^{-1}. Maize grain is therefore likely to be critical in determining Se intakes to the average SSA diet despite being low in terms of Se concentration according to local food composition tables. For example, in Malawi, whole-grain maize flour contained 25 μg Se kg^{-1} in Zomba District (Donovan et al., 1991) and 49 μg Se kg^{-1} in Mangochi District (Eick, 2007).

This study aimed to determine the contribution of maize grain to dietary Se intake in rural Malawi and establish whether maize grain Se concentration is dependent on soil Se concentration and/or other soil factors such as pH and organic matter content. Malawi was chosen because: (1) a large proportion of the population engages in subsistence farming and their diets are dominated by maize; (2) dietary Se intakes and Se status are likely to be low among rural populations (Donovan et al., 1991, Donovan et al., 1992, van Lettow et al., 2004, van Lettow et al., 2005); (3) there is a high national prevalence of immunological disorders (e.g. HIV/AIDS) and other morbidity symptoms (e.g. diarrhoea) which are associated with low micronutrient status (Kupka et al., 2004, Kupka et al., 2009, Fairweather-Tait et al., 2011); (4) the national government operates a national Farm Input Subsidy Programme (Dorward and Chirwa, 2011) which provides the opportunity to consider agronomic biofortification by incorporating trace quantities of Se in compound fertilisers. Such a strategy to alleviate suboptimal dietary Se intakes was adopted at a national scale in Finland in 1984 and is feasible in other contexts (Broadley et al., 2006, Broadley et al., 2010, White and Broadley, 2009).

3.2 Materials and Methods

3.3.1 Sourcing pre-existing data to estimate dietary Se intakes

Dietary Se intake in Malawi was estimated using data for national food consumption and Se concentration in dietary components. Food consumption data were sourced from the most recently published Food and Agriculture Organization (FAO) data for 2007 (FAO, 2011). Food Se concentration data for Malawi for those products contributing the majority of the national average diet were taken from Donovan *et al.* (1991) and Eick (2007) (Table 3.1). Donovan *et al.* (1991) measured Se concentrations in 37 food products collected in rural areas of Zomba District using neutron activation analyses. The results were reported on a fresh weight basis. Eick (2007) measured Se in 40 food products collected in Mangochi District, near Lake Malawi, using inductively coupled plasma-mass spectrometry (ICP-MS); these data were reported on both fresh and dry weight bases. Whilst both studies reported Se concentrations for fish, neither reported Se concentrations for other meat products. Therefore, Se concentration values for minced meat (beef, pork, lamb) and whole-meat (chicken) products from UK food composition tables (Food Standards Agency, 2002) were used.

3.2.2 Selecting sites for maize and soil sampling

A non-structured sampling strategy was adopted based on extension planning systems in Malawi. This approach provided representative spatial coverage of maize grain and soil types for the major crop production areas. The national extension system comprises Agricultural Development Divisions (ADDs; n=8), Districts (n=28), Extension Planning Areas (EPAs; n=195) and Sections (n=~2,300). The first round of sampling was undertaken in May 2009 at 73 sites within 27 EPAs, representing seven of the eight ADDs. Six of these sites were under standard maize cultivation at Research Stations in the Blantyre, Lilongwe, Machinga, Mzuzu, Salima and

Shire Valley ADDs. The remaining 67 sites were selected as farmers' fields which represented the major soil types used for maize production. Based on high concentrations in maize grain from a single site in Shire Valley ADD (Mikalango EPA) sampled in 2009, a further 15 field sites were sampled from the Shire Valley ADD in 2010 within three EPAs (Dolo, Magoti and Mikalango). The locations of sample sites are presented in Chapter 2, Figure 2.1.

3.2.3 Collecting maize grain and soil samples

For each of the selected fields (typically 1-2 ha), eight whole maize cobs and corresponding soils from the top of the planting ridges were sampled and pooled to produce composite samples of grain and soil. A 'W' transect was used across the field, with samples collected at four main corners and the four mid-points. Whole cobs were harvested when ripe and transported to Chitedze Research Station, where they were shelled, oven-dried to 13% moisture at 66 °C and milled. Soil was sampled to a depth of 0.15 m using a soil auger. Composite soil samples were transported to Chitedze Research Station where they were air-dried and sieved to ≤2 mm.

3.2.4 Plant and soil analysis

The procedures for plant analyses are described in Section 2.1.3.1, while those for determining total Se, KH_2PO_4-extractable Se and other soil properties) are described in Sections 2.1.3.2 and 2.1.3.3

3.2.5 Data integration

Soil types (Fig. 3.1a), District population distribution (Fig. 3.1b) and median grain Se of each 10 soil type (Fig. 3.2b) were used to compute median intake in each District (Table 3.2). Grain Se and soil type were Integrated to extrapolate grain Se in >75% of the land of Malawi.

3.3 Results

3.3.1 Estimating Se intake from dietary sources in Malawi using published data

National average food consumption patterns and published Se concentration data for food were used to estimate standardised baseline Se intakes for two districts in rural Malawi. Mean dietary Se intakes of 39.8 and 24.4 µg Se person^{-1} d^{-1} were estimated for Mangochi and Zomba Districts, respectively, with Se intake from all non-maize sources being 22.4 and 15.5 µg Se person^{-1} d^{-1}, respectively (Table 3.1). Thus, maize was the single major foodstuff contributing to dietary Se intake.

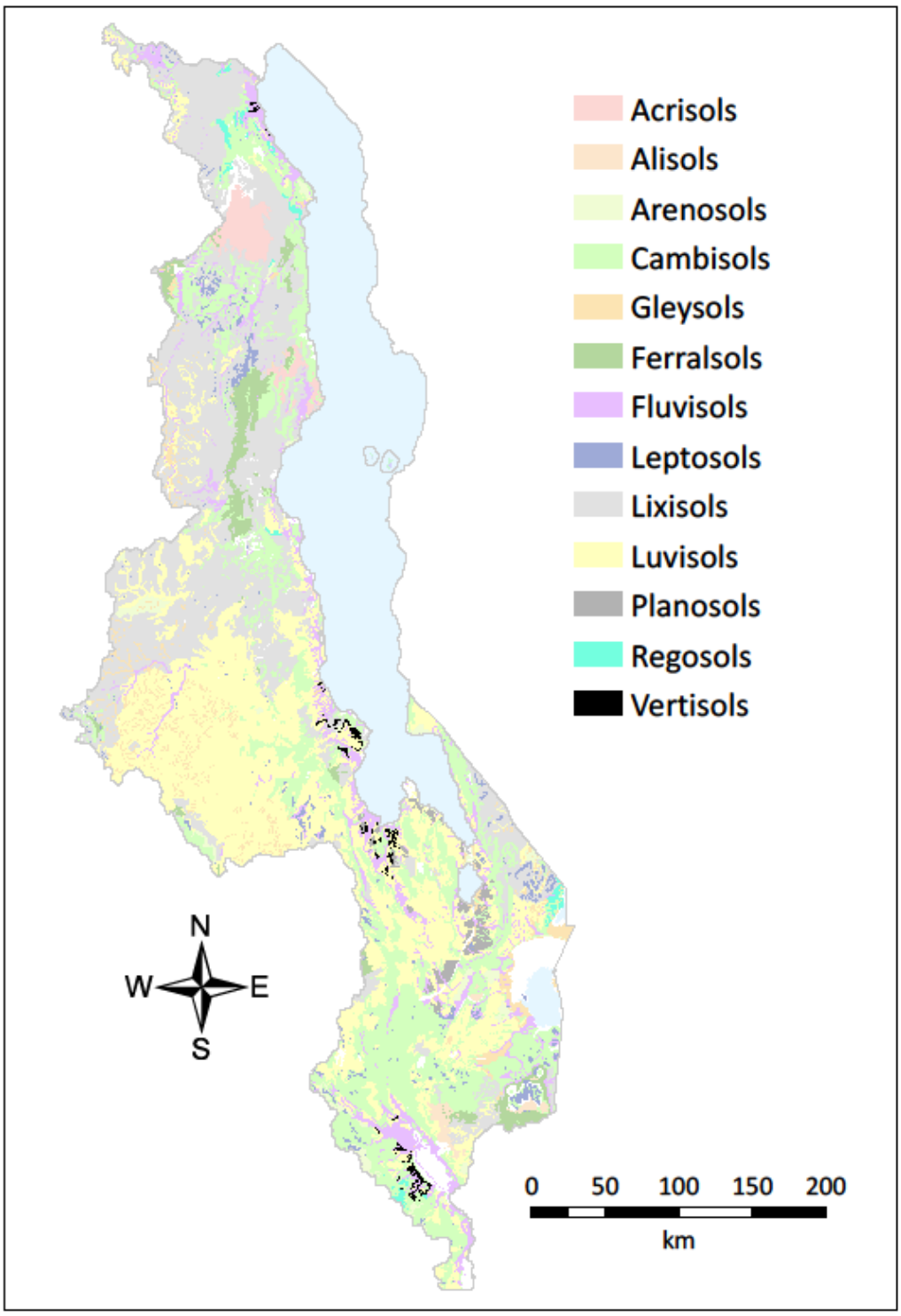

Figure 3.1a. Soil map of Malawi based on Green and Nanthambwe (1992); Figure produced using ArcGIS (v. 9.3, ESRI, Redlands, CA, USA).

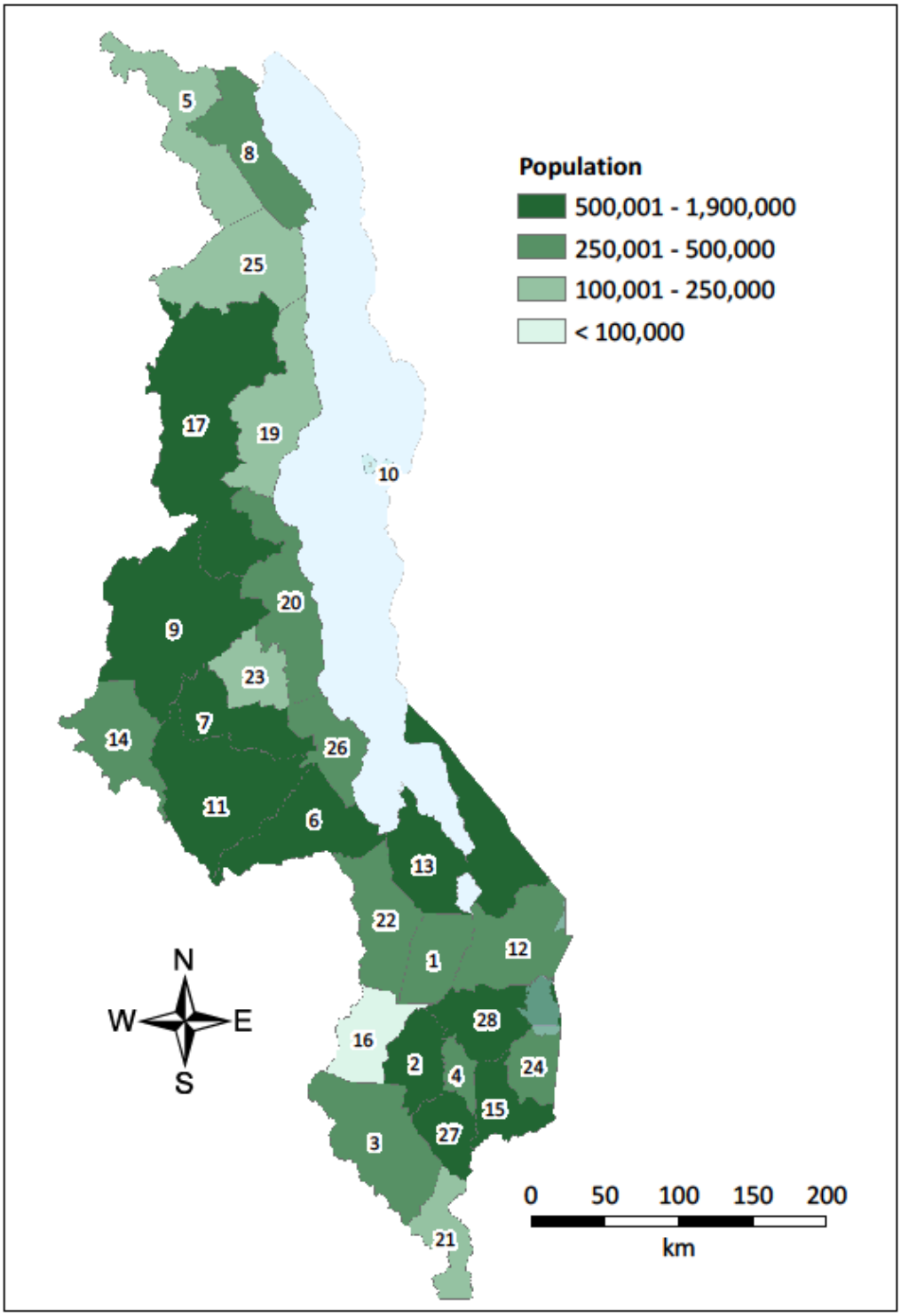

Figure 3.1b. Population distribution in Malawi at a district level; for numbering see Table 3.2 (NSO, 2008). Figure produced using ArcGIS (v. 9.3, ESRI, Redlands, CA, USA).

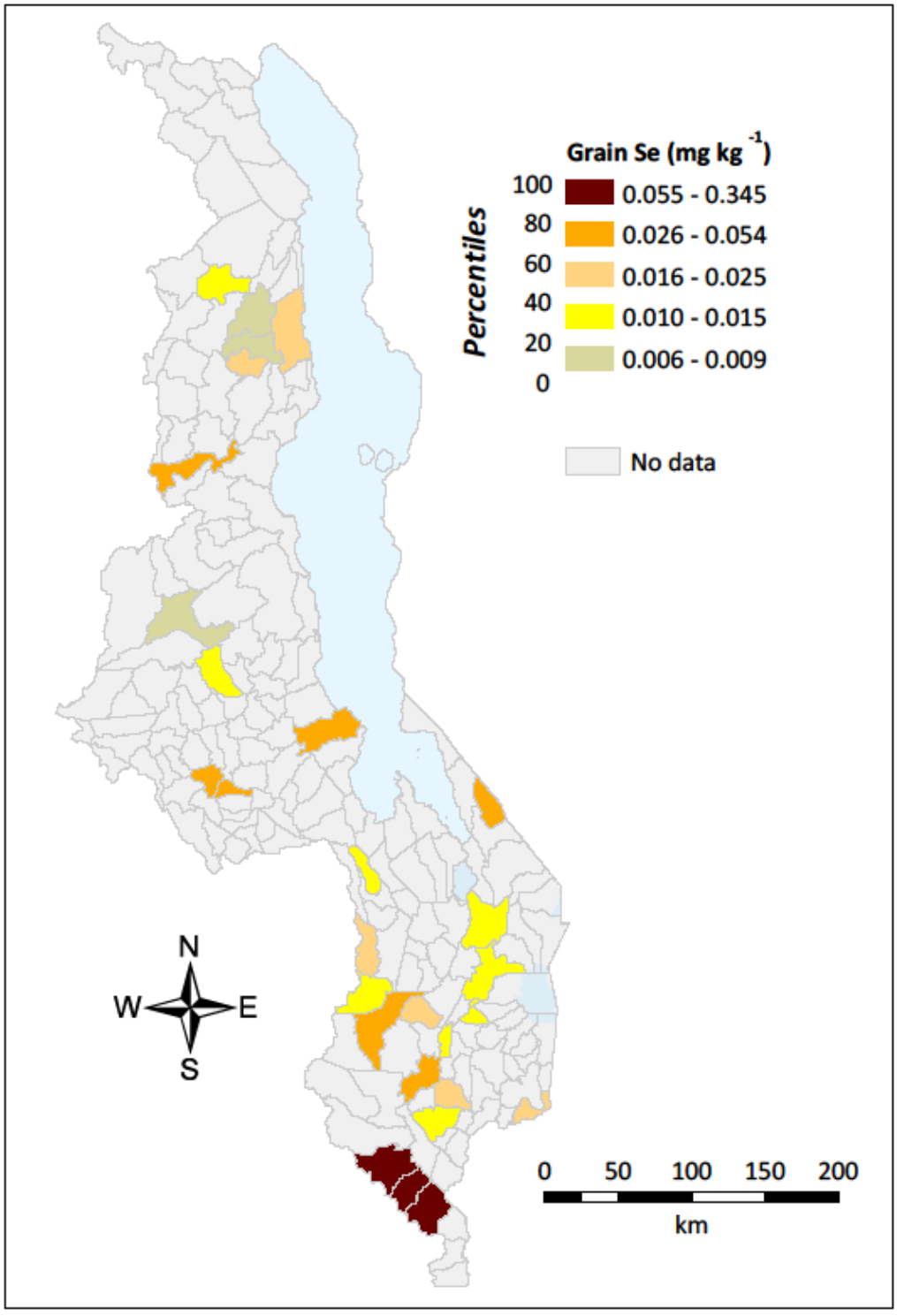

Figure 3.2a. Median grain Se concentrations shown on an Extension Planning Area (EPA) basis. Figure produced using ArcGIS (v. 9.3, ESRI, Redlands, CA, USA).

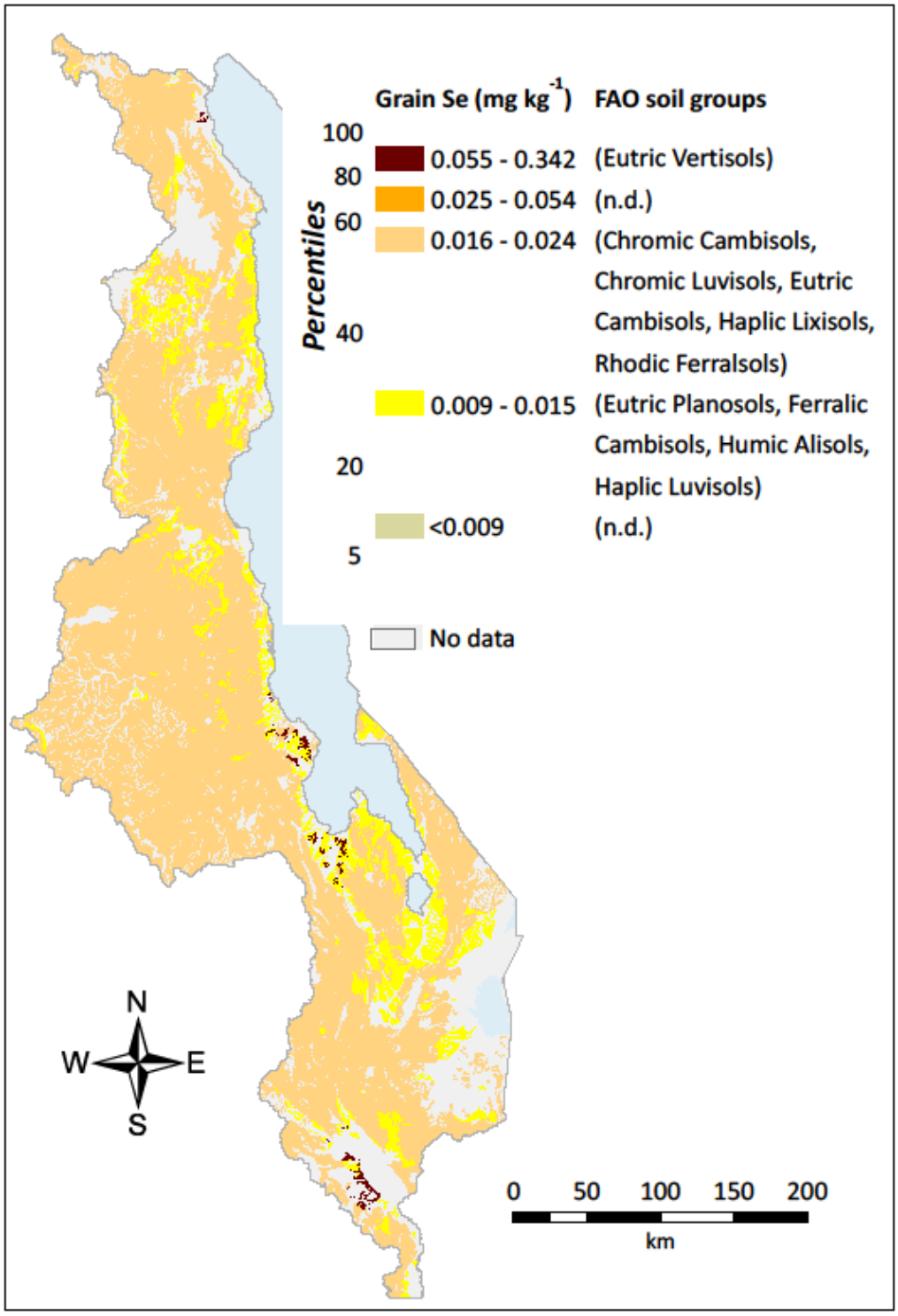

Figure 3.2b. Extrapolated grain Se concentration, based on median grain Se concentration for each soil type (two quintiles of grain Se concentration are not represented by median values for each soil type are indicated with n.d. in the legend). Figure produced using ArcGIS (v. 9.3, ESRI, Redlands, CA, USA).

Food consumption data are based on national *per capita* supplies, which will overestimate food intake due to wastage during storage, preparation and cooking (FAO, 2011); Se intakes are therefore likely to be lower than the estimated values above. The use of a single national metric for food consumption masks within-country variation in Se intake due to different food consumption patterns. For example, fish consumption is likely to be higher in Mangochi, near Lake Malawi, than in Zomba District.

A median Se intake of 45 µg person d^{-1} (inter-quartile range= 28-30) for Mangochi District was reported by Eick *et al.* (2009), based largely on the same food composition data, using dietary recall surveys and questionnaires. Adult Se intake was not reported in Zomba District (Donovan et al., 1992), but Se intake by children aged 4-6 was in the range 15 to 20 µg person d^{-1}. The link between fish consumption and dietary Se intake has previously been associated with higher income levels in Burundi (Benemariya et al., 1993). The greater dietary Se intake estimate at Mangochi than at Zomba in the present study reflects the differences in the Se concentration of edible crop portions reported by Donovan *et al.* (1991) and Eick (2007). For example, the Se concentration of whole-grain maize flour was 49 µg Se kg^{-1} in Mangochi compared to 25 µg Se kg^{-1} in Zomba. If food consumption patterns were identical in both Districts, maize would account for 46% and 36% respectively of dietary Se intake in Mangochi and Zomba. Moreover, the Se concentration of mango, banana, pigeonpea and kidney bean was *c*. 2 to 6-fold higher in Mangochi than in Zomba. Assuming there were no systematic differences in terms of sample collection, preparation or analysis between Donovan *et al.* (1991) and Eick (2007), these consistent differences in crop Se concentration between Districts are most likely to be due to soil factors rather than cultivar differences (Lyons et al., 2005a, Broadley et al., 2006, White and Broadley, 2009, Broadley et al., 2010). However, the combined Se intake from fruit, vegetables, other cereals and starchy staples was still less than that from maize in both Mangochi and Zomba. Selenium intake from animal sources other than fish is likely to be low, based on the limited contribution of these food sources to the typical Malawian diet. However, as there are gaps in Se concentration

data for these categories in local food composition tables, this conclusion requires validation.

3.3.2 Integration of data

Using the integration of the data of the District population distribution (Fig. 3.1b), soil types (Fig. 3.1a) and grain Se across soil types in each District, median Se intake was computed for each District (Table 3.2). The Median Se intake per person across Districts ranged from 5.2 to 16.6 μg d^{-1}. The highest intakes were from Salima and Chikwawa because of the presence of vertisols while most of the Districts were less than 10μg d^{-1}.

3.3.3 Variation in the Se concentration of maize grain in Malawi

To determine the wider contribution of maize grain to the dietary intake of Se in Malawi, samples of soil and grain were collected nationwide. In 2009, Se concentration in maize grain from 73 sites ranged from 0.0045 to 0.533 mg kg^{-1} with a median concentration of 0.016 mg kg^{-1}; over 70% of the samples had lower Se concentrations than those reported by Donovan *et al.* (1991). However, there was a disjunct distribution of grain Se concentrations as 69 samples contained <0.08 mg Se kg^{-1}, whereas a single sample from Lisungwi EPA contained 0.146 mg Se kg^{-1} and one sample from Mikalango EPA had 0.533 mg Se kg^{-1}. The sample from Mikalango was from a crop growing on a Eutric Vertisol (Green and Nanthambwe, 1992) with a pH of 7.9. Therefore, in 2010, a further 15 samples were collected from other Shire Valley Eutric Vertisol sites in the Mangoti, Dolo and Mikalango EPAs with soil pH values ranging from 6.97-8.02. In 2010, grain Se ranged from 0.173-0.413 mg Se kg^{-1} for 13 of the sites, although two sites in Mangoti had lower concentrations of 0.0054 mg Se kg^{-1}. Grain Se concentration data for all sites are shown in Figure 3.3a while mean grain Se concentrations expressed on an EPA basis are presented in ascending order in Figure 3.3b. Based on a mean *per capita* consumption of 0.354 kg d^{-1} and an overall median grain Se concentration of 0.019 mg Se kg^{-1} from

all 88 sites, the estimated median Se intake from maize was 6.7 μg person^{-1} d^{-1} although individual values ranged from 1.6 to 189 μg Se person^{-1} d^{-1}.

3.3.4 Soil factors affect maize grain Se concentration

Mean and median total soil Se concentrations were 0.1941 and 0.1623 mg kg^{-1}, respectively (Fig. 3.3c, d) and there was a *c*. 12-fold variation in values between 0.0521 to 0.6195 mg Se kg^{-1}. Mean and median KH_2PO_4-extractable soil Se concentrations were 0.0056 and 0.0046 mg Se kg^{-1}, respectively (Fig. 3.3e, f) and there was again a *c*. 12-fold variation in values between 0.0013 and 0.0158 mg kg^{-1}. There was no obvious link between grain and soil Se concentrations when data were expressed on a mean EPA basis (Fig. 3.3b, d, f). However, KH_2PO_4 extractable Se with grain Se seems to show positive relationship (Fig. 3.3b, f). Total soil Se was a poor indicator of Se availability and the critical value of total soil Se of 0.6 mg kg^{-1} below which supplies are regarded as being deficient (Gupta, 2010) requires revision as the results showed that low grain Se concentrations were obtained on soils which had a high total soil Se concentration (Fig. 3.3b, d). Multiple single regression analyses were therefore conducted between total and KH_2PO_4-extractable soil Se concentrations, soil pH and soil organic matter (Fig. 3.4). At pH >6.5, there was a strong correlation between grain Se concentration and soil pH. There were also weaker positive correlations between total soil Se, KH_2PO_4-extractable Se and soil organic matter and a weak correlation between KH_2PO_4-extractable, but not total, soil Se concentration and grain Se concentration.

Table 3.1. Average daily food supply and Se intake in two areas of Malawi, Mangochi and Zomba (based on Donovan *et al.,* 1991; Eick, 2007; FAO, 2011).

Foodstuff	Supply *per capita*[a]		Selenium concentrations reported in the literature (µg kg^{-1})				Se intake (µg $person^{-1}$ d^{-1})	
	(g d^{-1})	(kcal d^{-1})	Eick (2007)	Notes/sources	Donovan*et al.,* (1991)	Notes/sources	Mangochi	Zomba
Maize	354	1126	49	"whole-grain flour"	25	unrefined, unfermented	17.36	8.86
Potatoes	275	192	2		2	n.d., use Eick (2007)	0.55	0.55
Cassava	197	136	2	no data (n.d.), assume ≈	2	n.d., use Eick (2007)	0.39	0.39
Wheat	17	49	79		79	n.d., use Eick (2007)	1.34	1.34
Rice (milled equivalent)	12	44	10		24		0.12	0.30
Sorghum	6	19	84		12	Unmilled	0.48	0.74
Millet	6	18	84	n.d., assume ≈ sorghum	80	Milled	0.48	0.46
Sugar (Raw equivalent)	33	117	0	n.d., assume zero	0	n.d., assume zero	0.00	0.00
Beans	13	44	22	"kidney bean"	7	"kidney bean"	0.29	0.09
Pulses, other	23	81	15	"pigeonpea" (155),	60	"pigeonpea" (56), (64)	3.70	1.40
Groundnut (shelled)	13	66	94	dry average (74, 113)	94	n.d., use Eick (2007)	1.20	1.20
Vegetable oils	7	62	0	n.d., assume zero	0	n.d., use Eick (2007)	0.00	0.00
Tomatoes	7	1	9	fresh average (10, 8)	9	n.d., use Eick (2007)	0.06	0.06
Onions	10	4	33	"white" (11) & "red" (54)	33	n.d., use Eick (2007)	0.33	0.33
Vegetables, other	42	9	36	"ch. cbge" (2), "pumpkin	7	"*Amaranthus*, okra, c'sava lvs"	1.51	0.29
Bananas	67	40	9		5		0.60	0.33
Plantains	56	50	9	assume ≈ banana	5	assume ≈ banana	0.51	0.28
Fruits, other	46	20	27	"mango"	4	"mango" average	1.24	0.18
Beverages, fermented	41	14	4	"thobwa gruel"	4	n.d., use Eick (2007)	0.16	0.16
Bovine meat	5	10	70	n.d., beef "raw mince"	70	n.d., beef "raw mince"	0.35	0.35
Mutton & goat meat	4	5	20	n.d., lamb "raw	20	n.d., lamb "raw mince"	0.07	0.07
Pig meat	5	19	14	n.d., pork "raw mince"	14	n.d., pork "raw mince"	0.65	0.65
Poultry meat	3	4	13	n.d., chicken "meat only"	13	n.d., chicken "meat only"	0.39	0.39
Eggs	3	4	15	"chicken egg"	20	"duck egg"	0.05	0.06
Milk (excluding butter)	10	6	10	n.d., cow milk data	10	n.d., cow milk data	0.09	0.09
Fish & seafood	14	9	57	"smoked fish", "usipa"	42	"smoked chambo", usipa (108,	7.86	5.75
Sub-total[b]		2149				Average Se intake (all sources)	39.80	24.35
Grand total (all food sources)		2172				Average Se intake (excl. maize)	22.44	15.49

[a]*Per capita* supply in 2007 (FAO, 2011) reported as kcal (1 kcal=4.19 kJ). Data represent means, calculated from total supply available for consumption, divided by total population living within the national borders. Actual intake is likely to be lower due to losses during storage, preparation and cooking. Energy values based on typically consumed crop fractions. [b]Some minor food groups are excluded in this table, although 98.9% of energy intake is represented. Beef, lamb, pork raw mince and chicken meat (Food Standards Agency, 2002) and cow milk (Debski *et al.,* 1987).

Table 3.2 Median Se intake from maize at a District level in Malawi based on distribution of population and soil type, intergrated with median grain Se concentration from each soil type.

	District	Population[a] (k)	Area represented by FAO soil type (km^2)[b]											Total area (km^2)	Median Se intake from maize ($\mu g\ person^{-1}\ d^{-1}$)
			Chromic	Chromic	Eutric Cambisol	Eutric Planosol	Eutric Vertisols	Ferralic Cambisol	Haplic Lixisol	Haplic Luvisols	Humic Alisol	Rhodic Ferralsol	Other soils		
1	Balaka	317	-	520	326	265	-	-		773	-	-	272	2155	5.3
2	Blantyre	999	259	321	1150	11	-	-	72	4	-	-	200	2017	5.2
3	Chikwawa	439	-	283	2002	-	188	-	-	287	-	-	2123	4883	13.1
4	Chiradzulu	291	79	328	159	-	-	-	-	15	-	-	165	748	5.4
5	Chitipa	179	32	329	184	-	-	147	2566	21	-	-	970	4248	7.3
6	Dedza	624	-	2223	583	50	32	-	68	138	-	-	684	3777	6.8
7	Dowa	557	6	2296	180	-	-	72	48	37	-	-	228	2868	5.7
8	Karonga	273	55	59	871	-	25	14	1291	33	-	-	1094	3441	7.8
9	Kasungu	616	-	2141	21	-	-	111	5088	-	-	-	702	8064	7.2
10	Likoma	10	-	-	-	-	-	-	-	-	-	-	-	-	-
11	Lilongwe	1897	-	4750	630	-	-	3	271	-	-	-	500	6154	5.7
12	Machinga	489	41	147	513	242	-	-	252	555	-	-	2110	3860	5.5
13	Mangochi	804	-	579	1971	232	44	-	1361	1187	-	-	1037	6409	6.8
14	Mchinji	457	-	1461	33	-	-	68	1007	-	-	-	576	3145	6.5
15	Mulanje	525	18	35	88	-	-	-	223	17	112	299	1104	1895	6.3
16	Mwanza	94	89	755	1130	-	-	-	145	14	-	-	192	2326	5.5
17	Mzimba	853	101	553	83	-	-	1055	6574	214	-	347	1546	10474	7.2
18	Neno	109	-	-	-	-	-	-	-	-	-	-	-	-	-
19	Nkhata Bay	214	-	-	6	-	-	995	1926	-	-	668	759	4354	6.8
20	Nkhotakota	302	231	743	383	-	4	224	1668	391	-	83	591	4318	6.6
21	Nsanje	238	-	236	743	-	1	-	-	167	-	-	822	1969	5.3
22	Ntcheu	474	-	1304	973	37	47	-	87	235	-	-	562	3246	7.5
23	Ntchisi	224	-	1016	46	-	-	93	748	-	-	-	34	1938	6.5
24	Phalombe	313	8	-	205	-	-	-	51	-	9	-	1276	1549	5.5
25	Rumphi	169	39	-	83	-	-	994	1597	-	-	40	1830	4584	6.7
26	Salima	340	-	350	183	-	133	2	433	375	-	-	637	2113	16.6
27	Thyolo	587	371	399	174	-	-	-	212	-	323	116	75	1669	5.6
28	Zomba	671	16	719	306	21	-	-	-	337	41	-	1730	3170	5.5
-	residual	-	-	1	1	-	-	-	3	-	-	-	3	8	
	Totals	13065	1346	21546	13026	859	473	3778	25691	4801	486	1553	21821	95380	
Grain Se, ($mg\ kg^{-1}$)	**lower 25%**		0.009	0.009	0.009	-	0.254	0.009	0.009	0.009	-	0.006			
	median		0.014	0.016	0.014	0.010	0.342	0.014	0.022	0.016	0.013	0.018			
	upper 25%		0.030	0.028	0.030	-	0.356	0.030	0.042	0.028	-	0.021			

[a]based on NSO (2008); [b]based on Green and Nanthanbwe (1992)
[c]based on 354 g maize $person^{-1}\ d^{-1}$ (FAO, 2011); assumes equal population distribution within a district; excludes maize grain production on "other soils"

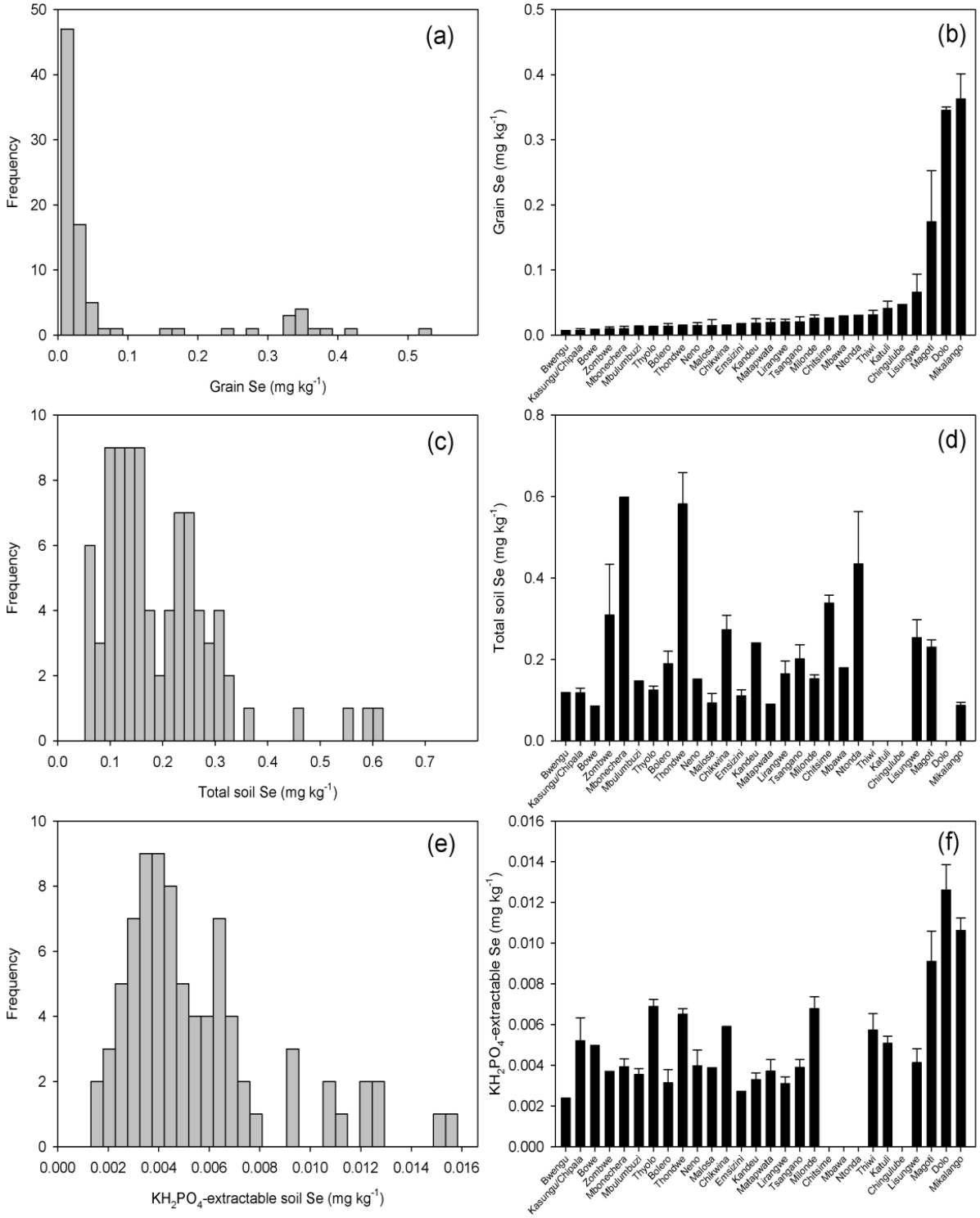

Figure 3.3. Concentrations of Se in maize grain (a,b) and soil (c-f) from farmers' fields surveys in Malawi. Soil Se is expressed as total Se (c,d) and KH_2PO_4-extractable (e,f) forms. Data are presented as frequency distributions (a,c,e) and on an Extension Planning Area (EPA) basis (mean ±s.e.m.).

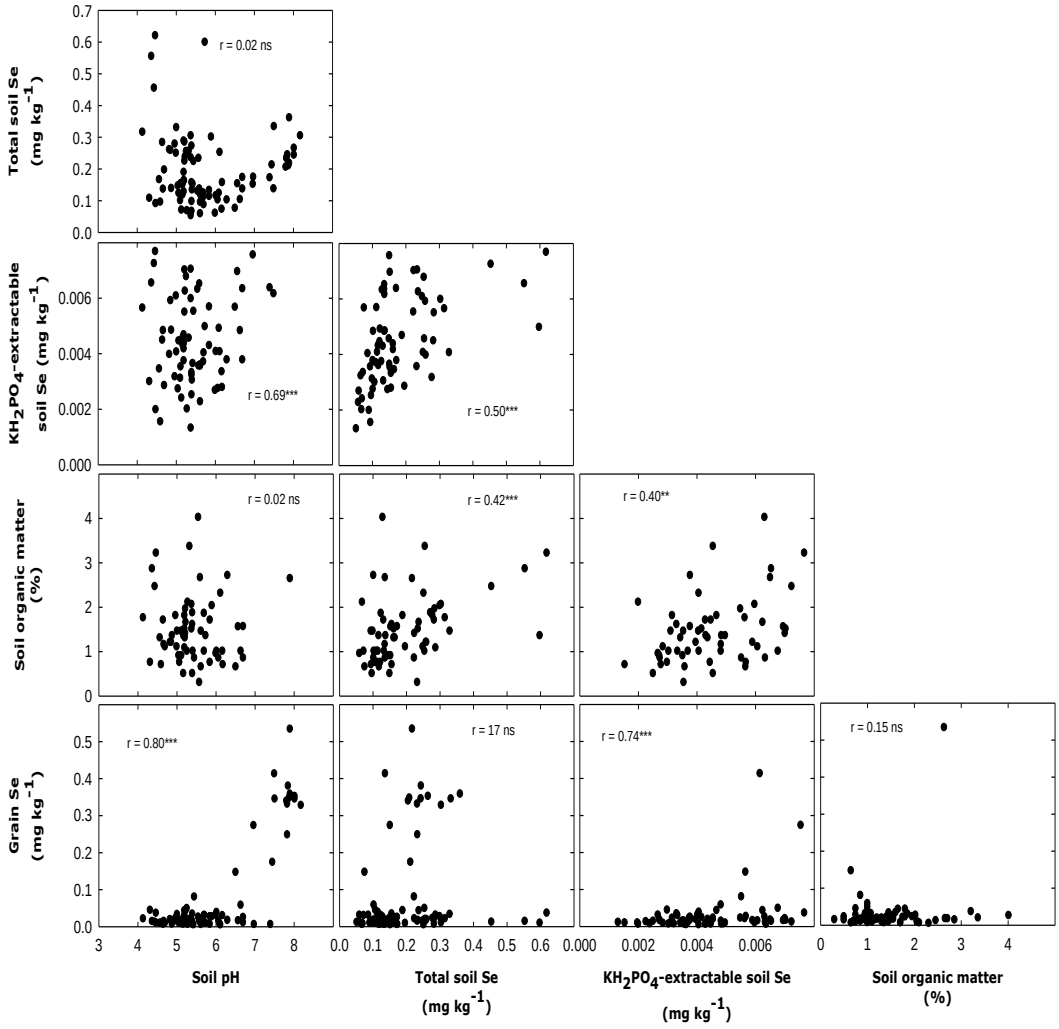

Figure 3.4. Relationships between soil Se (total and KH_2PO_4-extractable forms) and soil pH, soil organic matter and maize grain Se concentration from a survey of farmers' fields in Malawi. Correlation coefficients are inset (n.s. $P>0.05$; ** $P<0.01$; *** $P<0.001$).

3.4 Discussion

Selenium is an essential element in the human diet, although suboptimal intakes are likely where food choices are narrow. Here, we show that suboptimal dietary intake (i.e. 20-30 μg Se person^{-1} d^{-1}) is widespread in Malawi, based on a spatial integration of Se concentrations of maize grain and soil surveys collected from 88 field sites, representing 10 primary soil types and >75% of the national land area. The median maize grain Se concentration was 0.019 mg kg^{-1} (range 0.005-0.533), giving an intake of 6.7 μg Se person^{-1} d^{-1} from maize flour based on national consumption patterns. Maize grain Se concentration was up to 10-fold higher in crops grown on soils with naturally high pH (>6.5) (Eutric Vertisols). Under these alkaline conditions, Se becomes considerably more available to plants due to the greater solubility of $Se^{(IV)}$ species and oxidation to $Se^{(VI)}$.

Using previously published soil maps and demographic data, new data for maize grain Se concentration, and geographical information systems (GIS) based approaches, it can be shown that dietary Se deficiency is likely to be widespread in Malawi (Fig. 3.2b; Table 3.2). To obtain these results, the median grain Se concentration for each of the 10 soil types sampled was calculated (Table 3.2), and the area represented by each of the 10 soil types within each District was estimated using ArcGIS (v. 9.3, ESRI, Redlands, CA, USA) using the most recent cartographic data for FAO soil series (Fig. 3.4b; Table 3.2). By integrating these data, it was possible to predict grain Se concentrations for >75% of the land area in Malawi (Fig. 3.2b; Table 3.2) and estimate dietary Se intake assuming a mean *per capita* consumption of 0.354 kg maize flour d^{-1}.

Predicted Se intake, adjusted for soil series at a District level, shows that 50% of the population of Malawi currently consumes <6 μg Se person^{-1} d^{-1}, 75% <7 μg Se person^{-1} d^{-1} and 90% <7.5 μg Se person^{-1} d^{-1} from maize sources (Table 3.2). Given that Se intake from all non-maize sources is likely to range between 15-22 μg Se person^{-1} d^{-1} (Table 3.1) and that some groups will obtain a larger proportion of their dietary energy intake from maize, suboptimal Se intake appears to be the norm in Malawi. This

extrapolation is based on the major assumption that soil-to-grain transfer is determined primarily by soil properties. Given the critical role of Se in human health, this assumption must now be tested by more detailed sampling and incorporation of other factors such as soil management, dietary choices, and biomarkers of Se intake and status among the population, within an appropriate geospatially-informed framework.

The marked difference in Se concentration between maize grown on the calcareous Eutric Vertisols of the Shire Valley and almost all other soil types in Malawi, may arise from a combination of factors. The most obvious is soil pH (Fig. 3.2) which had a profound influence on Se uptake at pH >6.5 but only a weak influence in more acidic soils. The pH-dependence of selenate and selenite adsorption on Fe oxides may partly explain this trend. Using Extended X-ray Absorption Fine Structure (EXAFS) data (Peak and Sparks, 2002) showed that specific bonding (inner-sphere co-ordination) of selenate (Se^{VI}; pKa_2 = 1.92) on Fe hydrous oxides declined between pH 3.5 and pH 6. By contrast, selenite (Se^{IV}; pKa_2 = 7.3) is specifically adsorbed beyond the 'point of zero charge' of Fe oxides (pH *c*. 7-8), whereas the adsorption envelope of $HSeO_3^-/SeO_3^{2-}$ on haematite shows a marked fall in sorption strength over the pH range 6-8 (Duc et al., 2006), as expected from the second pKa value (7.3) of selenious acid (Vuori et al., 1989). The pH value at which Se uptake increases corresponds closely with the value at which selenate adsorption on Fe oxides ceases and (the selenite sorption envelope declines). Studies of sequential extraction results showed that 80-100% of the adsorbed Se was recovered as Se bound to Al and Fe (Nakamaru *et al.,* 2005), providing clear evidence that Fe and Al oxides and hydroxides are responsible for fixation of soil Se under low pH. An additional factor may be the dependence of inorganic speciation on Eh-pH relations. Thus, it is clear from Eh-pH predominance diagrams (Séby et al., 2001) and recognised from studies of solubility (Masscheleyn et al., 1990) that selenate is the dominant form of available inorganic Se under oxic and alkaline soil conditions. Other studies of Se solubility and bioavailability have also identified the importance of competition by phosphate ($H_2PO_4^-/HPO_4^{2-}$) for soil adsorption sites (Vuori et al., 1989) and sulphate (SO_4^{2-}) for root uptake

(Stroud et al., 2010), both of which depend on individual soil properties such as pH and mineralogy.

Finally, it must also be recognised that very little soil Se (*c*. 3%) exists in forms which can be extracted using KH_2PO_4, and that cycling between organic and inorganic forms may also influence Se uptake during the growing season. However, KH_2PO_4 proved effective in determining the quantity of soil Se available for uptake into grain and explaining why a correlation between grain Se and soil pH was observed. It is also possible that transformations between organic and inorganic forms of Se within the soil may contribute to the profound differences in Se uptake by maize seen in the Eutric Vertisols of the Shire Valley. Although Eutric Vertisols only occupy *c*. 0.5% of the land area of Malawi, it will be important to take these and other local variation in soil chemistry into account if agronomic biofortification strategies such as those previously adopted in Finland are to be successfully adopted (Broadley et al., 2006, Broadley et al., 2010, White and Broadley, 2009).

CHAPTER 4: Agronomic biofortification of maize with selenium in Malawi

4.1 Introduction

Selenium (Se) is an essential element for humans and is derived primarily from dietary sources (Fairweather-Tait *et al.,* 2011). Habitual suboptimal dietary Se intake leads to reduced Se status, which is associated with a range of adverse health outcomes including cardiovascular disorders, impaired immune functions, and some cancers. In Malawi, where subsistence agriculture is widespread and food choices are relatively narrow, there is evidence of widespread suboptimal dietary Se intakes (Donovan *et al.,* 1992; Eick *et al.,* 2009; Chapter 3) and status (van Lettow *et al.,* 2004). In Malawi, over 50% of dietary calorie intake (2,172 kcal person^{-1} d^{-1}) is derived from maize grain, equating to 0.354 kg person d^{-1} based on trade and production statistics (2007 data; FAO, 2011). Consumption of animal products with higher Se concentrations (fish, meat, offal, fats, milk and eggs) accounts for just 64 kcal person^{-1} d^{-1} (FAO, 2011). From nationwide surveys of farmers' fields, the median maize grain Se concentration of 0.019 mg Se kg^{-1} (range 0.005-0.533) represents an intake of only 6.7 µg Se person^{-1} d^{-1} from maize based on national consumption patterns (Chapter 3). Low Se concentrations in edible crop material produced in Malawi are due to the widespread occurrence of highly weathered acid soils with low plant-available Se concentrations. In these soils, most Se is likely to be present in organic and mineral-occluded forms which are unavailable to plants, with most of the remainder being present as $Se^{(IV)}$ species which are adsorbed strongly to soil colloids and are not taken up readily by roots compared to $Se^{(VI)}$ (Chapter 3).

Suboptimal Se intake can be addressed through dietary diversification, food imports, supplements, food fortification and biofortification (Broadley *et al.,* 2006, 2010; Rayman, 2004, 2008; Fairweather-Tait *et al.,* 2011). Dietary diversification is an attractive option in terms of general protein, mineral

and *Vitamin* intake. In Burundi, greater consumption of fish, meat and offal among more affluent groups has been linked to higher Se intakes (Benemariya *et al.,* 1993). However, access to diverse diets is not possible in many socio-economic contexts. Similarly, despite the clear links between the Se composition and the geographic origin of staple foods such as wheat and rice (Thompson, 2004; Williams *et al.,* 2009; Johnson *et al.,* 2010; Fairweather-Tait *et al.,* 2011), altering trade patterns is undesirable in many contexts. Supplementation of diets or foodstuffs with inorganic or organic forms of Se is again feasible (Rayman, 2004), although the production and equitable distribution of Se supplements are logistically challenging and expensive, and robust controls are required to minimise risks of toxicity. The potential for genetic biofortification of crops through breeding is not yet clear. Lyons *et al.* (2005) screened cereal grain Se composition among modern wheat (*Triticum aestivum* L.), durum wheat (*Triticum dicoccum* (Schrank) Schubl.), wheat landraces, ancestral diploid relatives (*Aegilops tauschii* (Coss.) Schmal.), barley (*Hordeum vulgare*), triticale (x *Triticosecale* Wittmack ex A. Camus.) and rye (*Secale cereale* L.), all grown on soils with low bioavailable Se concentrations. A lack of breeding potential was noted, with cereal grain Se composition being associated primarily with non-genetic factors, as has also been seen in UK bread wheat (n=150; Zhao *et al.,* 2009). However, variation in grain Se composition among non-cultivated varieties and at higher bioavailable soil Se concentrations indicates that future breeding efforts may yet be possible (Lyons *et al.,* 2005; Garvin *et al.,* 2006; White and Broadley, 2009). In terms of agronomic biofortification, the Se concentrations of all fractions of cereal grains can be increased easily when Se is applied as selenate (Broadley *et al.,* 2010; Hart *et al.,* 2011). In a public health setting, Se fertilisation has already been adopted at a national scale in Finland in 1984 following primary legislation. This led to immediate increases in the Se concentrations of Finnish foods and dietary Se intakes (Eurola *et al.,* 1991; Broadley *et al.,* 2006).

The present study aimed to determine the potential for increasing grain Se concentration in maize in Malawi using fertiliser-based approaches. Malawi was chosen because there is evidence of widespread low Se intakes and

status among the population due to the low plant-available Se concentrations of the soils and lack of diversity within the typical diet (Chapter 3). Furthermore, to secure maize yields, Malawi has operated a Farm Input Subsidy Programme (FISP) since 2005/6 (Dorward and Chirwa, 2011), under which fertiliser is distributed to small-scale farmers *via* a voucher system. FISP involves major commitments of financial and human resources through the national extension service system and represents a potential public health intervention route, as adopted previously in Finland.

4.2 Materials and Methods

The details are given in section 2.3.

4.3 Results

4.3.1 $Na_2SeO_{4(aq)}$ experiments

A single high volume drench of $Na_2SeO_{4(aq)}$ was applied to maize crops at six sites in each of two years (12 experimental units). The Se concentration of both maize grain and stover increased at all sites in both years in response to Se fertilisation (Figs. 4.3 & 4.4; Table 4.5).

The relationship between crop Se concentration and Se application rate was approximately linear, with R^2>0.87 for all grain and stover fractions in all experimental units except the stover fraction under irrigation at Kasinthula in 2009/10 (Table 4.5). For each g Se ha^{-1} applied, maize grain Se concentration increased by 11-29 µg Se kg^{-1} and stover Se concentration by 3-21 µg Se kg^{-1} (Table 4.6). Across all experimental units, crop yield varied from 2112-7009 kg grain ha^{-1} and 3169-16458 kg stover ha^{-1}, with a strong effect of site in each year (P<0.001; Table 4.3). However, there were no significant effects of Se application on grain or stover yield in any of the experimental units (P>0.05).

4.3.2 NPK+Se experiments

A granular NPK+Se compound was applied to maize crops at six sites in each of two years (12 experimental units), with split applications included as a treatment sub-factor. The relationship between crop Se concentration and Se fertilisation rate was approximately linear (Figs. 4.5 & 4.6; Table 4.5), a similar response to the liquid drench experiment. For the grain fraction, R^2>0.90 for all sites and both years except Ngabu in 2008/09 (R^2=0.82) and Chitala in 2009/10 (R^2=0.73). For the stover fraction, R^2>0.90 except for Makoka (R^2=0.58) and Ngabu (R^2=0.71) in 2008/09. For each g Se ha^{-1} applied, grain Se concentration increased by 11-33 µg Se kg^{-1} and stover Se concentration by 5-20 µg Se kg^{-1} (Table 4.5). Remarkably, consistent increases in crop Se concentration could be detected following application of as little as 1.5 g Se ha^{-1}.

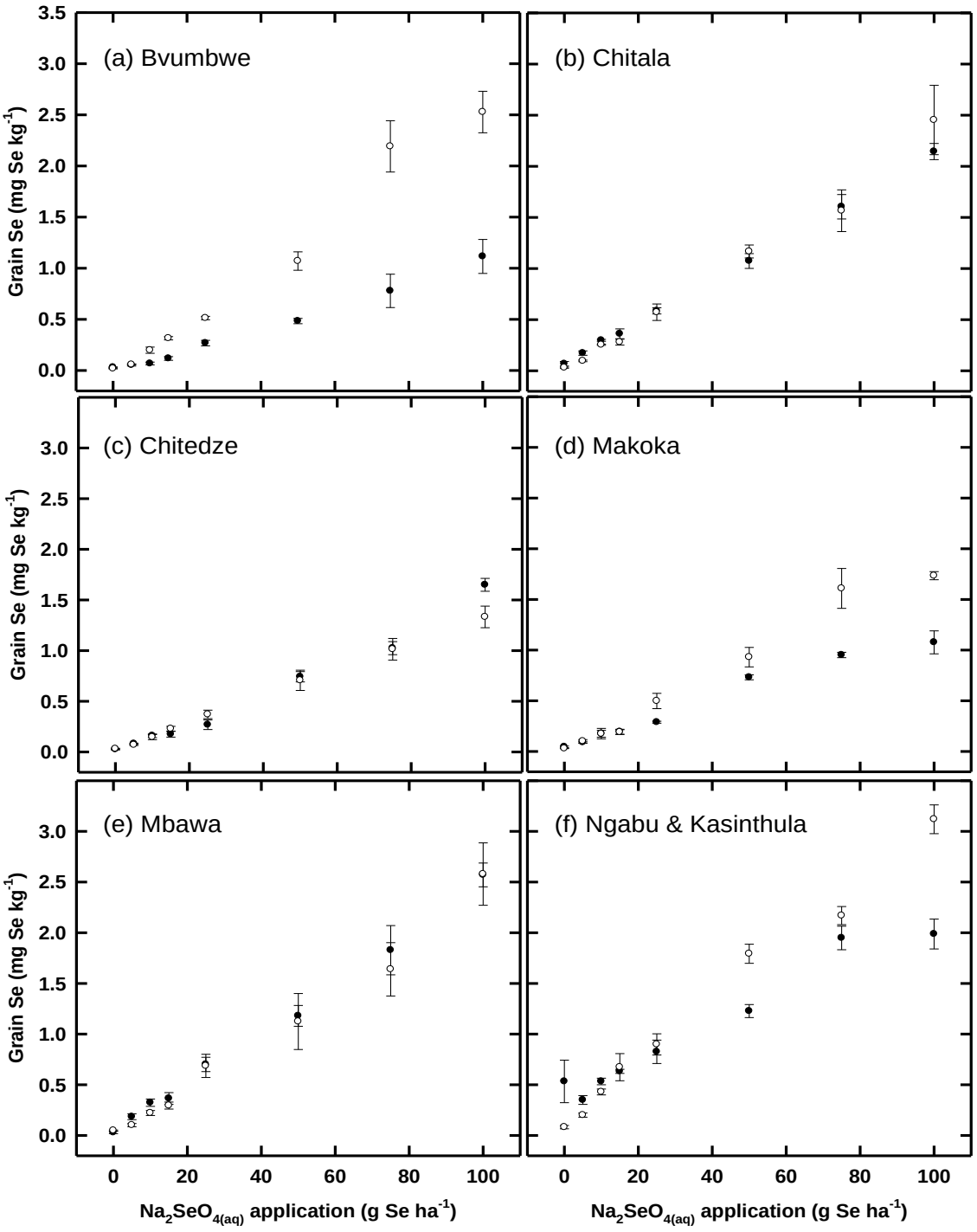

Figure 4.3. Effect of Se application on grain Se concentration at Bvumbwe (a), Chitala (b), Chitedze (c), Makoka (d), Mbawa (e) and Ngabu (f) for two seasons, 2009 (filled symbols) and 2010 (open symbols) using sodium selenate liquid. Double standard errors of the mean are shown.

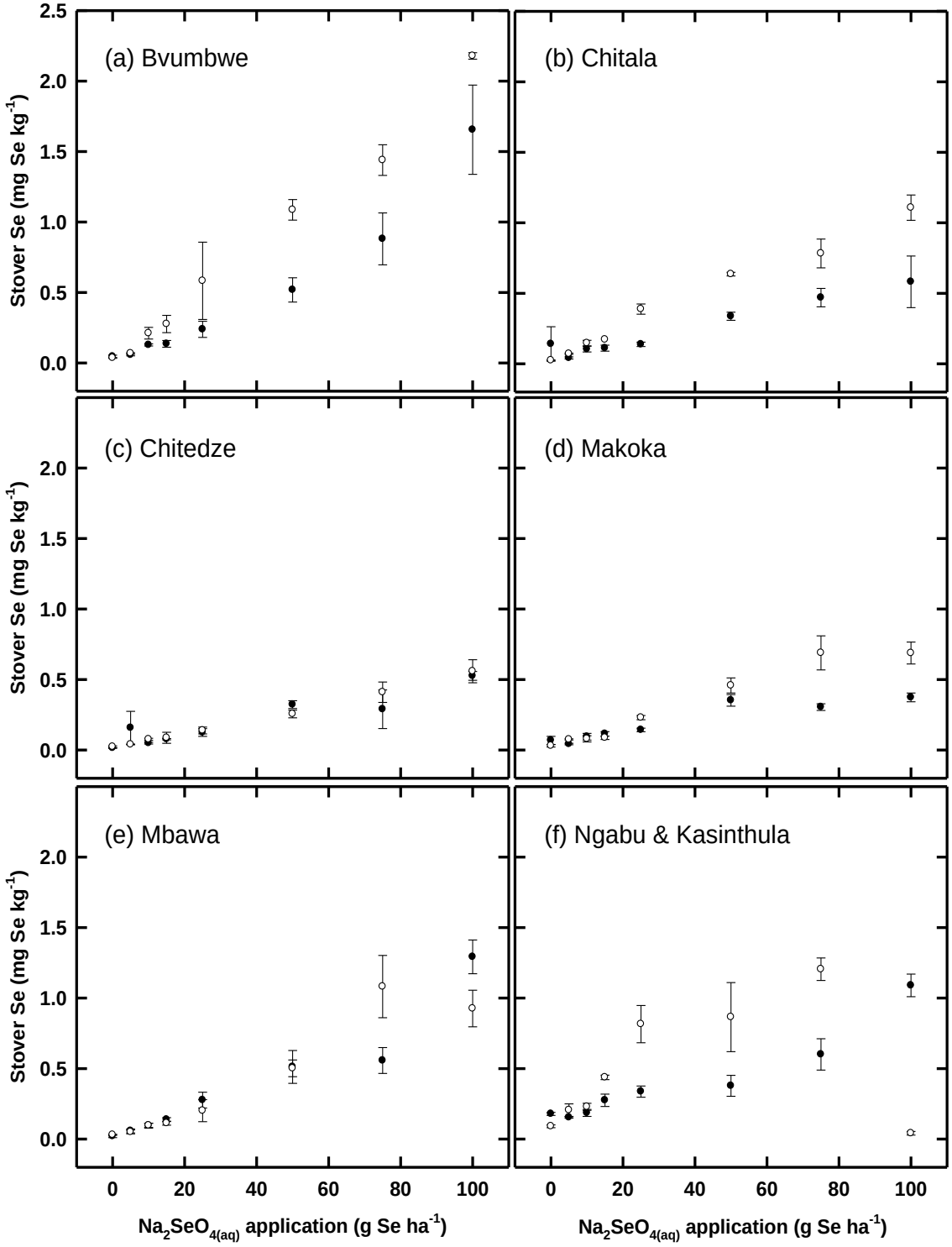

Figure 4.4. Effect of Se application on stover Se concentration at Bvumbwe (a), Chitala (b), Chitedze (c), Makoka (d), Mbawa (e) and Ngabu (f) for two seasons, 2009 (filled symbols) and 2010 (open symbols) using sodium selenate liquid. Double standard errors of the mean are shown.

Across all experimental units, crop yield varied from 2598-7637 kg grain ha^{-1} and 3961-18807 kg stover ha^{-1}, with a strong effect of site in each year (P<0.001; Table 4.4). Again, there were no significant effects of Se application on grain or stover yield in any of the experimental units (P>0.05).

Across all experimental units and fertiliser application rates, the timing of application affected grain Se concentration (Fig. 4.4). Although the significance of this effect was marginal in 2008/09 (P=0.06), grain Se concentration was higher in the late (top dressing) Se treatment than in the (basal) application plots at five of the six sites, with an overall difference of 13%. The effect of timing was highly significant in 2009/10 (P=0.009). Grain Se concentration was higher following late Se application compared to early Se application at all six sites, with an overall difference of 33% (Fig. 4.7).

4.3.3 CAN+Se experiments

A granular CAN+Se compound was applied to two maize genotypes, a local hybrid (SC627) and an open pollinated variety (ZM623), at three sites in each of two years (six experimental units). As there was no significant effect of variety on grain or stover Se concentration, the data for both varieties were combined for subsequent analyses. The relationship between crop Se concentration and Se fertilisation rate was approximately linear (Fig. 4.8; Table 4.5), similar to the liquid drench and NPK+Se experiments. For grain fractions, R^2>0.97 at all sites and years except for Ngabu in 2008/09 (R^2=0.17). For stover fractions, R^2>0.92 except for Ngabu (R^2=0.09) in 2008/09. For each g Se ha^{-1} applied, maize grain Se concentration increased by 4-33 µg Se kg^{-1} and stover Se concentration by 1-21 µg Se kg^{-1} (Table 4.5).

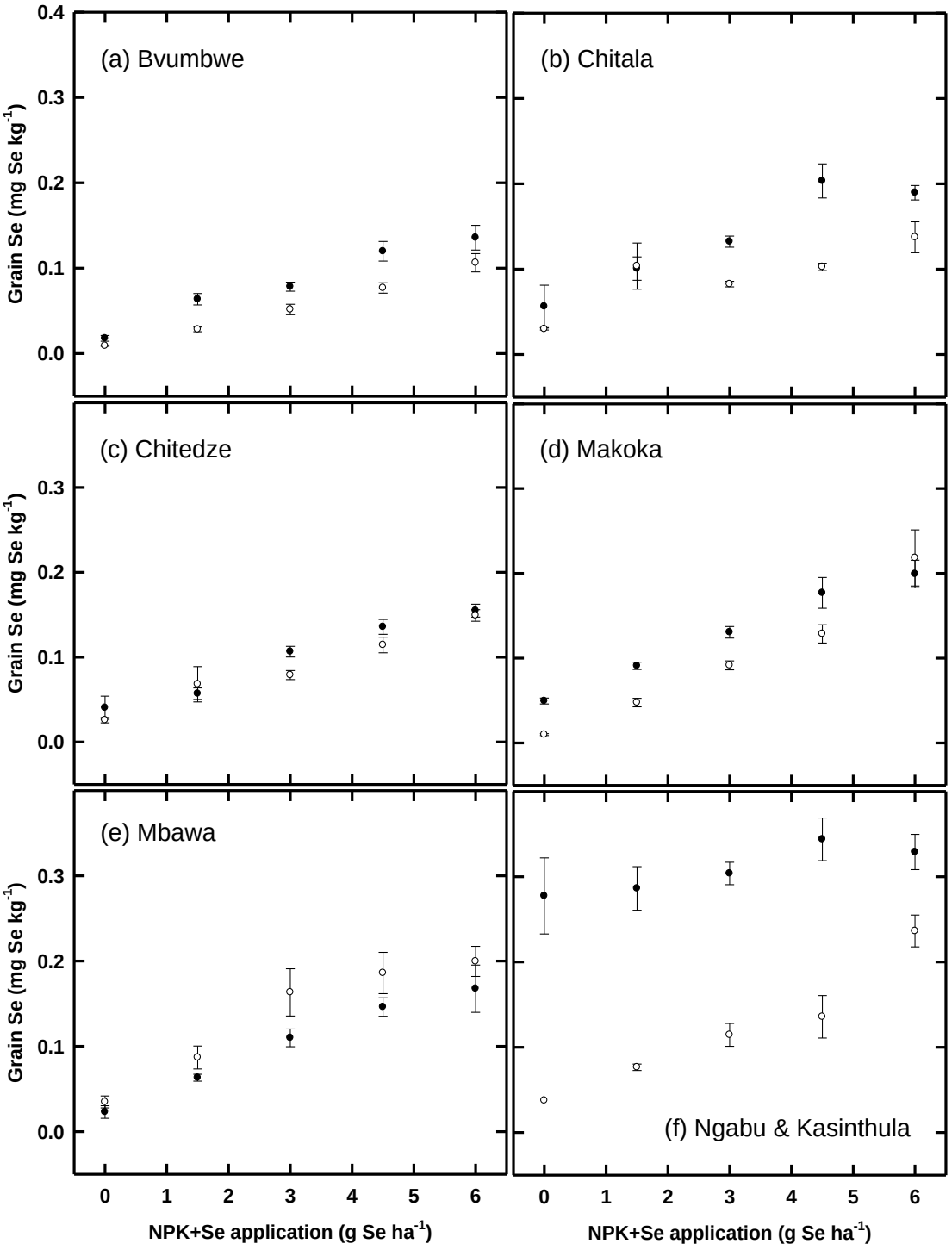

Figure 4.5. Effect of Se application on grain Se concentration at Bvumbwe (a), Chitala (b), Chitedze (c), Makoka (d), Mbawa (e) and Ngabu (f) for two seasons, 2009 (filled symbols) and 2010 (open symbols) using NPK+Se granular fertiliser. Double standard errors of the mean are shown.

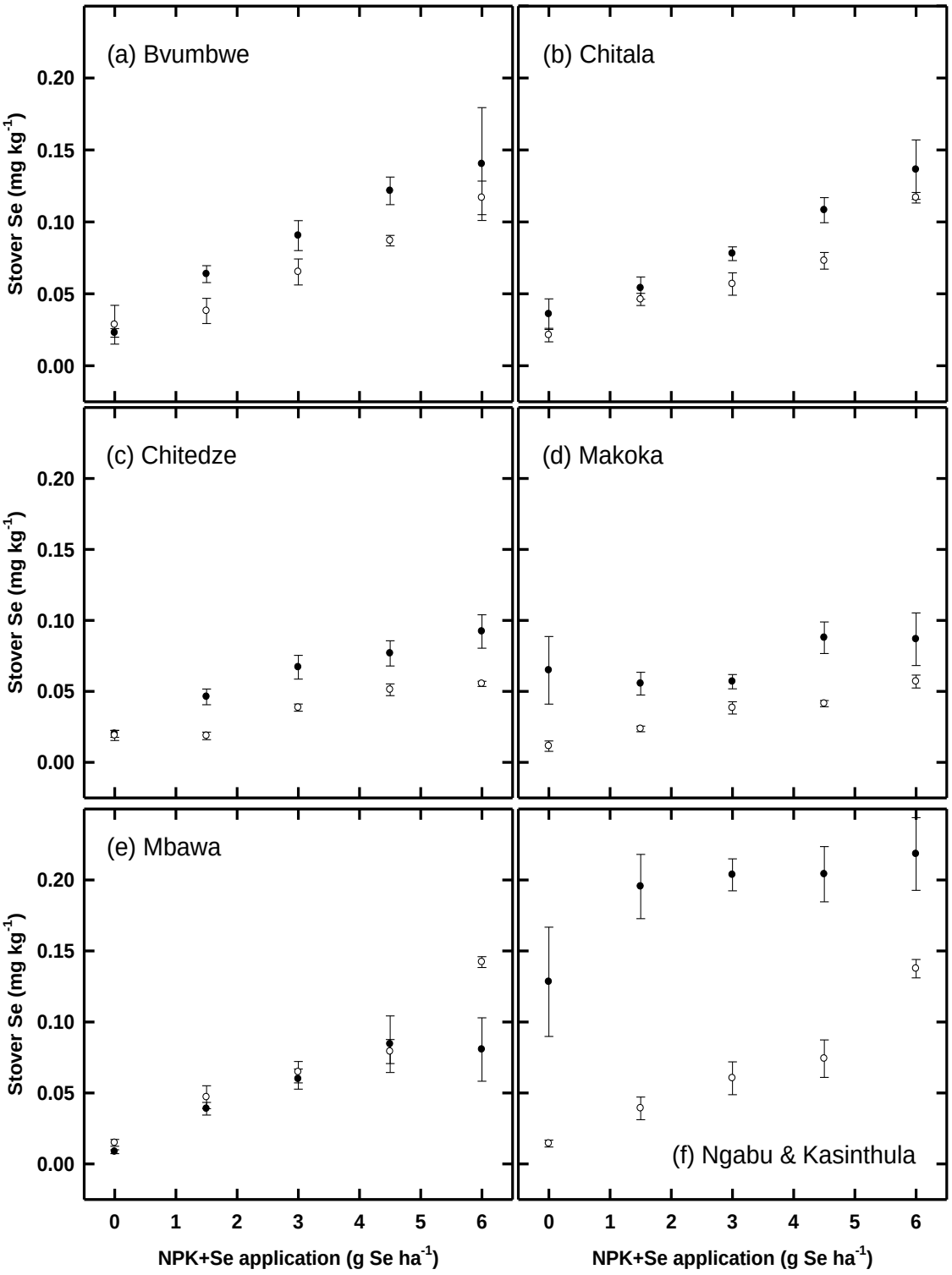

Figure 4.6. Effect of Se application on stover Se concentration at Bvumbwe (a), Chitala (b), Chitedze (c), Makoka (d), Mbawa (e) and Ngabu (f) for two seasons, 2009 (filled symbols) and 2010 (open symbols) using NPK+Se granular fertiliser. Double standard errors of the mean are shown.

Table 4.3. Yield data and treatment effects for liquid fertilisation experiments.

Experimental site	Grain yield (kg ha^{-1})		Stover yield (kg ha^{-1})	
	2008/09	2009/10	2008/09	2009/10
Bvumbwe	4141	3050	7333	4279
Chitala	6498	5242	15792	14875
Chitedze	6527	9369	5475	7117
Makoka	7009	5560	6542	11192
Mbawa	3906	3058	4758	4408
Ngabu	2764	Na	16458	na
Kasinthula	Na	2112	Na	3169
Site	$F_{5,141}$=116; P<0.001	$F_{5,133}$=175; P<0.001	$F_{5,141}$=306; P<0.001	$F_{5,133}$=127; P<0.001
Se treatment	**$F_{7,141}$=1.54; P=0.159**	**$F_{7,133}$=0.64; P=0.719**	**$F_{7,141}$=1.02; P=0.423**	**$F_{7,133}$=1.02; P=0.421**
Site/Se treatment	$F_{35,141}$=1.01; P=0.465	$F_{35,133}$=0.93; P=0.590	$F_{35,141}$=1.13; P=0.308	$F_{35,133}$=0.59; P=0.963

Table 4.4. Yield data and treatment effects for NPK+Se fertilisation experiments.

Experimental site	Grain yield (kg ha^{-1})		Stover yield (kg ha^{-1})	
	2008/09	2009/10	2008/09	2009/10
Bvumbwe	4206	3208	8870	4591
Chitala	7068	4759	16037	13667
Chitedze	5802	7637	5230	5670
Makoka	6955	7520	7000	18807
Mbawa	5684	2641	7328	3961
Ngabu	2598	Na	15285	na
Kasinthula	na	3890	Na	5835
Site	$F_{5,159}$=72.2; P<0.001	$F_{5,150}$=103; P<0.001	$F_{5,159}$=208; P<0.001	$F_{5,150}$=293; P<0.001
Se treatment	**$F_{4,159}$=2.14; P=0.079**	**$F_{4,150}$=1.16; P=0.331**	**$F_{4,159}$=0.61; P=0.659**	**$F_{4,150}$=2.24; P=0.067**
Site/Se treatment	$F_{20,159}$=0.68; P=0.840	$F_{20,150}$=0.65; P=0.865	$F_{20,159}$=0.36; P=0.995	$F_{20,150}$=0.70; P=0.825
Se treatment/split	$F_{4,159}$=1.76; P=0.140	$F_{4,150}$=1.17; P=0.325	$F_{4,159}$=0.62; P=0.65	$F_{4,150}$=2.25; P=0.066
Site/Se treatment/split	$F_{20,159}$=1.79; P=0.026	$F_{20,150}$=1.01; P=0.454	$F_{20,159}$=1.17; P=0.286	$F_{20,150}$=0.64; P=0.874

Table 4.5 Regression analysis outputs for all experiments, based on overall experiment means for each Se application level

Experimental Site	Se source	Se application levels	2008/09						2009/10					
			SLOPE (mg Se kg^{-1} g^{-1} Se ha^{-1})		*INTERCEPT (mg Se kg^{-1})*		R^2		*SLOPE (mg Se kg^{-1} g^{-1} Se ha^{-1})*		*INTERCEPT (mg Se kg^{-1})*		R^2	
			Grain	Stover	Grain	Stover	Grain	Stover	Grain	Stover	Grain	Stover	Grain	Stover
Bvumbwe	Na_2SeO_4	8	0.011	0.015	-0.018	-0.065	0.99	0.94	0.027	0.021	-0.077	-0.001	0.982	0.99
	NPK+Se	5	0.019	0.020	0.025	0.029	0.97	0.99	0.016	0.015	0.006	0.022	0.99	0.98
	CAN+Se	5	0.008	0.006	0.025	0.049	0.99	0.99	0.018	0.016	0.022	-0.006	0.99	0.96
Chitala	Na_2SeO_4	8	0.021	0.005	0.068	0.055	1.00	0.954	0.023	0.011	-0.018	0.044	0.99	0.99
	NPK+Se	5	0.025	0.017	0.063	0.031	0.91	0.99	0.014	0.015	0.048	0.019	0.73	0.94
Chitedze	Na_2SeO_4	8	0.016	0.004	-0.033	0.039	0.98	0.88	0.013	0.005	0.026	0.012	1.00	1.00
	NPK+Se	5	0.021	0.012	0.037	0.025	0.97	0.97	0.020	0.007	0.029	0.015	0.98	0.92
	CAN+Se	5	0.010	0.005	0.024	0.015	1.00	0.92	0.016	0.007	0.006	0.014	0.97	0.98
Makoka	Na_2SeO_4	8	0.011	0.003	0.054	0.066	0.98	0.87	0.019	0.008	0.004	0.029	0.98	0.96
	NPK+Se	5	0.026	0.005	0.052	0.055	0.99	0.58	0.033	0.007	0.000	0.013	0.96	0.97
Mbawa	Na_2SeO_4	8	0.025	0.011	0.036	-0.021	1.00	0.91	0.024	0.011	-0.017	-0.008	0.99	0.91
	NPK+Se	5	0.025	0.013	0.028	0.017	0.99	0.91	0.029	0.019	0.048	0.012	0.92	0.93
Ngabu /	Na_2SeO_4	8	0.017	0.008	0.394	0.113	0.96	0.90	0.029	0.003	0.138	0.366	0.99	0.09
Kasinthula	NPK+Se	5	0.011	0.013	0.276	0.152	0.82	0.71	0.030	0.019	0.029	0.009	0.93	0.92
	CAN+Se	5	0.004	0.001	0.358	0.217	0.17	0.09	0.033	0.021	0.075	0.01	1.00	0.94

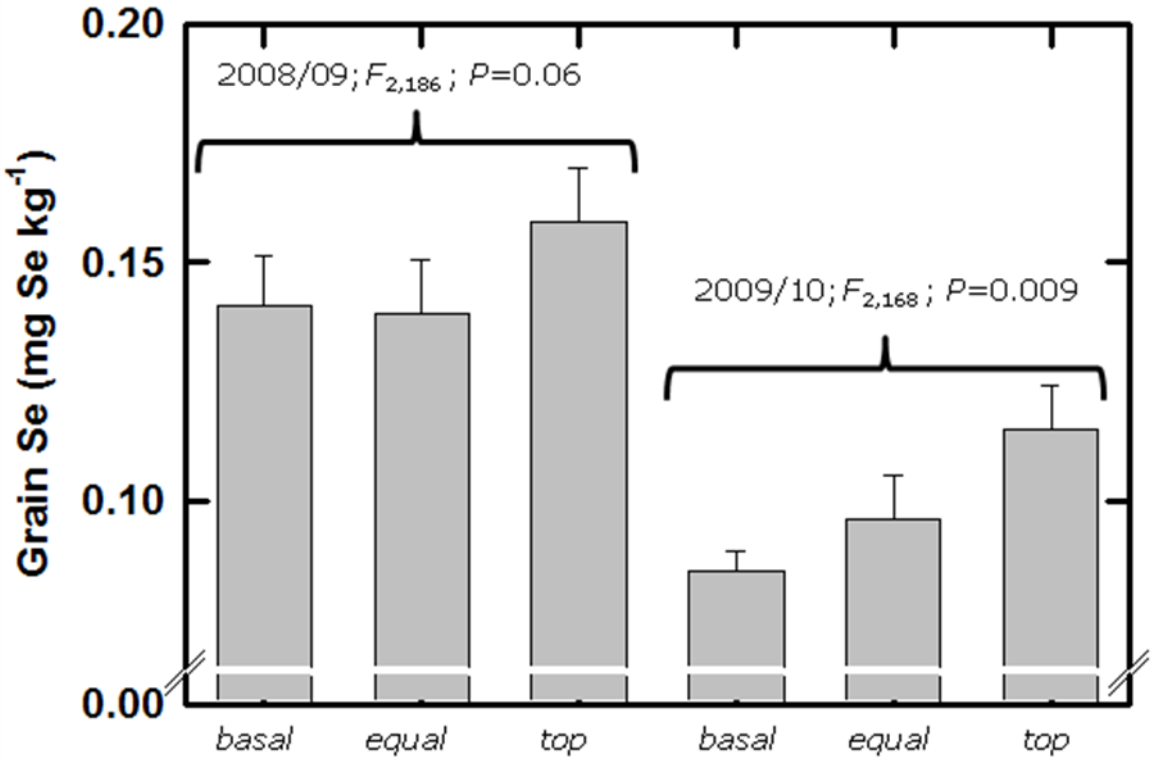

Figure 4.7. The effect of time of application (basal or top dressing) on grain Se concentration in 2008-2009 and 2009-2010. Single standard errors of the mean are shown.

Across all experimental units, crop yield varied from 2638-8311 kg grain ha^{-1} and 4773-15200 kg stover ha^{-1}, with a strong effect of site in both years (P<0.001; Table 4.6). There were no significant effects of Se application on grain or stover yields in any of the experimental units (P>0.05), similar to the other forms of Se. There were significant variety*site interaction terms for grain and stover yields in 2008/09, but not in 2009/10 (Table 4.6).

Table 4.6. Yield data for CAN+Se fertilisation experiments.

Experimental Sites	Grain yield (kg ha^{-1})				Stover yield (kg ha^{-1})			
	2008/09		2009/10		2008/09		2009/10	
	SC627	ZM623	SC627	ZM623	SC627	ZM623	SC627	ZM623
Bvumbwe	5930	4332	4720	3964	10044	7422	6947	5947
Chitedze	5209	5498	8098	8311	4773	5324	7040	6258
Ngabu	2638	3648	na	na	15200	11911	na	na
Kasinthula	na	na	4013	3996	na	Na	6021	5994
Site	$F_{2,58}$=24.9; P<0.001		$F_{2,58}$=86.6; P<0.001		$F_{2,58}$=72.4; P<0.001		$F_{2,58}$=1.13; P=0.329	
Se treatment	**$F_{4,58}$=1.03; =0.401**		**$F_{4,58}$=0.59; P=0.668**		**$F_{4,58}$=0.90; P=0.470**		**$F_{4,58}$=0.53;P= 0.716**	
Variety	$F_{1,58}$=0.12; P=0.725		$F_{1,58}$=0.42; P=0.522		$F_{1,58}$=9.52; P=0.003		$F_{1,58}$=2.87; P=0.095	
Site/Se treatment	$F_{8,58}$=0.60; P=0.777		$F_{8,58}$=0.53; P=0.832		$F_{8,58}$=1.04; P=0.420		$F_{8,58}$=0.61; P=0.766	
Site/Variety	$F_{2,58}$=7.61; P=0.001		$F_{2,58}$=1.02; P=0.368		$F_{2,58}$=4.19; P=0.020		$F_{2,58}$=0.69; P=0.508	
Se treatment/Variety	$F_{4,58}$=1.41; P=0.243		$F_{4,58}$=0.07; P=0.990		$F_{4,58}$=0.37; P=0.828		$F_{4,58}$=0.05; P=0.994	
Site/Se treatment/Variety	$F_{8,58}$=1.16; P=0.336		$F_{8,58}$=0.37; P=0.935		$F_{8,58}$=1.14; P=0.354		$F_{8,58}$=0.47; P=0.870	

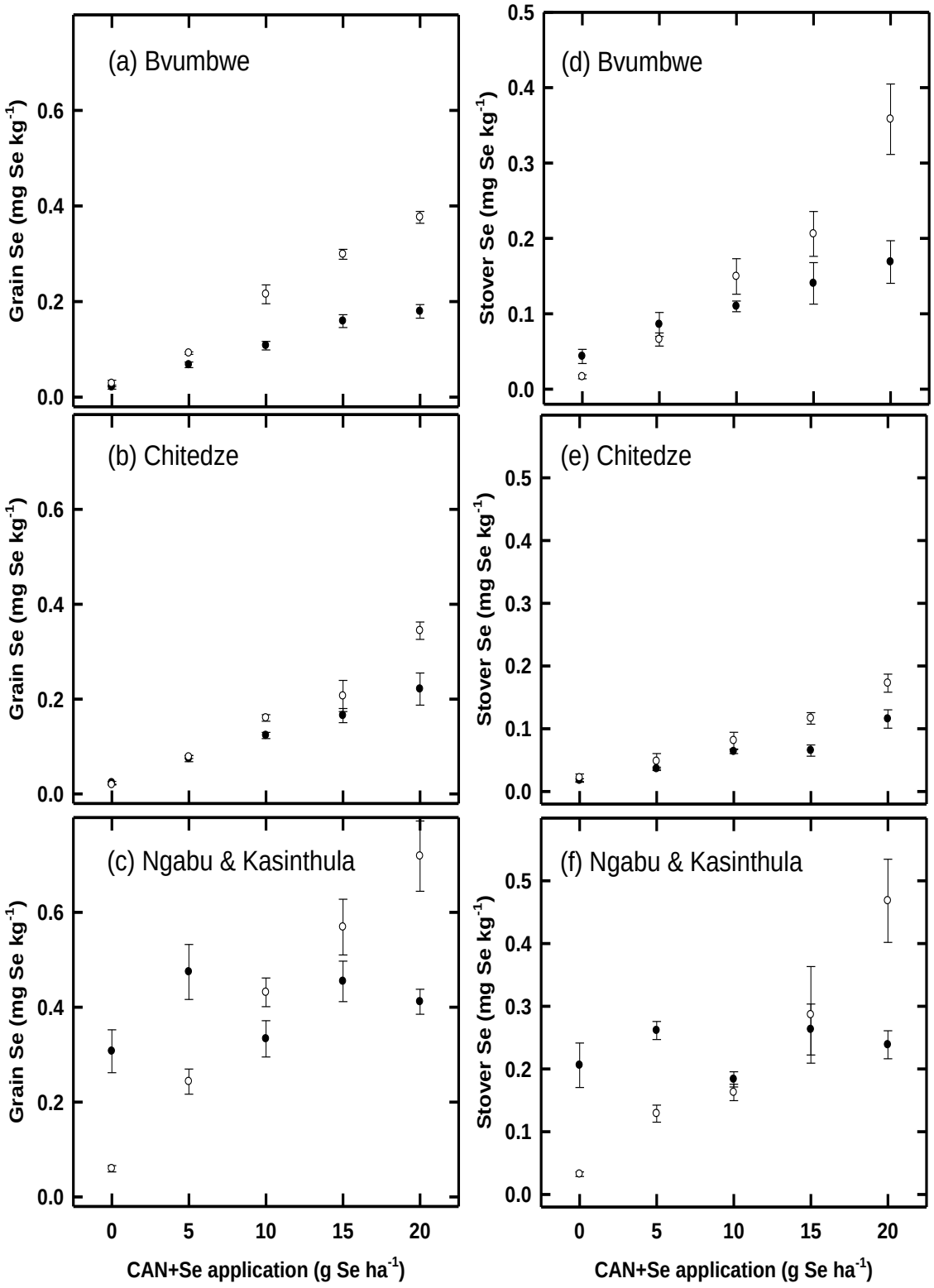

Figure 4.8. Effect of Se application on grain and stover Se concentrations at Bvumbwe (a, d), Chitedze (b, e), and Ngabu (c, f) respectively for two seasons, 2009 (filled symbols) and 2010 (open symbols) using CAN+Se granular fertiliser. Double standard errors of the mean are shown.

There were significant differences between seasons in grain Se concentration at Bvumbwe, Makoka and Ngabu (Fig. 4.3; Table 4.5). This occurred because maize at Bvumbwe was attacked by Grey Leaf Spot (*Cercospora maydis* L.) in 2009, resulting in premature death of some of the leaves during grain filling. This is likely to have affected translocation of Se from leaves and stems to the grain, with the result that the increase in grain Se content resulting from application of Se-containing fertiliser was 0.011 mg Se kg^{-1} g^{-1} Se applied in 2009 compared to 0.027 mg Se kg^{-1} g^{-1} Se applied in 2010 (Table 4.5). At Makoka, heavy rainfall occurred immediately after application of the spray containing Se began; as spraying continued, Se uptake may well have been affected as some of the applied Se would have been lost through surface run off and subsequent infiltration into the soil profile (Fig. 4.9).

Figure 4.9. Heavy rainfall occurred soon after spraying of sodium selenate began at Makoka in 2009.

In 2009, the experiment at Ngabu was rain-fed, but in 2010 the plots were irrigated because of poor planting rains at this site (Fig. 4.10). However, there were no significant differences between seasons in the increase in grain Se content per unit of applied Se at Chitala, Chitedze and Mbawa (Fig. 4.3), suggesting that Se uptake by maize is comparable throughout Malawi.

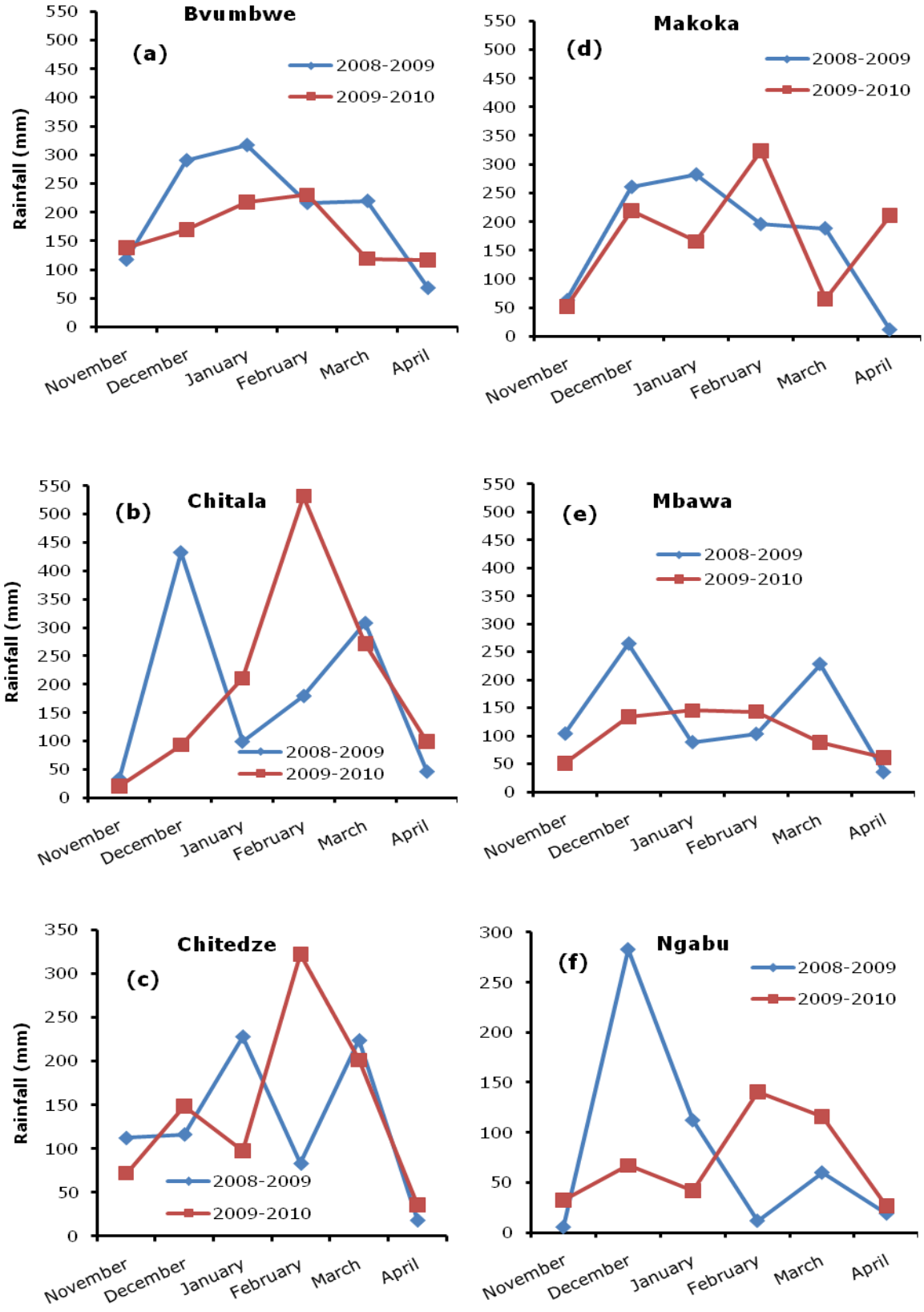

Figure 4.10. Total monthly rainfall (mm) for all experimental sites during the 2008-2009 and 2009-2010 cropping seasons.

4.3.4 Total above-ground recovery of Se

Percentage Se recovery is shown in Table 4.7. Total above-ground Se recoveries using sodium selenate were 13.7, 21.5, 12.6, 9.7, 15.0 and 17.9 % in 2009 and 17.3, 28.4, 11.5, 19.5, 12.2 and 7.1 % in 2010 at Bvumbwe, Chitala, Chitedze, Makoka, Mbawa and Ngabu, respectively. The NPK+Se total above-ground Se recovery by maize crop was 25.7, 44.9, 18.5, 21.6, 23.7 and 22.7% in 2009 and 13.1, 27.2, 16.7, 38.0, 16.5 and 24.9% in 2010 at Bvumbwe, Chitala, Chitedze, Makoka, Mbawa and Ngabu, respectively. The CAN+Se recoveries were 9.3, 7.9, 2.6 in 2009 and 9.0, 8.9 and 12.9% in 2010 for Bvumbwe, Chitedze and Ngabu, respectively. Recovery varied between sites, Se sources and seasons, ranging from 2.6 to 44.9%. The highest recoveries were at Chitala in both seasons. NPK+Se gave the highest Se recovery, followed by sodium selenate and CAN+Se.

Table 4.7. Percentage above-ground recovery of Se in maize crops at harvest calculated from the linear response.

Site	Se source	2008/09			2009/10		
		Grain	Stover	Total efficiency%	Grain	Stover	Total efficiency %
Bvumbwe	Na_2SeO_4	0.05	0.11	**16**	0.08	0.09	**17**
	NPK+Se	0.08	0.18	**26**	0.05	0.07	**12**
	CAN+Se	0.04	0.05	**9**	0.08	0.10	**18**
Chitala	Na_2SeO_4	0.14	0.08	**22**	0.12	0.16	**28**
	NPK+Se	0.18	0.27	**45**	0.07	0.21	**27**
Chitedze	Na_2SeO_4	0.10	0.02	**13**	0.12	0.04	**16**
	NPK+Se	0.12	0.06	**18**	0.15	0.04	**19**
	CAN+Se	0.05	0.03	**8**	0.13	0.05	**18**
Makoka	Na_2SeO_4	0.08	0.02	**10**	0.11	0.09	**20**
	NPK+Se	0.18	0.04	**22**	0.25	0.13	**38**
Mbawa	Na_2SeO_4	0.10	0.05	**15**	0.07	0.05	**12**
	NPK+Se	0.14	0.10	**24**	0.08	0.08	**15**
Ngabu /	Na_2SeO_4	0.05	0.13	**18**	0.06	0.01	**7**
Kasinthula	NPK+Se	0.03	0.20	**23**	0.12	0.11	**23**
	CAN+Se	0.01	0.01	**3**	0.13	0.13	**26**

4.3.5 Effects of traditional maize processing on flour Se concentration

The studies of several traditional maize processing practices revealed that Se concentration was higher in whole grain flour (Mgaiwa) when Se applications exceeded 25 g Se ha^{-1} than in Gramil and Woyera flour ($P<0.001$; Fig. 4.11), but there was no significant difference between flour types at application rates <25 g Se ha^{-1} (Fig. 4.11). A significant interaction between the grain processing practice and the rate of Se application was detected ($P<0.005$) as the procedure employed significantly affected flour Se concentration at 50 g Se ha^{-1} of application ($P<0.005$), but not at lower rates of application (Fig. 4.11).

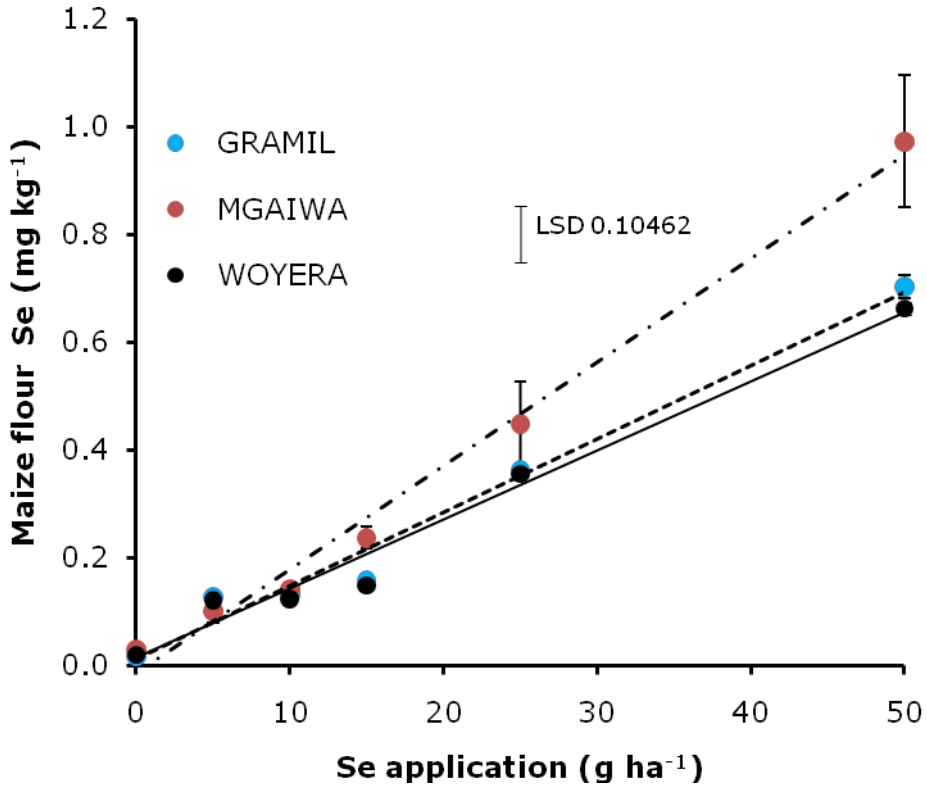

Figure 4.11. Effect of three grain processing methods and Se application rates on grain Se concentration. Double standard errors of the mean are shown.

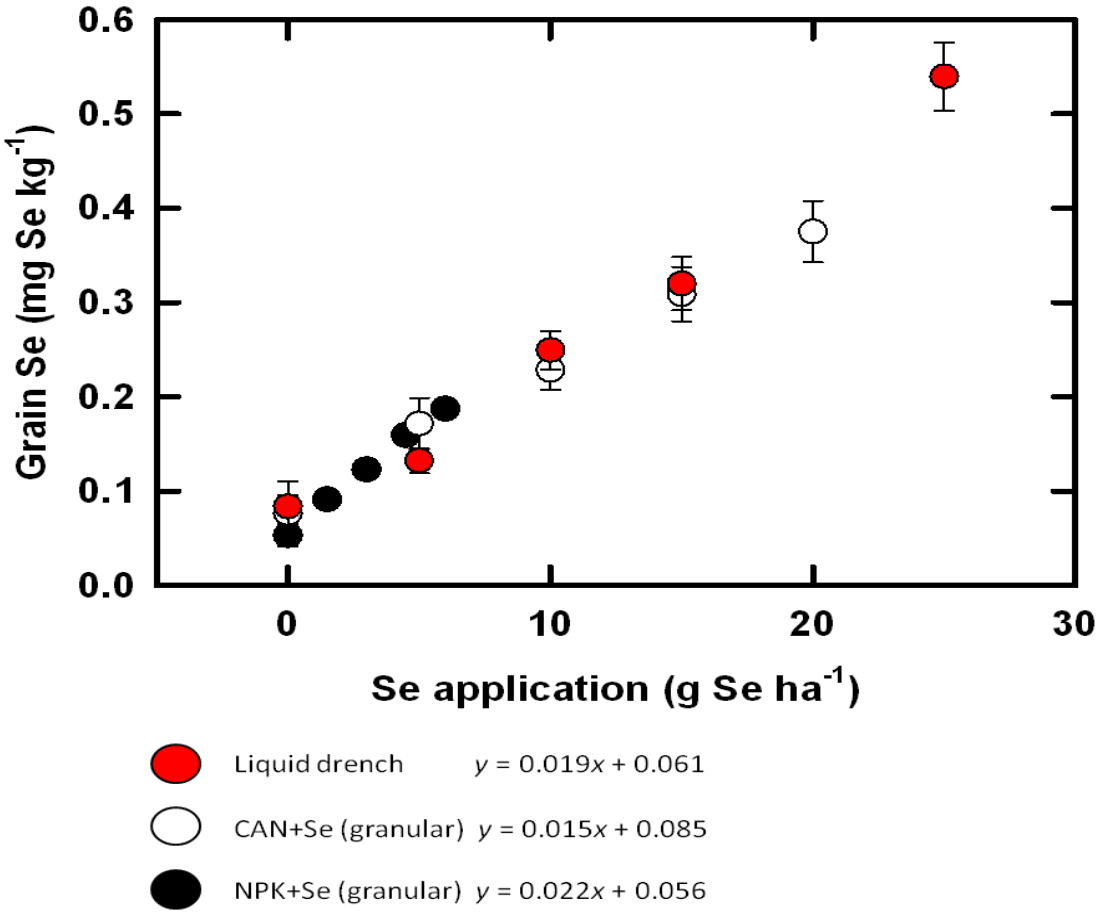

Figure 4.12. Relationship between mean grain Se concentration and Se application rates for all three sources averaged over all sites. Double standard errors of the mean are shown.

4.4 **Discussion**

The observed increase in grain and stover Se concentrations in response to Se fertiliser are consistent with reports of increased Se concentrations in wheat grain following applications of sodium selenate or granular fertiliser containing Se (Lyons et al., 2003, Grant et al., 2007, Curtin et al., 2008, Broadley et al., 2010). Similar increases were also reported in Finland, where the Se concentration of bread baked using wheat was increased from 0.03 to 0.35 mg Se kg^{-1} DW (Eurola, 2005) and the UK (Broadley et al., 2010). The results shown in table 4.6 indicate that the local grain Se concentrations as indicated by intercept in NPK+Se differed among sites

(P<0.001), being highest at Ngabu, followed by Chitala, Makoka, Mbawa, Chitedze and Bvumbwe. Although, there were no effects of Se application on yield, other studies have suggested possible beneficial effects of Se treatment on crops, particularly at low concentrations; for example, Se applications have been observed to delay senescence and improve utilisation of short wavelength solar radiation (Hartikainen et al., 2000, Hartikainen, 2005). Although there was no evidence of an increase in biomass following Se application, a 43% increase in seed production was observed in *Brassica napus* L. (Lyons et al., 2009). Further research reported that a low Se application rate of 0.1 mg kg^{-1} stimulated the growth of senescing seedlings, whereas 1.0 mg kg^{-1} reduced yield in young plants (Xue et al., 2001). Potato plants supplied with Se had a higher starch concentration in their upper leaves and tubers than untreated plants (Turakainen et al., 2004).

As anticipated, Se recovery varied between experimental sites as they were located in different agro-ecological zones, with the result that soil pH, organic matter content and texture, total rainfall and its distribution, the prevalence of diseases and yield potential all differed between sites (Table 4.7). The results show that foliar and soil application of granular fertiliser both provided similar Se recovery by maize, although the efficacy of foliar-applied Se was subject to environmental factors such as rainfall. Application of sodium selenate or calcium nitrate enriched with Se provided recovery values ranging from 8-20%, consistent with the value of 18% reported (Lyons et al., 2004). Total Se recovery ranged from 8.7-43% for the NPK + Se (Top Stock) treatments. Curtin *et al.* (2008) reported recovery of 20% when Se was applied as a top dressing, but recovery was <5% when Se was applied as seed treatment. Although Se recovery of 5% (Stephen et al., 1989) and <10% have also been reported (Eurola, 2005), Broadley *et al.* (2010) reported above-ground Se recovery of 20-35% in wheat, in agreement with the present study. The uptake of applied selenate by crops declines within weeks of application, even when large amounts are applied, and uptake by cereals in the second year after application was minimal (Gissel-Nielsen and Bisbjerg, 1970, Mikkelsen et al., 1989). Other studies indicate that 80-95% and 94-98% of the Se applied each year was not

absorbed by grass and maize respectively (Laing et al., 2009). The method of application was shown to affect the efficiency of Se recovery as foliar application was several times more efficient than application to the soil (Aspila, 2005, Curtin et al., 2006). By contrast, Lyons *et al.* (2004) reported that foliar application was less effective than application to the soil.

Agronomic biofortification of maize with Se is, in theory, a feasible option for increasing dietary Se intake in Malawi as grain Se increased by 19.7, 20.7 and 14.8 μg Se kg^{-1} grain for each g Se ha^{-1} applied as $Na_2SeO_{4(aq)}$, NPK+Se and CAN+Se, respectively (Fig. 4.12). However, if agronomic biofortification is to be adopted, the process has to be reliable and cost-effective, in terms of health benefits and resource-use efficiency, compared to alternative strategies such as the use of mineral supplements.

Selenium intake in Malawi is estimated to be <6 μg Se $person^{-1}$ d^{-1} for 50% of the population and <7.5 μg Se $person^{-1}$ d^{-1} for 90% of the population from maize sources (Chapter 3). These intake data are based on extrapolated soil and maize grain Se concentration data from a preliminary survey, combined with average *per capita* maize consumption. Based on limited published data for Malawi, average Se intake from non-maize sources is likely to range between 15-22 μg Se $person^{-1}$ d^{-1} (Chapter 3). However, many individuals will obtain a much larger proportion of their dietary energy from maize than average *per capita* maize consumption patterns suggest, and suboptimal Se intake is clearly widespread. From the present study, an application of 5 g Se ha^{-1} to maize crops would increase average dietary Se intake in Malawi by 26.3-36.6 μg Se $person^{-1}$ d^{-1}. Such levels would increase dietary Se intake to accepted reference values of 50-70 μg Se $person^{-1}$ d^{-1} (Fairweather-Tait *et al.,* 2011). The risk of overdose, based on current upper safe intake limits of 400 μg Se $person^{-1}$ d^{-1} (Department of Health, 1991; Institute of Medicine, 2000), would appear to be minimal at these application levels, even for individuals with diverse diets. However, any public health intervention involving widespread agronomic biofortification with Se would clearly require careful monitoring to ensure beneficial health outcomes. Whilst it is widely accepted that Se intake <30 μg Se d^{-1} is suboptimal for most adults, there remain

considerable gaps in our knowledge of the relationships between Se intake, plasma Se concentrations and selenoenzyme activities, and definitive health outcomes (e.g. immune functioning), especially among individuals of very low-Se status in SSA. This situation must be now addressed *via* controlled intervention experiments as a matter of urgency.

The traditional maize processing results are closely comparable to those reported by Garvin *et al.* (2011) and indicate that partitioning of Se within the grain may change at higher Se application rates, with more being partitioned to bran, thereby increasing losses associated with de-hulling the grain. Fermentation and soaking in water did not affect the Se concentration of the flour as no differences were detected between the Gramil and Woyera flour types for all rates of Se application (Fig. 4.11). The results also indicate that processing biofortified maize grain from plants which received a lower Se application rate of 5 g Se ha^{-1} would not affect the Se concentration of the flour. Processing did not affect Se concentration and application of 10 g Se ha^{-1} increased total Se in white and wholemeal bread by 155 and 185 µg kg^{-1}, respectively, but there were minimal losses of Se during grain processing and bread production (Hart et al., 2011, Garvin et al., 2011). Garvin *et al.* (2011) also reported that there was no evidence of Se losses during bread-making using flour with either a low or high Se concentration.

In terms of reliability, the linear response of crop Se concentration to all forms and application rates of Se was striking and consistent for most sites. Thus, the response for grain Se concentration was linear (R^2>0.90) in 27 of the 30 trials. For stover Se concentration, the response was also linear (R^2>0.87) in 26 of the trials. For those trials where the linear response was less strong, four still had highly significant R^2 values of 0.58-0.82. The three non-significant linear responses were at the Ngabu or Kasinthula sites. In addition to low rainfall at Ngabu in 2008/09, both sites have soils of the calcareous Eutric Vertisol FAO classification with $pH_{(water)}$ values of 7.4 and 7.9 respectively (Green and Nanthambwe, 1992; Chapter 3). At these pH levels, soil-to-grain transfer of native Se is up to 10-fold greater than under the normal acid conditions seen at Luvisol sites (Chapter 3). This is likely to

be due to a decrease in the sorption strength of $Se^{(IV)}$ in the pH range 6-8 (Duc *et al.,* 2006) and the potential oxidation of $Se^{(IV)}$ to $Se^{(VI)}$ at high pH, which is more available for crop uptake (Vuori *et al.,* 1989; Masscheleyn *et al.,* 1990; Séby *et al.,* 2001). Although Eutric Vertisols comprise *c*. 0.5% of the land area of Malawi, soil types representing a further 23% have not yet been sampled (Chapter 3). Given the critical role of soil properties in determining grain Se concentration, there is a pressing need for structured geochemical sampling of soils and grain in Malawi before agronomic biofortification strategies are implemented. Geochemical data should be combined with information on other factors including rainfall, soil management and crop yield. Within this geochemical context, the overall agronomic efficiency of the process must also be carefully monitored and optimised, to ensure the sustainable use of global Se reserves (Haug *et al.,* 2007; Broadley *et al.,* 2010).

As observed previously for field-grown wheat, maize grain and stover yields were unaffected by Se applications up to 100 μg Se ha^{-1} (Broadley *et al.,* 2010). These observations are consistent with other field studies of wheat (Ducsay and Ložek, 2006; Grant *et al.,* 2007; Curtin *et al.,* 2008), despite evidence that plant growth may be stimulated by increased Se supply under controlled environment conditions (Hartikainen and Xue 1999; Xue and Hartikainen 2000; Turakainen *et al.,* 2004; White *et al.,* 2004; Lyons *et al.,* 2009; Ríos *et al.,* 2009). Se-induced growth stimulation in plants has been attributed to increased resistance to oxidative stress and the stimulation of sulphur transport and assimilation pathways. Further studies are needed to assess these phenomena in a wider field context.

In terms of input-costs, the distribution of fertilisers to smallholder farmers and villages and the cost of exogenous Se supplies must be weighed against the projected health benefits at an individual and population level. The distribution and use of fertilisers at the smallholder farmer level is widespread in Malawi. In 2005, following poor maize yields, the Malawi Government introduced an Agricultural Input Subsidy Programme (AISP, since renamed FISP). Under FISP, small-scale farmers are given vouchers for mineral fertilisers and hybrid maize seed *via* national extension services

on an annual basis (Denning *et al.,* 2009; Dorward and Chirwa, 2011). In terms of fertilisers, FISP imports *c*. 0.2 Mt yr^{-1} of fertilisers and distributes these according to economic need. FISP involves a major commitment of financial and human resources, representing 6.6% of GDP in 2008/9, i.e. an annual spend of >$250 m. At a household level, a fertiliser 'coupon' is worth >10% of annual income for almost half of the population. An independent review of FISP recently concluded that it has led to a doubling of maize production and led to wider economic growth and poverty reduction (Dorward and Chirwa, 2011). The opportunity to distribute Se-enriched fertilisers *via* FISP is analogous to the precedent set when the Finnish Government passed primary legislation in 1983 to incorporate Se in compound fertilisers from 1984. The fact that the sector was largely under state control facilitated this initiative and led to rapid increases in the Se concentrations of all foodstuffs, dietary Se intake and the Se status of individuals (Eurola *et al.,* 1991; Broadley *et al.,* 2006). The Finnish programme has continued to the present day.

In terms of exogenous Se, the mean annual price of commercial-grade Se over the five year period 2005-2009 has ranged from *c*. $50 to 110 USD kg^{-1} (USGS, 2011). If 5 g Se ha^{-1} is deemed to be a suitable target for all Se imported under FISP and assuming that a 25% N-containing product was applied at rate of 50 kg N ha^{-1}, each metric tonne of fertiliser would require incorporation of sufficient Se to treat 5 g ha^{-1}, i.e. 25 g Se t^{-1} fertiliser. This equates to 5000 kg Se to enrich all fertiliser used in the FISP at an additional cost of *c*. $250-550k yr^{-1} for the period 2005-2009 (*c*. 1.6-3.5 US cents $person^{-1}$ yr^{-1}). Clearly there are additional technical and compliance costs associated with the incorporation of Se into granular fertiliser, while Se-enriched fertilisers distributed under FISP may not reach all individual farmers. However, a distribution method based on fertilisers is likely to be more equitable than a supplementation programme which targets certain demographic groups (e.g. children), especially as most individuals in Malawi are likely to be vulnerable to Se malnutrition. It is difficult to envisage a more cost-effective, equitable, or immediate method for alleviating Se malnutrition among the population of Malawi than one based on agronomic biofortification.

4.5 Conclusions

Adequate intake of selenium in the Malawian diet is essential if the country is to mitigate the effects of prevalent diseases such as HIV and AIDS, cancer and heart disease, among others. The feasibility of agronomic biofortification of maize with Se in Malawi has proved successful, and both sodium selenate and Se-enriched granular fertilisers proved equally effective in increasing grain Se concentration. Application of 5 g Se ha^{-1} in the form of sodium selenate or granular fertiliser both provided grain Se concentrations that would deliver adequate selenium intake in Malawi. The results also confirmed that top dressings containing Se were superior to basal dressings in increasing grain Se concentration, and that splitting selenium applications did not affect grain concentration. As Malawi has implemented a fertiliser subsidy, the introduction of Se-enriched granular fertiliser is feasible as a top dressing. Application at 5 g Se ha^{-1} would achieve adequate intake of Se in Malawi. This approach is strongly recommended because it provides a close linkage between the agriculture, nutrition and health areas to support efforts to find sustainable solutions to micronutrient malnutrition; agriculture is likely to become the intervention tool of choice in this fight (Welch and Graham, 2005). The processing methods examined did not affect the Se concentration of flour at the lower rates of Se application, but whole grain flour outperformed the other two flour types in terms of Se concentration at the highest rate of Se application. Agronomic biofortification of maize with Se in Malawi would be at much lower rates of up to 5 g Se ha^{-1} and therefore would not experience significant differences in the Se concentration of flour resulting from the use of different maize grain processing procedures.

CHAPTER 5: Assessing residual availability of selenium applied to maize crops

5.1 Introduction

Agronomic biofortification has been shown to increase Se concentration in various crops, and thereby elevate dietary intake of Se (Broadley *et al.*, 2010, Eurola *et al.*, 2004, Eurola *et al.*, 2005, Lyons *et al.* 2005). Finland introduced agronomic biofortification of their food crops with selenium in 1984 and other countries have since adopted similar policies. Few studies have been conducted to investigate the fate of applied Se in a cropping systems or residual effects of applied Se, Sager and Hoesch, (2006) reported 0.7 to 4.7% of applied Se was transferred to the grain in the field and observed residual effects of applied Se in the second year summer barley crop but the residual effect was not detected in the soil. Stroud *et al.* (2010) reported that speciation of Se extracted by KH_2PO_4 showed that selenate was not detectable before and after harvest but selenite which accounted for 13–70% of extractable Se and the remainder was considered to be soluble organic Se. Martens and Suarez (1997) reported proportion of the total Se extracted by KH_2PO_4 from top soil to be in the range of 1.1–3.4%. Gissel-Nielsen, 1984 reported that the residual extractable Se was too low to produce crops with sufficient Se for human nutrition. However, there is a need to investigate the fate of applied Se within the soil-plant system (Keskinen et al., 2009) to protect against possible contamination of aquatic systems and maximise the efficiency of Se use in biofortification programmes.

Residual Se availability can be assessed simply by growing a second crop, in the following growing season, and determining Se uptake as a function of the original Se application. Alternatively, the remaining 'available' Se in the soil can be measured at harvest of the first (Se fertilised) crop. In practice this normally involves determination of the reactive inorganic Se in the soil and is accomplished by extraction with a competing anion, such as phosphate (Stroud et al., 2010). However, the majority of Se in soil is

covalently bound to humus carbon (Gissel-Nielsen *et al.*, 1984, Masscheleyn *et al.*, 1990, Johnson *et al.*, 1996, Fordyce, 2005, Johnson *et al.*, 2010). It is therefore doubtful that chemical extraction of soil can unambiguously quantify the 'plant-available' Se pool because it makes no allowance for cycling of Se between labile organic and inorganic forms during the course of the growing season. Furthermore, partly as a consequence of organic-inorganic cycling, extraction alone cannot distinguish between Se from native soil sources or fertiliser originally applied in field experiments (Goodson et al., 2003). However, labelling with stable isotopes allows direct measurement of uptake from fertiliser and native soil pools, and can also provide information on the longer term fate of the applied fertiliser (Zapata and Hera, 1995). Stable isotopes are usually preferred as tracers because the use of radioactive isotopes is often limited by their short half lives and associated risk of radiation exposure (Janghorbani, 1981). Stable Se isotopes have been used in human metabolic studies (Finley, 1995, González Iglesias et al., 2007, Janghorbani et al., 1981, Young et al., 1982) and in determining the isotopically exchangeable fraction of inorganic Se in soils (Collins et al., 2006). However, to date, they have not been used in field trials to determine the source apportionment of Se uptake by crops or examine residual availability to subsequent crops.

Studies conducted in Malawi have demonstrated the success of agronomic biofortification of maize with selenium (Chapter 4) and the results indicate that application of 5 g Se ha^{-1} could deliver adequate dietary Se intake throughout the country. However, there is currently no information on the fate of the applied Se, including the extent to which fertiliser selenate mixes with soil Se pools and is retained in a bioavailable form beyond the year of application. Such information could inform future biofortification strategies and identify future research areas aimed at improving Se availability in soils in Malawi. To investigate these processes three strategies were adopted as an adjunct to the field trials described in Chapter 4:

1. Soil samples were taken at final harvest (year 1, 2009) from all plots of the Se liquid trial (0 – 100 g ha^{-1}) described in Chapter 2 and phosphate-extractable Se measured (Section 2.3.6) to determine the remaining reactive inorganic Se.

2. To directly determine uptake of residual Se from the sodium selenate liquid trial plots, maize was re-planted in December 2010, but without application of Se.
3. Isotopically labelled sodium selenate (Selenate-^{74}Se; 10 g ha^{-1}) was applied to maize in plots at the same two test sites, Chitedze and Mbawa, in January 2010, to directly determine the relative contributions from fertiliser and native soil pools to Se uptake by grain and stover - both in the year of application (2010) and in the following year (2011) as a residual effect.

5.2 Materials and Methods

5.2.1 Measurement of residual 'available' Se by phosphate extraction.

All plots at each of the six sites used in the $Na_2SeO_{4(aq)}$ Se uptake trial described in Chapter 4, Section 4.2.3 (liquid application of Na selenate; 0 – 100 g ha^{-1}) were sampled for topsoil (0 – 15 cm) at final harvest in 2009. Soil samples were air dried, sieved to < 2 mm and extracted with 0.016 M KH_2PO_4 (Chapter 2, Section 2.2.3.3). Extractable Se concentration (mg kg^{-1}) was assayed by ICP-MS (Chapter 2, Section 2.2.3.2) and, for comparison with plant uptake, was also converted to units of g ha^{-1} assuming 2500 t ha^{-1} within the topsoil.

5.2.2 Uptake of residual Se by a subsequent maize crop.

A maize crop was planted in the $Na_2SeO_{4(aq)}$ experiment plots at Chitedze and Mbawa in December 2010, the year following the initial trial, to assess the residual availability of applied Se to a following maize crop. The pre-existing ridges were not disturbed but were weeded before planting the second crop on the ridge. The plants were fertilised as previously, but with no addition of Na_2SeO_4. Grain yield was recorded at harvest (2010) and sub-samples of grain were processed for Se analysis as described previously (Chapter 2, Sections 2.3.4 and 2.3.7).

5.2.3 Source apportionment of applied Se using ^{74}Se-labelled sodium selenate.

5.2.3.1 Preparation of ^{74}Se-labelled selenate for field application.

Elemental ^{74}Se (40 mg; >99.9% IA) obtained from Isoflex® (Moscow, Russian Federation) was converted to selenate following a procedure adapted from Collins *et al.* (2006). The Se^0-selenium was weighed, dissolved in 5 mL of 2.0 M KOH with 5 mL of 30 % H_2O_2 and heated at 90 ^oC in a conical flask. The solution was evaporated to dryness and immediately re-dissolved in 10 mL of peroxide solution. The evaporation and re-dissolution was repeated twice more and the salt was then re-dissolved in 10 mL of 2% HNO_3 and transferred to a 100 mL volumetric flask. The conical flask was repeatedly washed with 10 mL aliquots of 2 % HNO_3 and the washings retained in the volumetric flask. To confirm conversion of elemental ^{74}Se to selenate, speciation analysis was undertaken by hyphenated LC-ICP-MS (Thermo Fisher Scientific Inc., Waltham, Madison, USA). In-line chromatographic separation of selenite and selenate was achieved using an anion exchange column (Hamilton PRP-X100, 250 x 4.6 mm, Nevada, USA); the mobile phase was 60 mM NH_4NO_3 adjusted to pH 9.0 with tetra methyl ammonium hydroxide (TMAH) as the mobile phase eluent.

Figure 5.1 shows a comparison of the ^{74}Se-selenate stock solution (b and c) with (^{78}Se) selenate and selenite speciation standards (a) containing 40 ppb Se (Certiprep). Conversion to ^{74}Se-selenate was virtually complete with negligible presence of selenite (Fig. 5.1c). The concentration of the ^{74}Se-selenate stock solution was 478 mg L^{-1}, measured by ICP-MS in H_2-cell CCT-KED mode (Section 5.2.3) following determination of the sensitivity (counts-per-second-per-micromol per litre'; CPS μM^{-1}) of the ^{74}Se isotope. This was achieved by extrapolation of measured sensitivity (CPS μM^{-1}) against isotope mass for all Se isotopes using conventional calibration standards (Certiprep, single element standard) at 20, 40 and 100 µg L^{-1} total Se (Fig. 5.2) assuming normal isotopic abundances and thereby converting isotope concentrations to µM units.

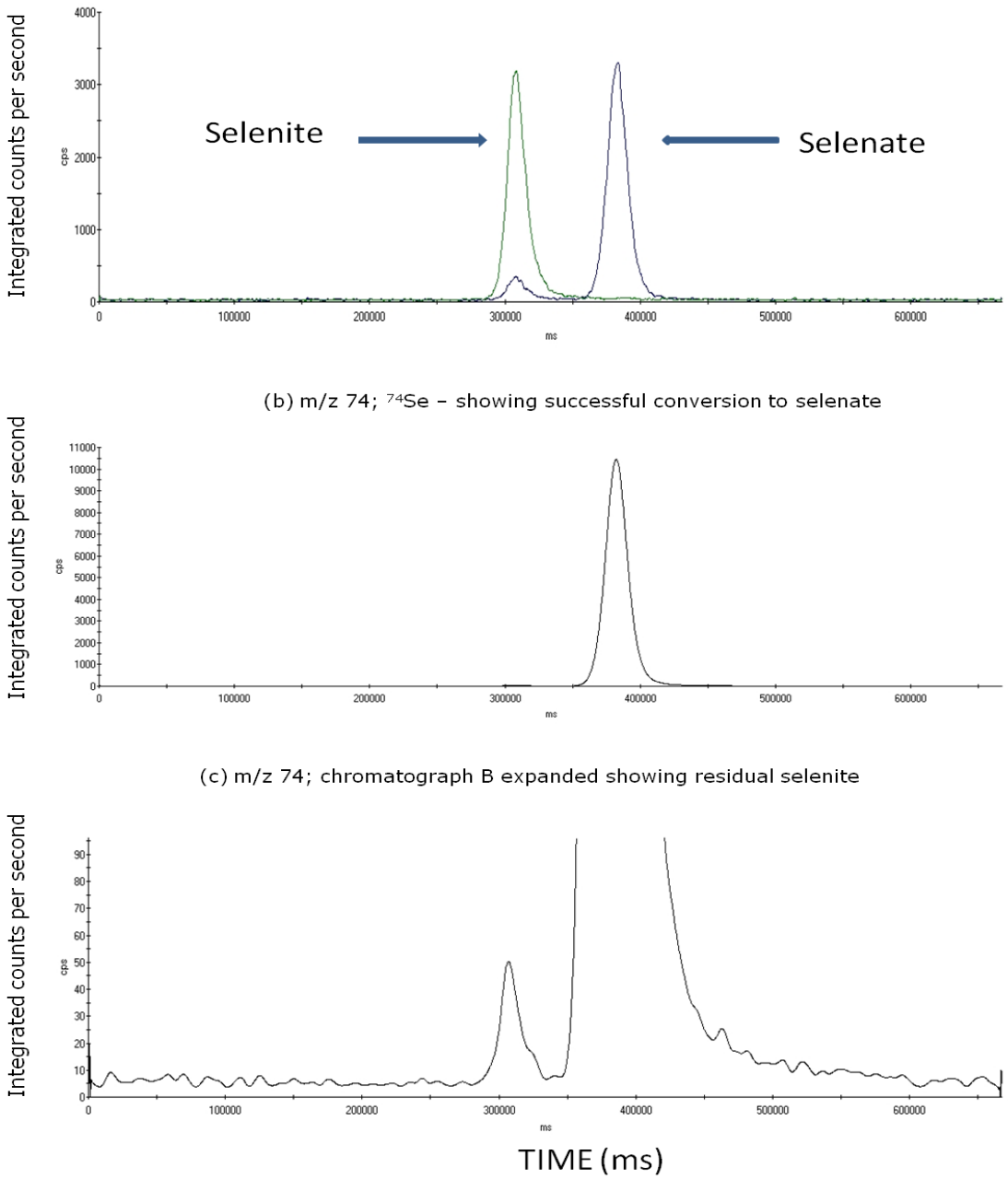

Figure 5.1. HPLC-ICP-MS chromatograph timecourses (ms) showing (a) selenite and selenate speciation standards measured at 78 m/z (^{78}Se), (b) ^{74}Se isotopic stock solution, measured at 74 m/z, demonstrating successful conversion to selenate and (c) chromatograph B with expanded y-axis (ICPS) to illustrate the very small degree of selenite contamination of the ^{74}Se-selenate stock solution.

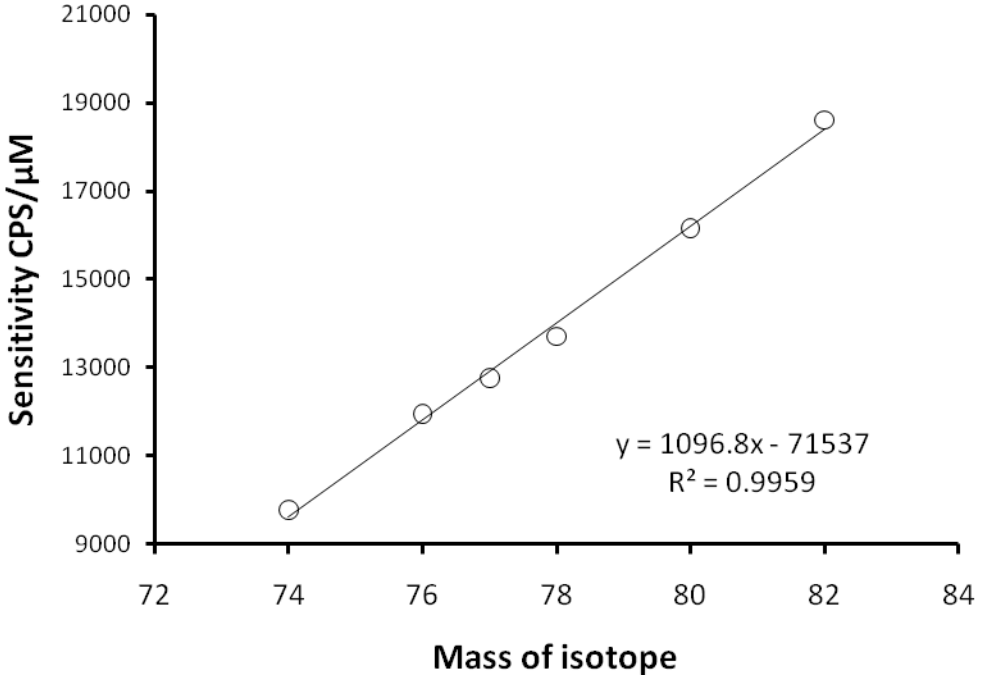

Figure 5.2. Sensitivity (counts per second (cps) per µmol L^{-1}) for all stable Se isotopes as a function of atomic mass.

The extrapolated sensitivity of the ^{74}Se isotope was used to calculate the concentration of the stock solution required for an application of 10 g ha^{-1} in the Chitedze and Mbawa trials.

5.2.3.2 Isotopically labelled field trial

A field experiment was laid out at both sites using a randomised complete block design with four replicates. Land was ploughed and the plots were surrounded by ridges to prevent soil erosion. The rows were 75 cm apart and maize was planted at 25 cm intervals within rows. The trial was planted on 15 December and 21 December 2009 at Chitedze and Mbawa respectively. All plots received 92 kg N ha^{-1}, 20 kg P_2O_5 ha^{-1} and 10 kg K_2O ha^{-1}. One level of ^{74}Se was applied as sodium selenate ($Na_2{}^{74}SeO_4$) solution at a rate equivalent to 10 g Se ha^{-1}. Plot size was 4 m^2 (2 m x 2 m); each of the two sites therefore required 16 mg Se (average atomic mass 78.96), equivalent to 14.98 mg ^{74}Se. This was delivered to Malawi as two aliquots (31.36 mL) of the ^{74}Se stock solution (478 mg L^{-1}), to be made up to a

working solution for application to the four plots at each site. Each aliquot of ^{74}Se-selenate (31.36 mL) was dissolved in 5 L water and sprayed on to the four replicate plots at the Chitedze and Mbawa sites. Selenium application was carried out at the top dressing stage, after approximately four weeks of crop growth on 19 January and 21 January 2010.

At harvest, the central 1 m^2 of each plot was used to estimate yield and provide soil and plant samples for analysis. Stover and grain were harvested from the net plot (middle row) and weighed. Sub-samples of grain and stover were dried, milled and analysed for ^{78}Se and ^{74}Se using ICP-MS (Section 5.2.3). Soil-derived Se (Se_{soil}) in all digested flour and stover samples was measured as 'total' Se using a calibration based on ^{78}Se and assuming normal isotopic abundances and no isotopic discrimination. Calibration standards for ^{74}Se were derived from the ^{74}Se-selenate stock solution. Total ^{74}Se concentrations in flour and stover were attributed to fertiliser ($^{74}Se_{fert}$) and soil sources assuming that total Se uptake measured from the ^{78}Se calibration (Se_{soil}) included normal background levels of ^{74}Se (0.89% isotopic abundance). All gravimetric assays of ^{74}Se were converted to an equivalent quantity of Se with a normal isotopic abundance by multiplying by 1.068 (79/74). The gravimetric proportion (%) of fertiliser-derived Se in flour and stover (Se_R) was calculated as:

$$Se_R = \frac{^{74}Se_{fert} \times 1.068 \times 100}{Se_{soil} + (^{74}Se_{fert} \times 1.068} \qquad \text{Eq. 5.1}$$

In the following growth season (December 2010) the maize was re-sown, without further Se application, but with standard fertiliser application, and the harvest and analysis of grain flour was repeated (May, 2011) to directly measure residual fertiliser Se uptake.

5.3 Results

5.3.1 Effect of sodium selenate application on residual soil Se

5.3.1.1 Changes in total soil Se concentration.

Total soil Se concentration was not expected to differ substantially with Se application. The maximum treatment (100 g ha^{-1}) could only produce a change in total soil Se content of 0.04 mg kg^{-1}, assuming no plant uptake, complete retention within the topsoil and a topsoil mass of 2500 t ha^{-1}. A small significant difference resulting from addition of 100 g ha^{-1} was only seen in the topsoil at Mbawa and Ngabu (Fig. 5.3 e, f); no similar effect was apparent in the sub soil.

5.3.1.2 Changes in phosphate-extractable Se concentration in soil.

Phosphate-extractable Se (g ha^{-1}) increased at all sites with increasing Se application (g ha^{-1}) with linear responses in all cases (Fig. 5.4). The residual soil Se concentrations, expressed as a percentage of the initial application, were estimated from regression slopes in Fig. 5.4. Thus concentrations of residual inorganic Se were 1.78, 0.70, 3.47, 3.58, 4.76 and 26.47% of initial applications for Bvumbwe, Chitala, Chitedze, Makoka, Mbawa and Ngabu, respectively. Although the six sites represent a small number of data from which to draw conclusions there was a broad relationship between residual Se and soil pH value (Fig. 5.5) which was similar to the pattern of Se availability seen in the farmers' fields study discussed in Chapter 3. Applied Se appears to remain in an available form to a much greater extent in the calcareous vertisols at Ngabu compared to the acidic soils which cover most of Malawi.

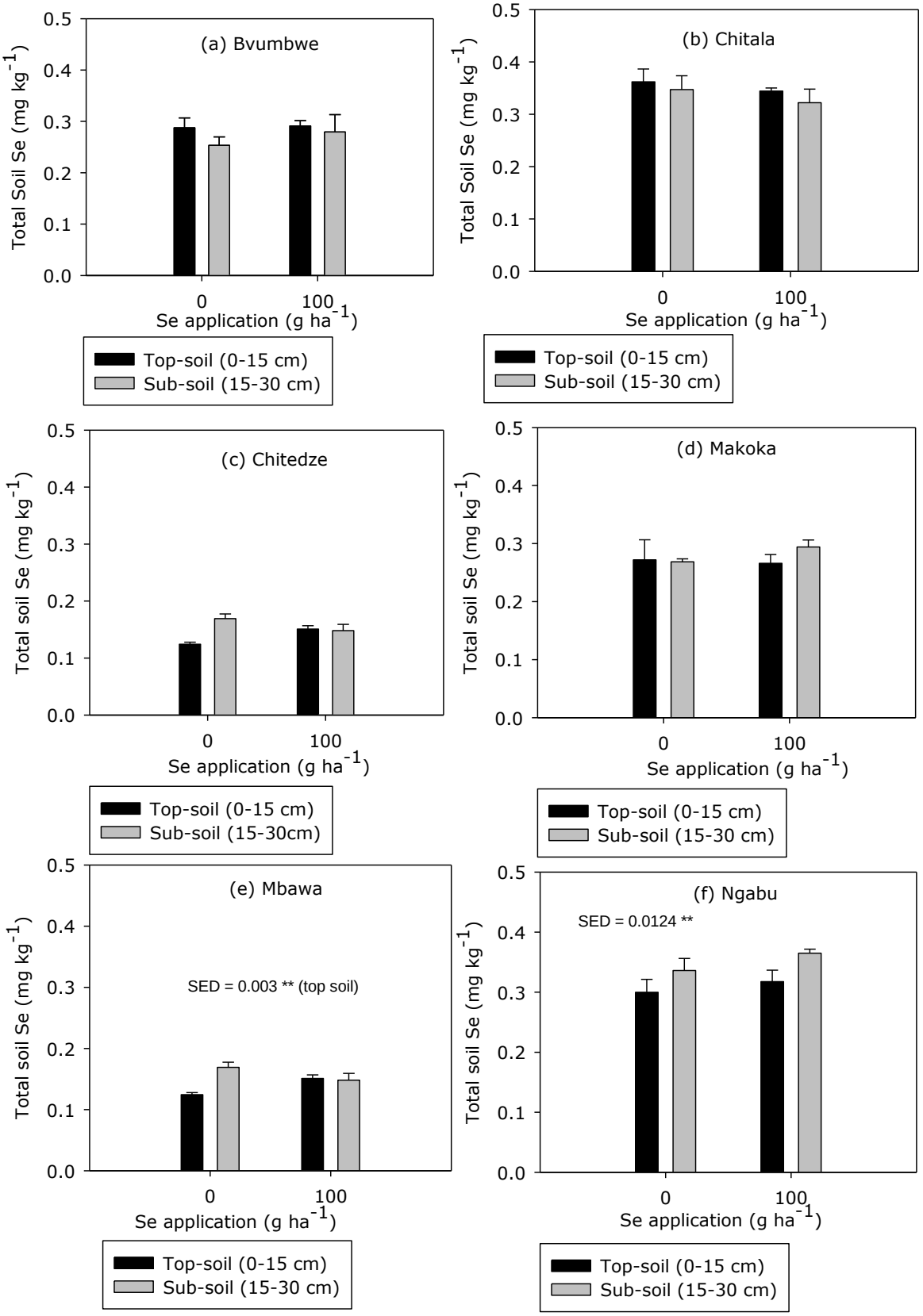

Figure 5.3. The effect of Se application on residual total soil Se at sodium selenate liquid trials sites: (a) Bvumbwe, (b) Chitala, (c) Chitedze, (d) Makoka, (e) Mbawa and (f) Ngabu. Single standard errors of the mean are shown.

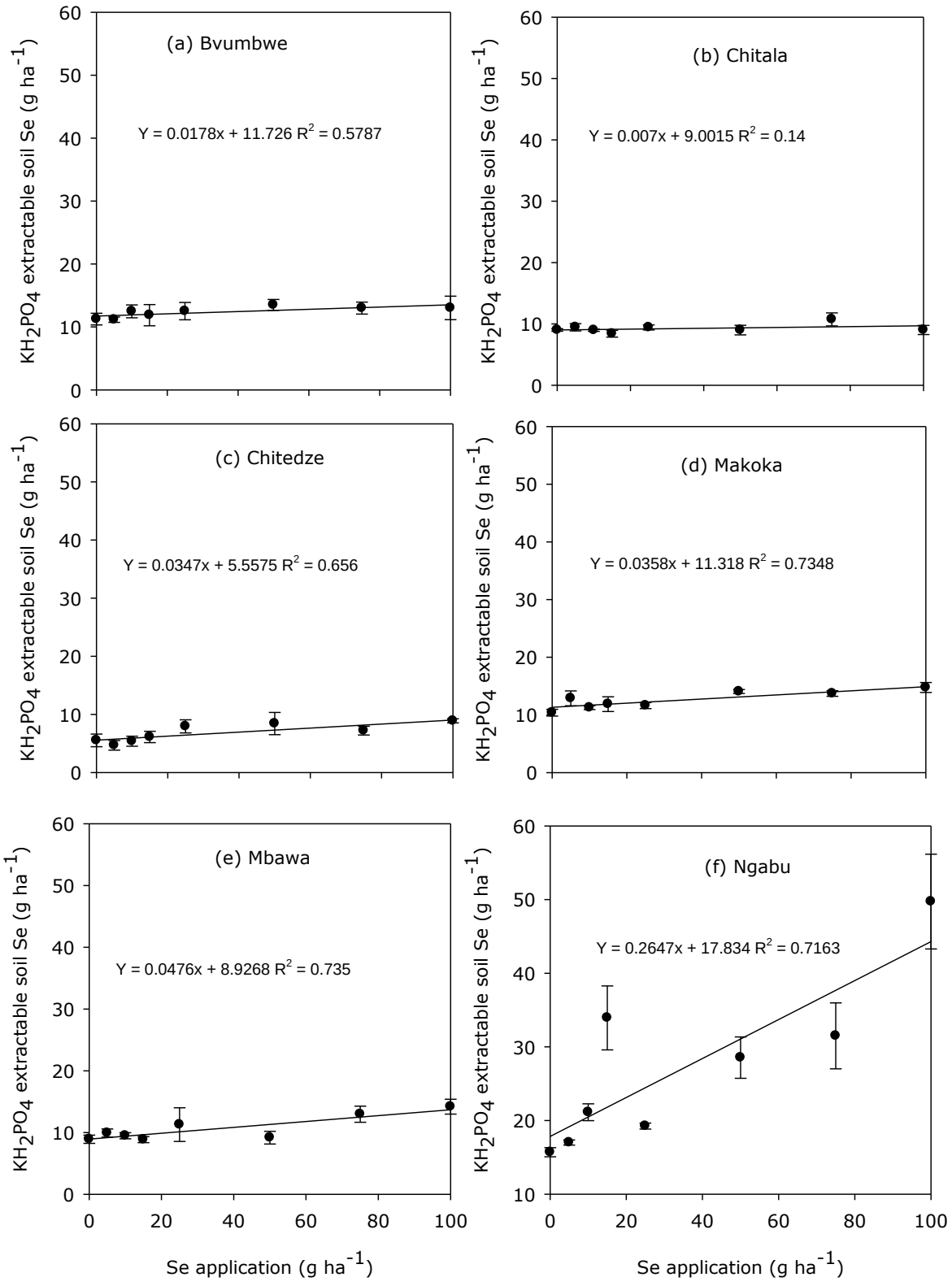

Figure 5.4. Relationship between residual soil Se extracted by KH_2PO_4 and initial Se application rate at sodium selenate liquid trials sites: (a) Bvumbwe, (b) Chitala, (c) Chitedze, (d) Makoka, (e) Mbawa and (f) Ngabu.

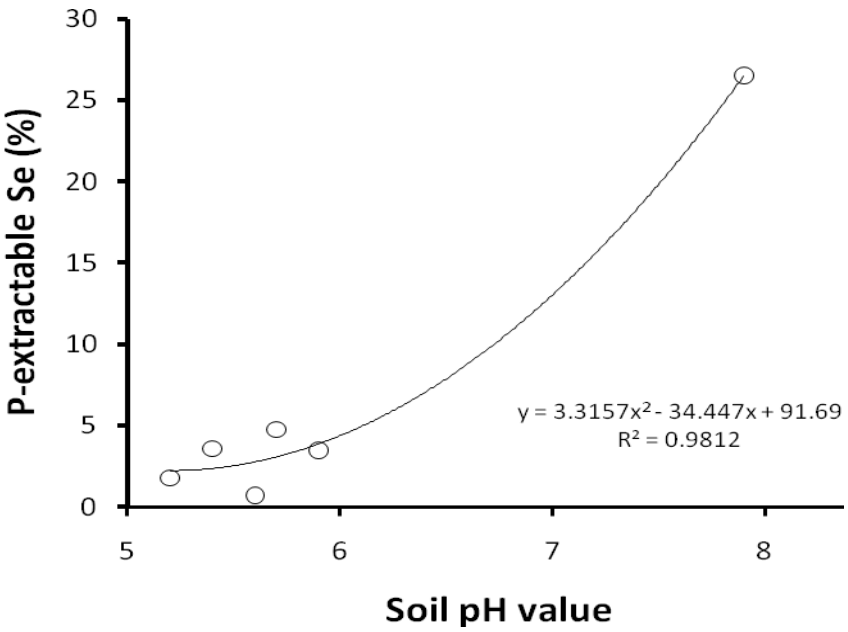

Figure 5.5. Relationship between (residual) phosphate-extractable Se at harvest, and soil pH value, for sodium selenate liquid trials sites (Chapter 3) at Bvumbwe, Chitala, Chitedze, Makoka, Mbawa and Ngabu.

5.3.2 Effect of residual soil Se on grain Se in a following crop at Chitedze and Mbawa

Uptake of residual Se was clearly seen in subsequent maize crops at Chitedze and Mbawa with responses of 0.303 and 0.586 µg Se kg^{-1} per g ha^{-1} respectively, (Figs. 5.6 a, c). The responses were linear and the correlation coefficients (R^2) were 0.907 and 0.6004 at Chitedze and Mbawa, respectively (Figs. 5.6 a, c). The grain Se concentration response was highly variable at Mbawa (large standard errors), probably due to substantial variation in crop yield between blocks and plots due to maize stripe virus disease which attacked the maize crop (Chapter 4). By comparison yields were much more consistent at Chitedze. However, when the data were re-plotted as uptake values (g ha^{-1}) (Figs 5.6 c, d), rather than concentrations, the Mbawa response followed a much more consistent trend. A comparison of Figs 5.6c and 5.6d strongly suggests that (i) uptake is limited by availability in soil, rather than plant demand, and/or (ii) uptake of residual available Se occurs at an early stage in plant development and so is less likely to be affected by subsequent growth limitations.

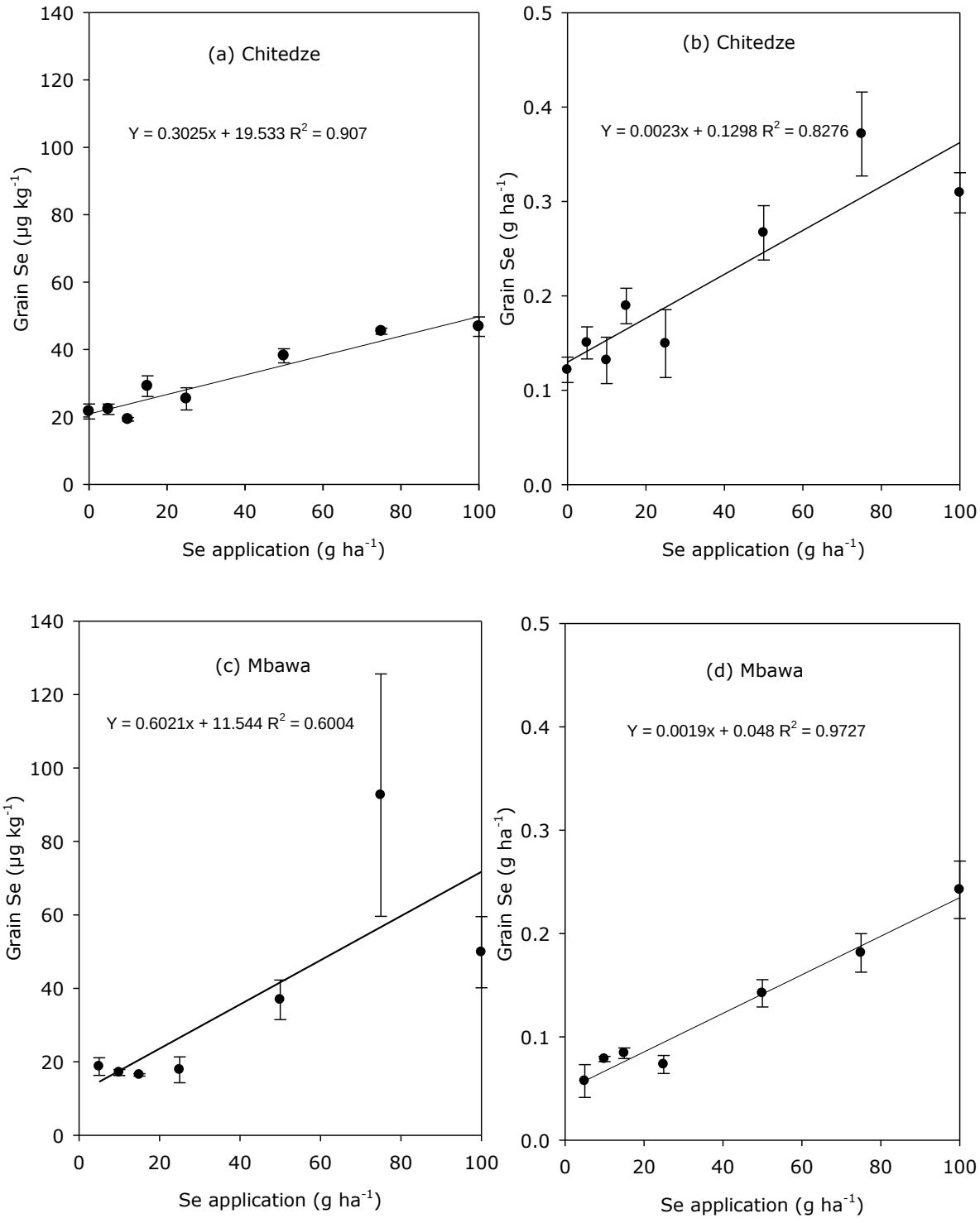

Figure 5.6. Residual effect of Se application as indicated by grain Se concentration in the subsequent year: (a) and (b) grain Se concentration and grain Se uptake at Chitedze; (c) and (d) grain Se concentration and grain Se uptake at Mbawa. Double standard errors of the mean are shown.

5.3.3 Selenium source apportionment using isotopic dilution with ^{74}Se.

5.3.3.1 Overview.

The grain and stover analysis for ^{74}Se was successful but the analysis of ^{74}Se in digested soil samples was compromised by very high levels of interference, partly from ^{74}Ge but mainly as a result of polyatomic species such as ^{57}Fe-^{17}OH or ^{56}Fe-$^{18}OH_2$ which were not completely removed in the hydrogen-cell hexapole. This effect was also seen, to a minor degree, in stover samples; these were contaminated by soil dust to a greater extent than grain flour. Table 5.1 shows the results obtained at harvest in the year of ^{74}Se-selenate application.

Table 5.1. Mean selenium concentration (mg kg^{-1}) and recovery (g ha^{-1}) in grain and stover at Chitedze and Mbawa; source apportionment between fertiliser and soil was determined by isotopic dilution of ^{74}Se applied as sodium selenate at 10 g Se ha^{-1}; standard errors of the mean are shown in brackets. Control plots did not receive ^{74}Se fertiliser.

Variable	**Chitedze**			**Mbawa**		
	^{74}Se plots Se from fertiliser	^{74}Se plots Se from Soil	***Control*** Se from soil	^{74}Se plots Se from fertiliser	^{74}Se plots Se from soil	***Control*** Se from soil
Grain Se conc (mg kg^{-1})	**0.056** (±0.0051)	**0.023** (±0.0019)	**0.019** (±0.0003)	**0.059** (±0.0066)	**0.013** (±0.0009)	**0.026** (±0.0008)
Stover Se conc (mg kg^{-1})	**0.031** (±0.0035)	**0.017** (±0.0009)	**0.017** (±0.0042)	**0.027** (±0.0050)	**0.008** (±0.0013)	**0.008** (±0.0008)
Grain Se (g ha^{-1})	**0.304** (±0.0310)	**0.125** (±0.0020)		**0.595** (±0.0180)	**0.131** (±0.0009)	
Stover Se (g ha^{-1})	**0.342** (±0.0035)	**0.177** (±0.0083)		**0.485** (±0.0046)	**0.135** (±0.0113)	
Total Se (g ha^{-1})	**0.646** (±0.1225)	**0.302** (±0.0057)		**1.08** (±0.1105)	**0.266** (±0.0060)	

5.3.3.2 Recovery and partitioning of fertiliser-^{74}Se in the year of application

The fertiliser-derived Se concentrations in grain and stover (Se_R; Eq. 5.1) differed significantly between the experimental sites (Fig. 5.7a), being higher in grain and stover at Mbawa (82, 77%) than at Chitedze (71, 65%) respectively ($P<0.05$; Fig. 5.3a). Similarly, the total (above ground) recovery of Se from fertiliser and soil sources is shown for both sites in Fig. 5.7c and emphasises the lower supply potential in the soil at Mbawa and the greater reliance on fertiliser Se at this site, compared to Chitedze. The total recoveries of applied Se (10 g Se ha^{-1}) in the crop were 0.65 and 1.08 g Se ha^{-1}, representing 6.5 and 10.8% of the applied Se at Chitedze and Mbawa respectively ($P<0.05$; Fig. 5.3b). The results are closer to the liquid experiments Se recovery conducted at Chitedze and Mbawa which gave above ground recovery ranging from 12.6 to 15.7% for the two sites (Chapter 4, Table 4.7). The differences in Se could be due to different Se application rates and crop yield, as was reported that low Se recovery at one site was a reflection of large yield differences between sites (Curtin *et al.,* 2006). Overall partitioning of Se to the grain and stover, from fertiliser and soil sources differed significantly between the sites ($P<0.001$; Fig. 5.3d), with the majority of the Se within the shoots being trans-located to the grain. The ratio of stover fertiliser derived Se to grain fertiliser derived Se or grain soil derived Se to stover soil derived Se was higher at Chitedze than at Mbawa. This indicates that Se translocation from stover to grain was more efficient at Mbawa than at Chitedze. However, most of the fertiliser derived Se was trans-located to grain while the soil derived Se remained in the stover at both of the sites and soil derived Se was higher at Chitedze both in the grain and stover than at Mbawa.

Comparison of the ^{74}Se fertilised and unfertilised control plots (Table 5.1 and Fig. 5.8) suggests that total Se uptake in control plots was similar to levels of soil-derived Se in maize grown in plots receiving 10 g ha^{-1} ^{74}Se-selenate. This suggests that there was minimal isotopic dilution of ^{74}Se within the soil Se pool prior to uptake and no evidence of possible 'priming effects' of native soil Se availability arising from the addition of Se fertiliser

at Chitedze (Figs. 5.8 a, c) and Mbawa (Figs. 5.8 b, d) although the Mbawa control plot seem to indicate Se application decreased soil derived Se in the maize grain but there was no effect in the stover. The results support the earlier findings that at Mbawa the fertiliser derived was higher than at Chitedze while the soil derived was higher at Chitedze. This may imply that Se uptake by the crop was rapid following fertiliser application, or that the added Se simply did not perturb native soil Se dynamics.

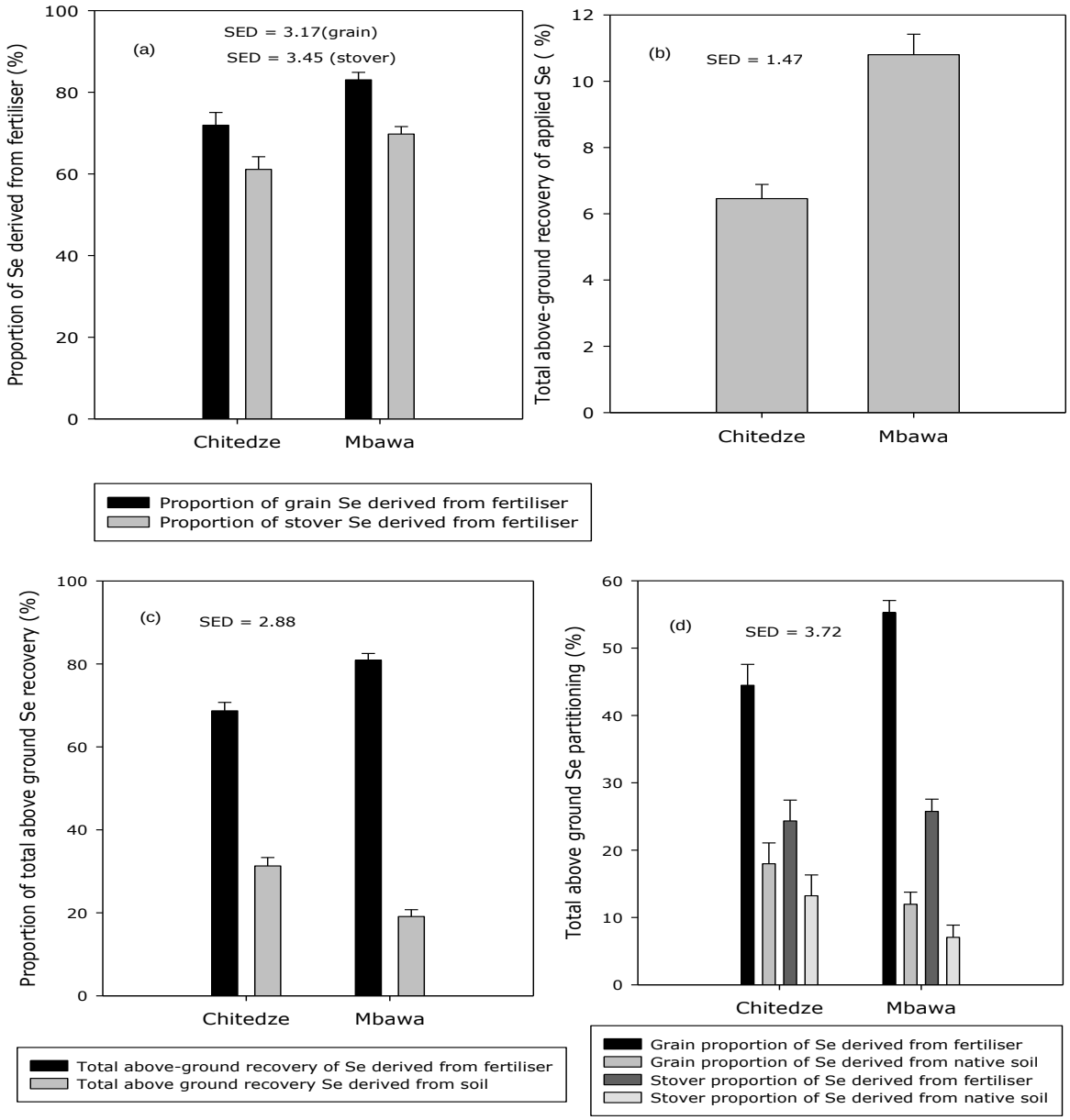

Figure 5.7. (a) Proportion of Se in grain and stover derived from fertiliser, (b) total above-ground recovery (% of 10 g ha^{-1} Se application), (c) total above-ground proportion (%) of Se derived from fertiliser and soil (d) partitioning of Se derived from fertiliser and native soil sources to grain and stover at Chitedze and Mbawa. Single standard errors of the mean are shown.

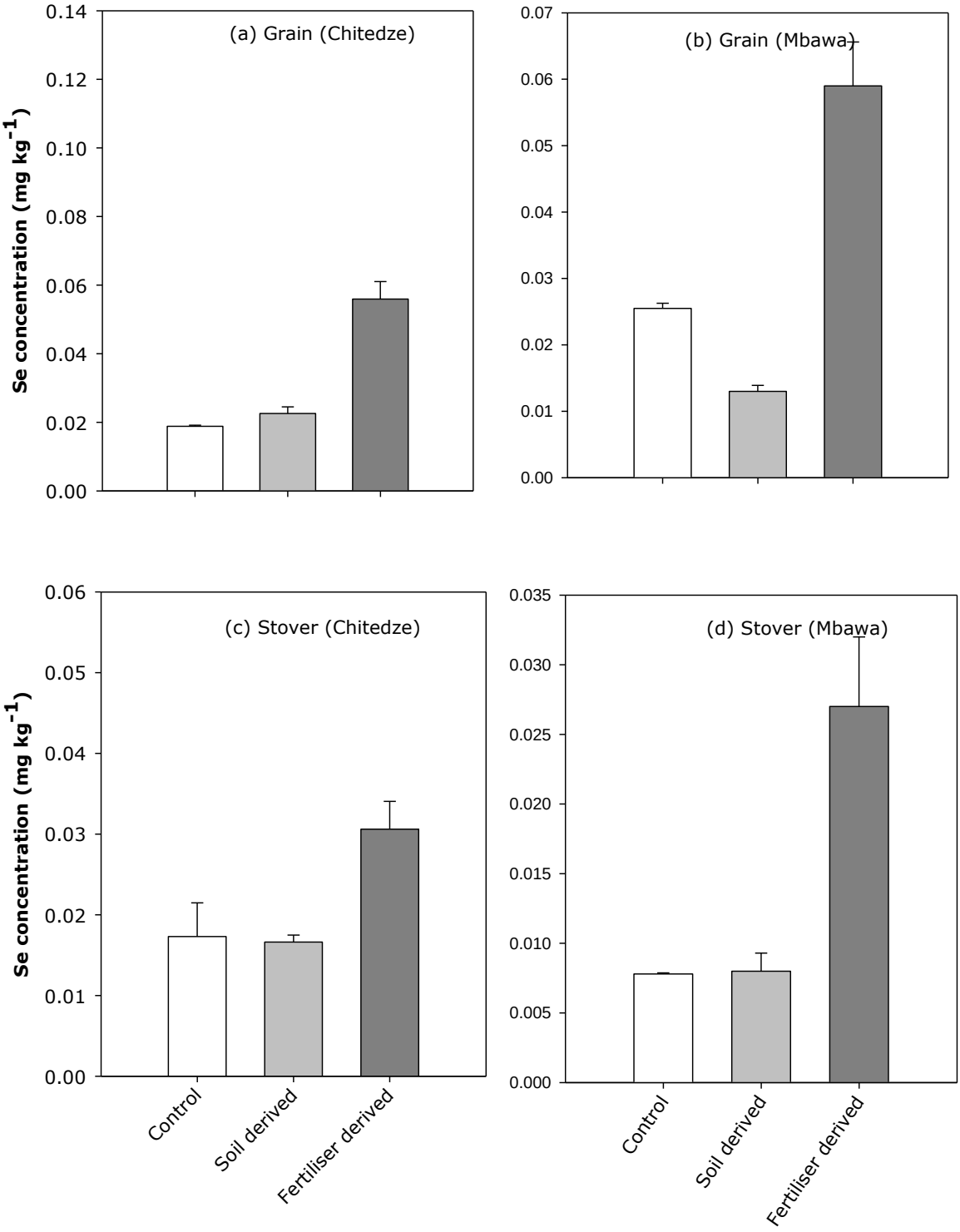

Figure 5.8. Source apportionment of Se in grain at Chitedze (a) and at Mbawa (b) and stover of maize at Chitedze (c) and at Mbawa (d), in control plots (zero Se-fertilisation, white bars) and plots treated with ^{74}Se at 10 g Se ha^{-1} (shaded bars). Note the differing y-axis scales for grain and stover. Single standard errors of the mean are shown.

5.3.3.3 Residual availability of ^{74}Se fertiliser in the year following application

Residual ^{74}Se uptake from the ^{74}Se-applied plots at Chitedze and Mbawa was measured in crops grown in the following year without Se fertiliser addition (Fig. 5.9). The degree of ^{74}Se isotopic enrichment over background was very small and not measurable in stover samples because of greater variability caused by Fe-based polyatomics arising from soil contamination (see Section 5.3.3.1). Uptake of ^{74}Se was slightly greater at Chitedze than at Mbawa but extremely small at both sites. There was only 0.50 µg ^{74}Se kg^{-1} from fertiliser in the grain flour at Chitedze and 0.087 µg kg^{-1} at Mbawa (Fig. 5.9 a). Thus, the proportion of grain flour Se arising from the original ^{74}Se fertiliser application was only 2.0% (±0.56) at Chitedze and 0.78% (±1.1) at Mbawa. It is probably unwise to draw any conclusions regarding differences between the sites with such low and highly variable data from Mbawa. Figure 5.9 shows grain Se data both in the year of application and in the residual year, for comparison. It is noticeable that soil derived Se was very consistent over the two years and, as already discussed (Table 5.1; Fig. 5.9 b, d), consistent in comparison with control plots also.

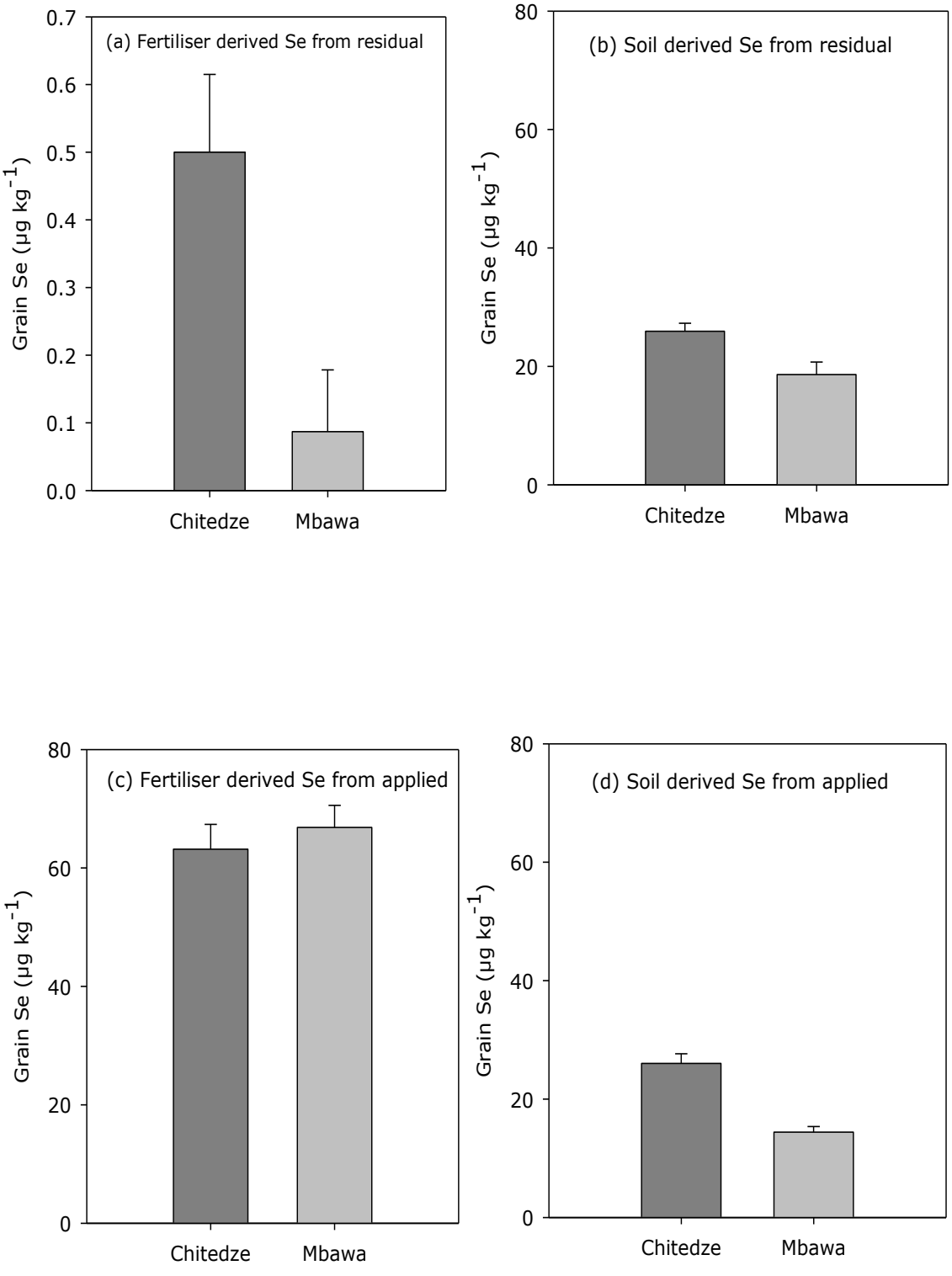

Figure 5.9. (a) Residual effect of ^{74}Se application on grain Se concentration originating from the fertiliser; (b) soil derived Se in the grain flour of the residual crop; (c) grain ^{74}Se concentration from fertiliser in the year of application; (d) soil derived portion Se in the year of application of the ^{74} Se isotope.

5.4.1 Soil control over residual Se

Residual fertiliser Se, measured as phosphate-extractable inorganic Se at harvest, appeared to be a function of soil properties. In particular, the trend with pH closely followed the availability of native soil Se seen in Chapter 3. Inorganic Se in acidic soils exists mainly as selenite which is strongly adsorbed by Fe or Al hydrous oxides and is not readily available for plant uptake (Elrashid et al., 1987, Geering et al., 1968). Duc *et al.* (2006) reported that the amount of selenite adsorbed on Fe oxides surfaces increases as the soil pH decreases in keeping with known patterns of weak acid anion adsorption on variable charged surfaces. Selenite is also known to compete with phosphate for adsorption on oxide surfaces and substitution into phosphate minerals (Hayes et al., 1987, Dhillon and Dhillon, 2000, Duc et al., 2003) thereby reducing or increasing Se availability for plant uptake depending upon phosphate status.

However, phosphate-extractable Se, measured at harvest in liquid selenate trial plots, was considerably greater than the offtake measured in a following crop. Uptake of grain Se derived from the applied fertiliser in a second crop was 0.0023 and 0.0019 g grain Se ha^{-1} per g Se applied ha^{-1} at Chitedze and Mbawa, respectively (Fig. 5.6), giving a recovery of 0.23 and 0.19% of the originally applied Se. By contrast, the proportions of KH_2PO_4-extractable Se derived from the applied Se (at harvest in the year of application) were 0.0347 and 0.0476 g soil Se ha^{-1} per g Se applied ha^{-1} at Chitedze and Mbawa (Fig. 5.4), representing 3.47 and 4.76% recovery of the original applied Se. Thus uptake in a second crop amounted to less than 5% of the residual fertiliser Se available in the topsoil at harvest of the preceding (Se fertilised) crop. This is similar to the low recoveries seen with respect to primary Se applications in the trials discussed in Chapter 4. In addition, the residual Se measured in soils at harvest of the initial liquid selenate trials would have been subject to further losses by leaching, and

possibly fixation into organic forms or within Fe oxides as selenite, prior to the sowing of a second crop 6 months later. Several studies have reported that applied Se is fixed within a few months of application, thereby becoming unavailable for uptake by plants (Gissel-Nielsen and Bisbjerg, 1970, Mikkelsen et al., 1989).

There is also considerable evidence from plant responses that Se uptake is limited by soil-controlled availability, rather than plant demand. For example, the responses to Se fertiliser seen in Chapter 4 were almost invariably linear on all soils with no sign of a demand limitation up to an application of 100 g ha^{-1}. Furthermore, the large variation in yield and grain-Se concentration in the residual crop at Mbawa nevertheless produced a remarkably consistent offtake trend (c.f. Figs 5.6c and 5.6d), again suggesting soil control over availability rather than limited plant demand.

5.4.2 Comparison of Mbawa and Chitedze

The significant differences in the amount of Se recovered between the two sites suggest that the soil at Mbawa was less able to supply native Se sources than that at Chitedze, or that the fertiliser-Se was conserved in a bio-available form for longer at Mbawa. Although total soil Se concentration was higher at Chitedze (0.300 mg kg^{-1}) than at Mbawa (0.124 mg kg^{-1}), KH_2PO_4-extractable Se was higher at Mbawa (0.0061 mg kg^{-1}) than at Chitedze (0.0032 mg kg^{-1}), suggesting that the applied Se remained in an available form for longer at Mbawa.

From the initial ^{74}Se trial, the proportions of Se in maize that originated from fertiliser, in the year of application, were 68.7% and 80.9% at Chitedze and Mbawa respectively ($P<0.001$; Fig. 5.7). The above ground plant recovery of the applied ^{74}Se-selenate was greater at Mbawa (10.8%) than at Chitedze (6.5%). These recovery levels, are similar to reported Se recoveries of 2-6% (Stephen et al., 1989); recoveries of 10% have been recorded in Finland (Eurola, 2005). However, higher recoveries of 18-20% have been reported when Se was applied as top dressing (Curtin et al.,

2008, Lyons et al., 2004). Comparing maize recovery of fertiliser Se applied at Mbawa (10.8%) with the level at Chitedze (6.5%) it appears again that that Se availability after fertiliser application depends on soil properties as there were significant differences between the soils at the two sites examined (Chapter 4, table 4.1). Thus, plants grown on soil which maintain a relatively higher level of Se availability (as phosphate-extractable Se), in this case Mbawa, also take up a greater proportion of newly applied Se.

The second crop sown on the isotopically labelled plots at Chitedze and Mbawa accessed very little of the original ^{74}Se-labelled fertiliser. Only 2.0% and 0.78% of the grain Se taken up originated from the fertiliser. If we assume that all of the original ^{74}Se (10 g ha^{-1}) was retained in the soil, neglecting plant uptake in year 1 and assuming negligible leaching losses, then this would constitute 1.3% and 3.0% of the topsoil Se at Chitedze and Mbawa respectively (assuming 2500 t soil ha^{-1}). Therefore, after one growing season the added Se appears to have been completely assimilated into the soil pool and was only proportionately represented in the maize. An alternative explanation could be that much of the ^{74}Se has been lost and in fact the remaining ^{74}Se is more bioavailable than soil Se. Unfortunately this question could not be resolved because (i) the ^{74}Se in the grain was present at extremely small concentrations with large standard errors and was therefore analytically suspect and (ii) it was not possible to measure residual ^{74}Se in the soil directly due to analytical interferences (Section 5.3.3.1).

CHAPTER 6: Dietary intake of macro- and other micronutrients from a maize-based diet in Malawi

6.1 Introduction

Humans require over 22 mineral elements, all of which can be supplied by an appropriate diet (White and Broadley, 2005). Adequate dietary intake of essential minerals is becoming increasingly evident because keeping a balance of all essential minerals in the human body is a key to maintaining a healthy population. For example, calcium (Ca) is essential for developing and maintaining healthy bones and teeth and assists in blood clotting, muscle contraction, and nerve transmission, oxygen transport, cellular secretion of fluids and enzyme activity, and optimal intake of Ca would help reduce risk of osteoporosis (Soetan et al., 2010). Magnesium (Mg) is one of the macro-nutrients which activates over 100 enzymes and helps nerves and muscles to function, while potassium (K) regulates heartbeat, maintains fluid balance and helps muscles to contract (McArdle et al., 2006).

Zinc (Zn) is a essential component of over 200 enzymes involved in digestion, metabolism, reproduction and wound healing and has a critical role in immune responses (Soetan et al., 2010). Copper (Cu) is essential for the normal formation of red blood cells and connective tissue, while iron (Fe) is needed for red blood cell formation and oxygen transport throughout the body. Manganese (Mn) is a key component of enzyme systems and supports brain function and reproduction, while molybdenum (Mo) contributes to normal growth and development as a key component of many enzyme systems including enzymes involved in detoxification. Chromium (Cr) helps in glucose metabolism and regulates blood sugar, while cobalt promotes the formulation of red blood cells and serves as a component of *Vitamin* B-12.

Cereal-based diets are typically deficient in Fe, Zn, Ca, Mg, Cu, iodine (I) and Se (White and Broadley, 2005). It has been estimated that, of the current global population of 6 bn people, 60-80% are Fe deficient, >30%

are Zn deficient, 30% are I deficient and *c*. 15% are Se deficient (Combs, 2001, Kennedy et al., 2003. Developing countries subsisting on cereal-based diets are deficient in Fe and Zn (Kennedy et al., 2003) because such diets are characterised by high intakes of staple foods and low intakes of vegetables, fruit, animal and fish products which are rich sources of minerals (White and Broadley, 2005). Deficient intake of Ca in rural populations in developing countries is prevalent because Ca concentrations in cereal grain are low and shift from bean-rich to cereal-rich diets (Graham et al., 2001). The mean intake of Ca, K and Mg in Sweden is 1110, 3320 and 285 mg d^{-1} respectively (Becker et al., 2011), confirming that there is an adequate intake of Ca in developed countries where diets are diversified. However, deficient intake of Cu has been reported in both the developed and developing World (Welch and Graham, 2002). Studies in Nigeria showed that Cu and Zn concentrations in maize were 2.33 and 33.4 mg kg^{-1} respectively, and mean estimated dietary intakes for adults were respectively 2.64 and 15.5 mg d^{-1} for Cu and Zn (Onianwa et al., 2001). These values are both higher than the RDA (Table 6.1).

Table 6.1. Required daily allowance (RDA), nutrient intake (RNI) and upper limits (UL) for specific mineral elements.

Mineral element	**RDA[1]**	**RNI[2]**	**UL[3]**
K (mg)	1600-3500	3500	-
Ca (mg)	1000-1200	700	2500
Mg (mg)	310-420	300	350
Fe (mg)	8.0-18.0	11.4	45
Zn (mg)	8.0-11.0	9.5	40
Mn (mg)	1.8-2.3	>1.4	11
Cu (mg)	0.9	1.2	10
Mo (µg)	45	50-400	2000
Cr (µg)	25-35	>25	-
Ni (µg)	100-300	-	-

Source: White and Broadley, 2005; [1]Required Daily Allowance, USA
[2]Required Nutrient Intake, UK (Broadley and White, 2010)
[3]Upper limits; [4]World Health Organization

The required nutrient intakes which are within the permissible RDA ranges shown in Table 6.1 should provide appropriate guidelines for any country. Among the essential mineral elements required for human health are Ca, Mg, K, Fe, Zn, Cu, Mo, Cr, Mn and Ni, and intake of these must come from an appropriate diet. It has been stated that accurate and adequate food composition data are essential to estimate the adequacy of intakes of essential nutrients and assess exposure risks resulting from the intake of toxic elements (Barberá et al., 1993). Minerals are essential for human health but can be toxic if taken in excess and therefore there is a need to maintain concentrations of minerals within the accepted ranges in order to attain adequate intakes and avoid toxic effects.

In addition to essential elements, there are also concerns over the potential adverse health effects of ingestion of some heavy metals, particularly Cd. Cadmium is regarded as possibly the most serious inorganic contaminant of the modern age because it is an accumulative poison whose danger lies on regular consumption of even low concentrations(CAOBISCO, 1996). At high Cd intake levels, potentially creating serious human health problems can arise since, and Cd is a carcinogen that affects the kidneys and generates various toxic effects in the body (Mudgal et al., 2010, Barberá et al., 1993). Elevated soil concentrations of Cd may result from the application of metal-rich sewage sludge, farmyard manure, phosphate fertilisers or atmospheric deposition, or may occur naturally where soils are derived from parent material which is rich in Cd (Alloway and Steinnes, 1999). A provisional Cd daily intake by humans of 70 μg d^{-1} has been set to avoid adverse health outcomes (Adams et al., 2004). Recently the European Union introduced legislation defining a maximum permissible concentration (MPC) of 0.1 mg Cd kg^{-1} in food stuffs and for cereals excluding wheat grain, bran, germ and rice (Adams et al., 2004).

The existing data for nutrient concentrations in the staple food crop, maize, in Malawi, where this crop is the main component of the diet, are inadequate. Reliable computation of the dietary intake of Ca, Mg, K, Fe, Mn, Zn, Cu, Cd, Cr, Mo and Ni requires data on the concentrations of these elements in maize grain. The present study therefore determined the

concentrations of these elements in grain and estimated dietary intake from maize.

6.2 Materials and Methods

Samples of maize grain collected from farmers' fields throughout Malawi were subjected to multi-elemental analysis using the sampling and analytical procedures described in Chapter 2. Mean *per capita* consumption of maize in Malawi from the most recent FAO data (FAO, 2011) was used to compute dietary intake.

6.3 Results

6.3.1 Macronutrients

Calcium concentration in grain ranged from 17.7 to 98.8 mg kg^{-1}, with a mean of 39.6 mg kg^{-1} (Fig. 6.1a). Estimated mean intake from maize ranged from 6.28 to 45.1 mg Ca d^{-1}, with a mean of 14.35 mg d^{-1} (Fig. 6.1d). This is much lower than the RDA of 1000-1200 mg d^{-1} reported in Table 6.1. The K concentration in maize grain ranged from 2416-3978 mg kg^{-1} with a mean of 2969 mg kg^{-1} (Fig. 6.1b). Potassium intake ranged from 855-1408 mg d^{-1} with a mean of 1051 mg d^{-1} (Fig. 6.1e). The Mg concentrations of grain ranged between 624-1106 mg kg^{-1}, with a mean of 843 mg kg^{-1} (Fig. 6.1c). Magnesium intake ranged from 221 – 392 mg d^{-1} with mean intake of 299 mg d^{-1} (Fig. 6.1f).

6.3.2 Micronutrients

Zinc concentration in grain ranged between 13.0-22.4 mg kg^{-1}, with a mean of 16.6 mg kg^{-1} (Fig. 6.2a); estimated Zn intake ranged between 4.6-7.9 mg d^{-1}, with a mean of 5.9 mg d^{-1} (Fig. 6.2d). Intake of Zn is therefore lower than the RDA of 8-11 mg d^{-1} set in USA and RNI of 9.5 mg d^{-1} set in the UK (Table 6.1).

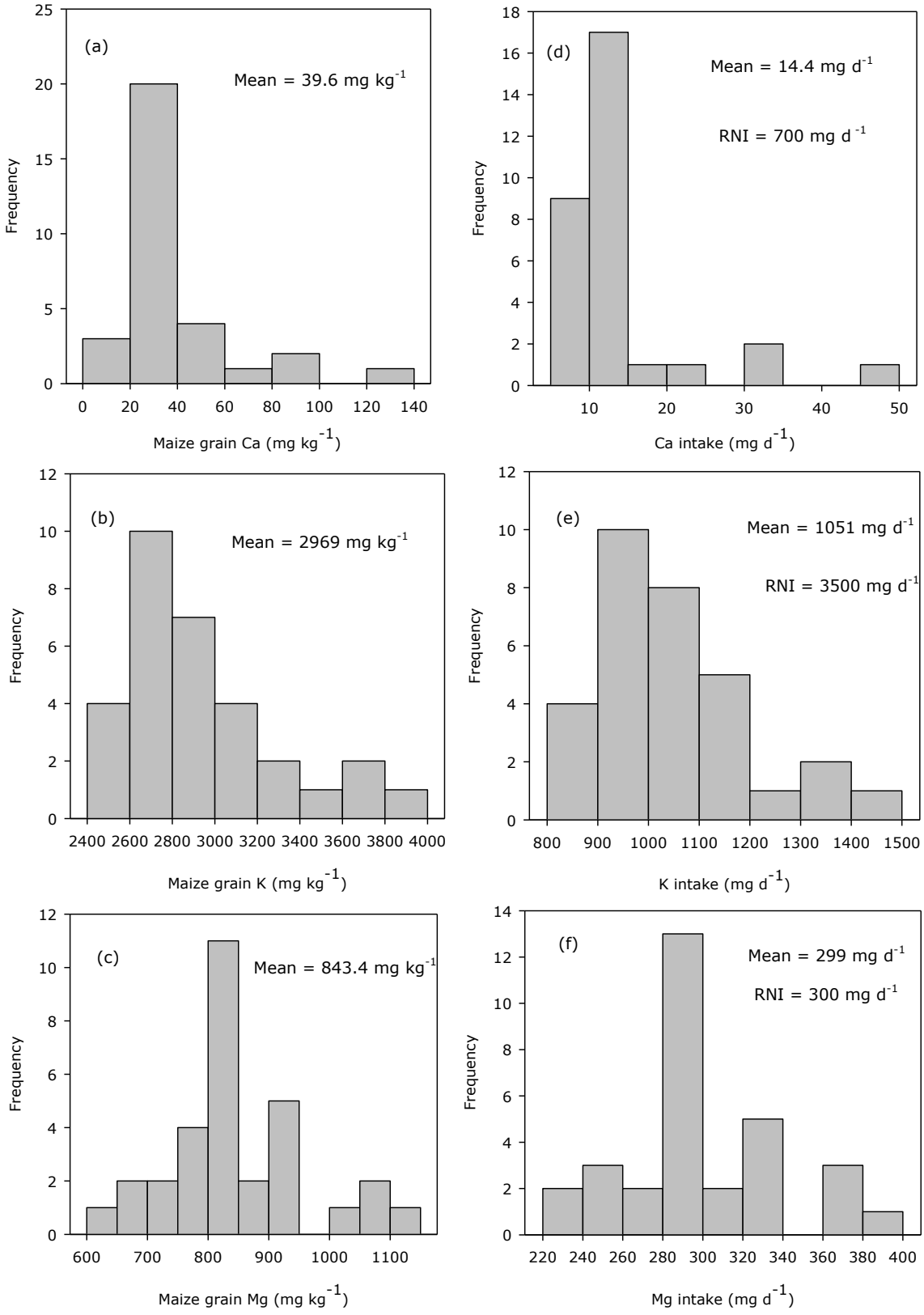

Figure 6.1. Grain Ca, K and Mg concentrations (a,b,c) and daily Ca, K and Mg intakes (d,e,f) based on mean *per capita* maize consumption (FAO, 2011). Values for mean grain concentration mean daily intake and RNI are shown.

Iron grain concentrations ranged from 9.1-181.5 mg kg^{-1} with a mean of 24.0 mg kg^{-1} (Fig. 6.2b), giving an estimated Fe intake range of 3.21-13.6 mg d^{-1} with a mean of 8.5 mg d^{-1} (Fig. 6.2e). Grain Cu concentration ranged from 1.11-2.51 mg kg^{-1} with a mean of 1.65 mg kg^{-1} (Fig. 6.2c). Estimated dietary intake from maize ranged between 0.392-0.888 mg Cu d^{-1} with a mean of 0.585 mg d^{-1} (Fig. 6.2f), below the RDA of 0.900 and 1.200 mg d^{-1} prescribed in the US and UK regulatory guidelines (Table 6.1). Grain Mn concentration ranged from 3.01-7.49 mg kg^{-1}, with a mean of 4.6 mg kg^{-1} (Fig. 6.3a), giving an estimated intake range of 1.07-2.7 mg Mn d^{-1}, with a mean of 1.63 mg d^{-1} (Fig. 6.3d). These values are within the RNI set for the UK (>1.4 mg d^{-1}) and the RDA in the USA (1.8–2.3 µg d^{-1}; Table 6.1).

Grain Co concentrations ranged from 0.006-0.119 mg kg^{-1} with a mean of 0.025 mg kg^{-1} (Fig. 6.3b). Cobalt intake in Malawi ranged from 2.0–42.2 µg d^{-1} with a mean of 8.16 µg d^{-1} (Fig. 6.3e). Grain Mo concentration ranged from 0.002-0.994 mg kg^{-1} with a mean of 0.27 mg kg^{-1} (Fig. 6.3c); the estimated dietary intake ranged from 0.57-334 µg d^{-1}, with a mean of 94.5 µg d^{-1} (Fig. 6.3f). Grain Cr concentration ranged from 0.004-0.366 mg kg^{-1} with a mean of 0.037 mg kg^{-1} (Fig. 6.4a). Estimated dietary intake of Cr in Malawi ranged from 1.24-129.2 µg d^{-1} with a mean of 11.48 µg d^{-1} (Fig. 6.4d).

Grain Ni concentration ranged from 0.05-0.926 mg kg^{-1} with a mean of 0.29 mg kg^{-1} (Fig. 6.4b). Estimated Ni intake ranged from 15.9-283 µg d^{-1} with a mean of 96.1 µg d^{-1} (Fig. 6.4e), within the RDA of 100-300 µg d^{-1} (Table 6.1). Grain Cd concentration ranged from 0.001-0.008 mg kg^{-1} with a mean of 0.0036 mg kg^{-1} (Fig. 6.4c). The range of estimated Cd intakes in Malawi was 0.20-2.74 µg d^{-1} with a mean of 1.18 µg d^{-1} (Fig. 6.4f).

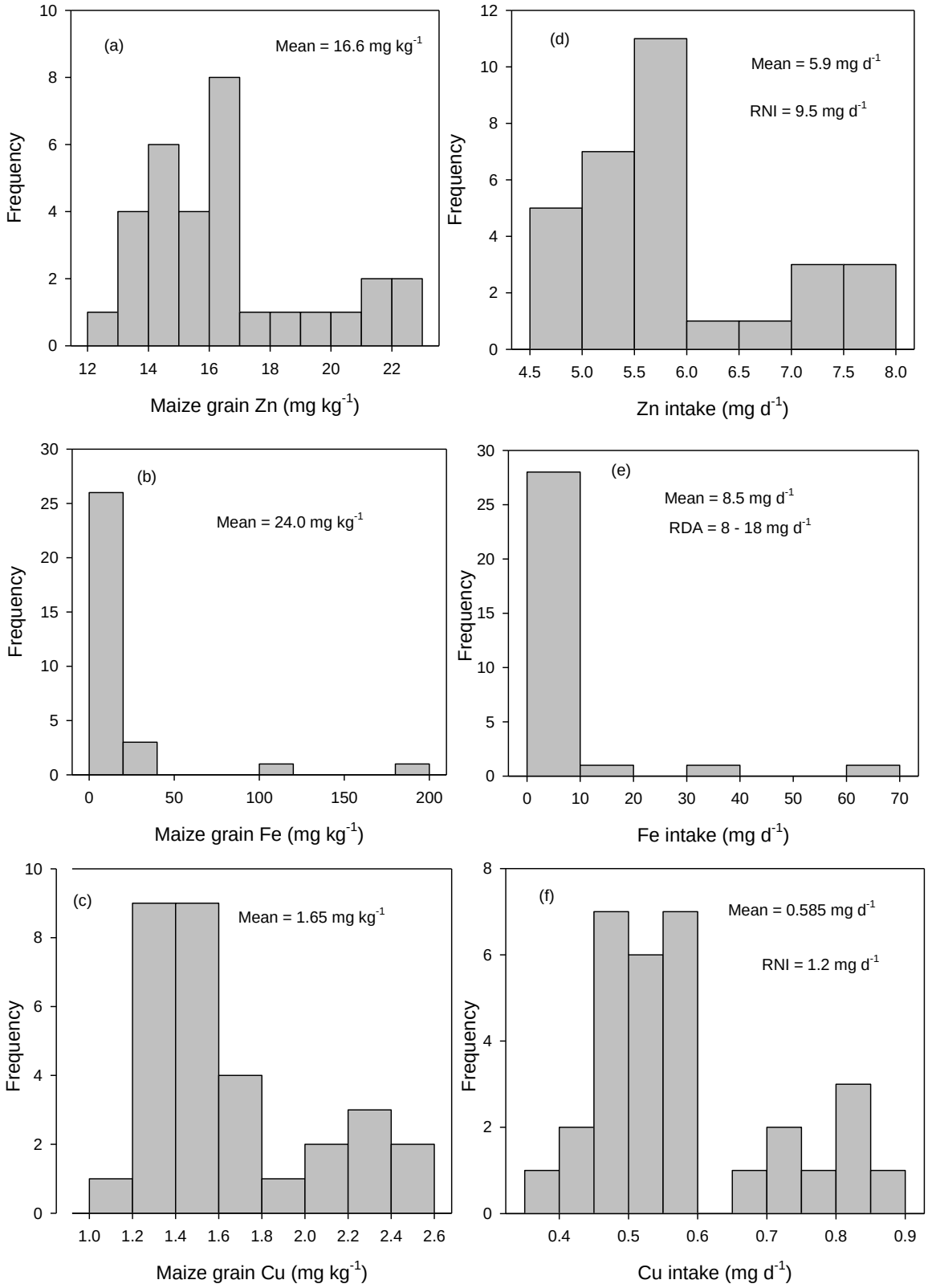

Figure 6.2. Grain Zn, Fe and Cu concentrations (a,b,c) and daily intake (d,e,f) based on mean *per capita* maize consumption (FAO, 2011). Mean concentration, mean daily intake, RNI and RDA are shown.

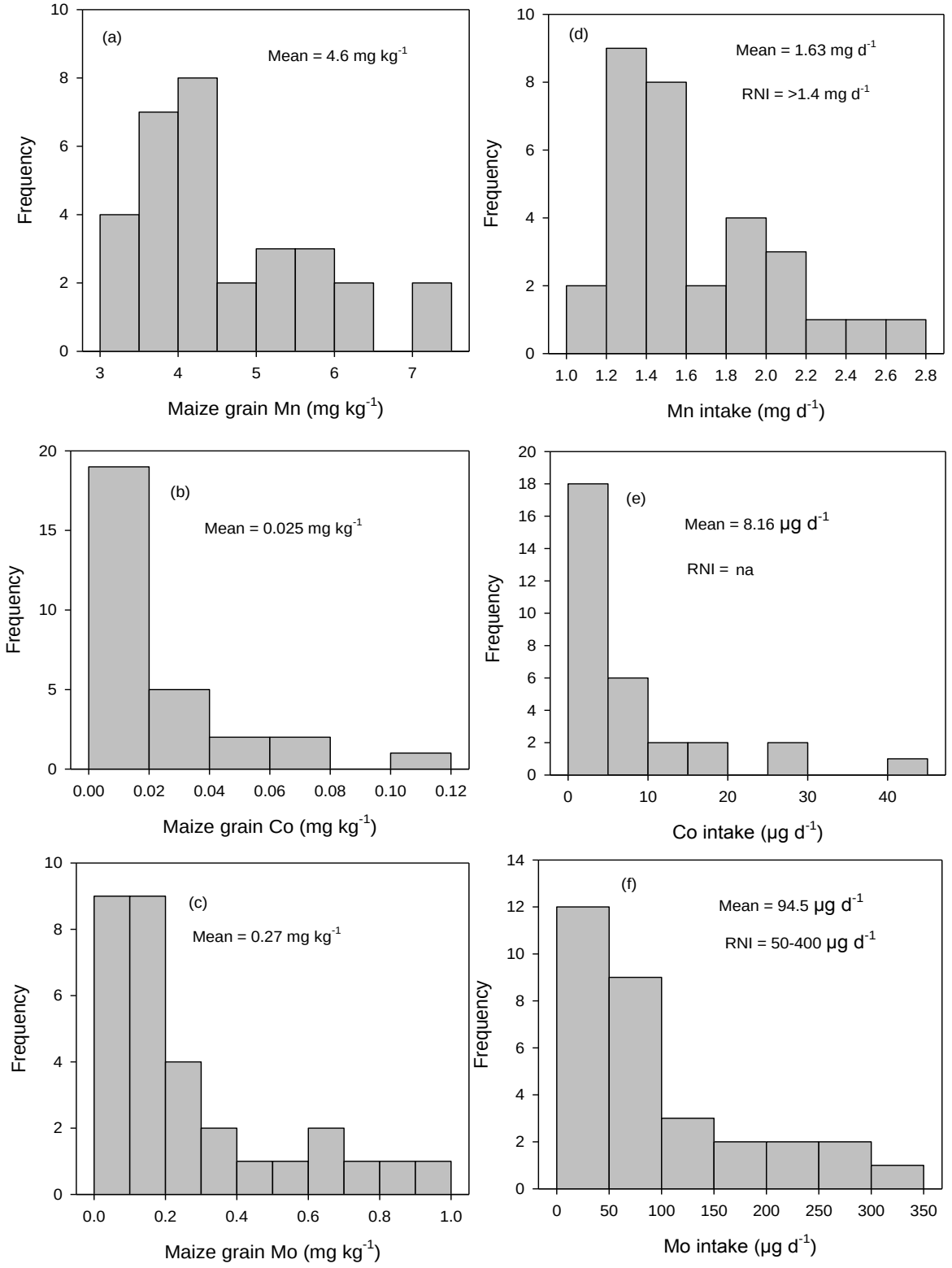

Figure 6.3. Grain Mn, Co and Mo concentrations (a,b,c) and daily intake (d,e,f) based on mean *per capita* consumption of maize (FAO, 2011). Mean grain concentration, dietary intake and RNI values are shown.

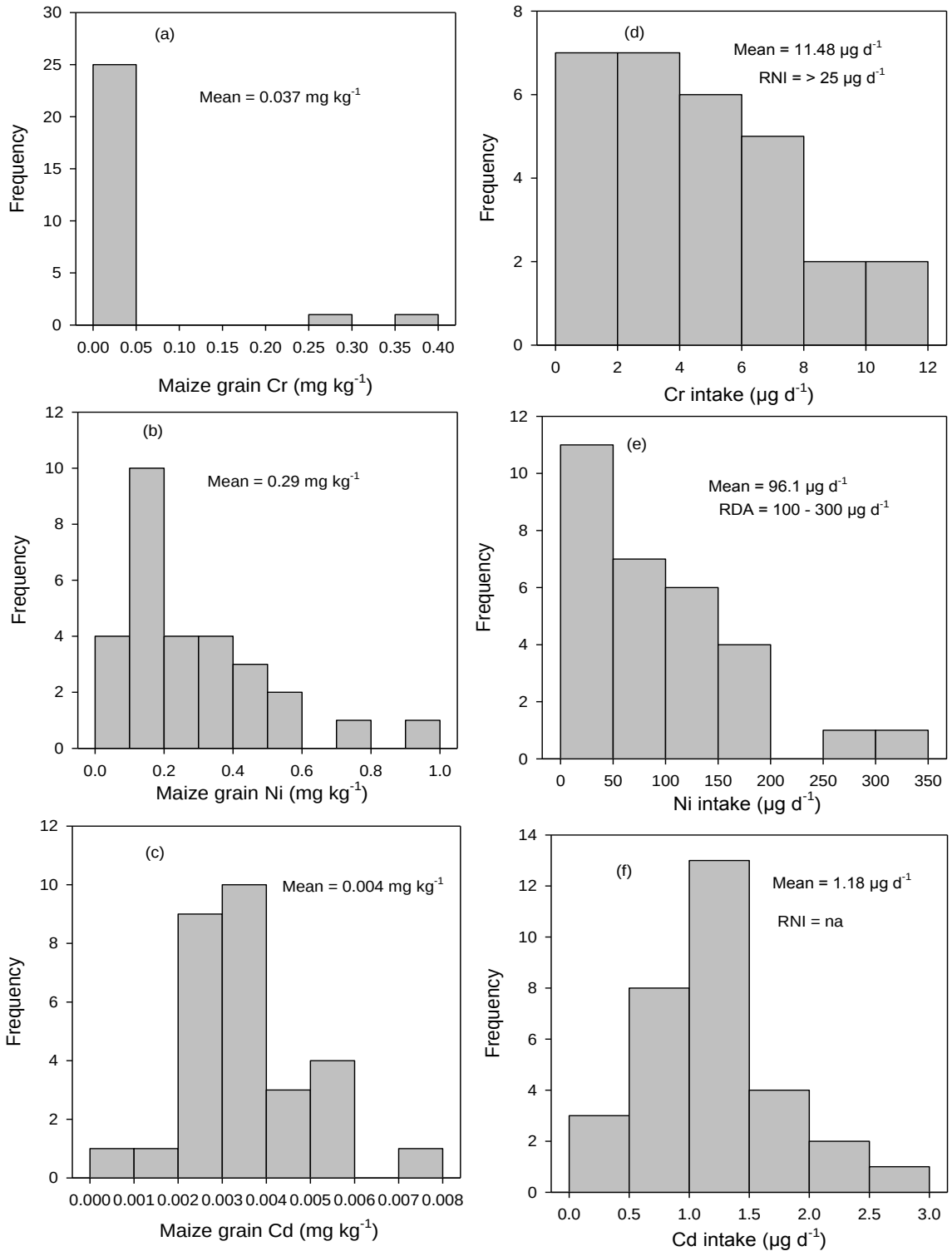

Figure 6.4. Grain Cr, Ni and Cd concentrations (a,b,c) and daily intake (d,e,f) based on mean *per capita* consumption of maize (FAO, 2011). Mean concentration, mean daily intake and RNI are shown; na = not available.

6.4 Discussion

Multi-element analysis of the maize grain, the staple food crop in Sub-Saharan Africa, has demonstrated its importance as a source of essential mineral nutrients to the population. Dietary intake of Ca is very low in Malawi; this requires urgent assessment and mitigation. Much higher concentrations of Ca ranging between 363–458 mg kg^{-1} have been reported for cereals in Sweden, providing a dietary intake of 1110 mg Ca d^{-1} (Becker et al., 2011). The results suggest that maize is inefficient in absorbing calcium even in soils with a high pH, in which Ca concentration is likely to be high. Deficient intake of Ca by rural populations in developing countries is common because their diets are predominantly cereal-based and therefore low in Ca (Graham et al., 2001, White and Broadley, 2005). The limited contribution of maize to national Ca intake seen in the present study supports previous reports that cereals are poor sources of calcium (Graham et al., 2001, White and Broadley, 2005). Although Ca concentration was very low in maize grain, analysis of vegetables grown in Malawi showed that these were very high in Ca, with concentrations ranging from 840-5140 mg kg^{-1} (Ferguson *et al.,* 1989). However, mean *per capita* consumption of all vegetables including tomatoes and onions in Malawi is 59 g d^{-1} (FAO, 2011), which would contribute only 49.6-303.3 mg d^{-1} of Ca. As the contribution from maize ranged from 6.3–45.1 mg d^{-1} depending on sampling location, it would appear that Malawi may not be meeting the RDA for calcium of 1000-1200 mg kg^{-1}. Potassium concentration in cereals ranged from 2020-2280 mg kg^{-1} in Sweden and K intake was 3320 mg d^{-1} based on Sweden diet (Becker et al., 2011), which is higher than in Malawi which was based on maize consumption alone, values are lower than the RDA of 1600-3500 mg d^{-1}. Mean intake of Mg is close to the computed intake of Sweden of 285 mg d^{-1} (Becker et al., 2011) and was within the RDA of 310-420 mg d^{-1} in the USA and the RNI of 300 mg d^{-1} in UK (Table 6.1). The results indicate that Malawi is adequate in Mg intake.

The intake of Zn and Cu from maize exceeds 60% of the RDA, indicating that other dietary sources may provide the balance if they can supply the remaining 40% of RDA for these elements. Consumption of fish may be

extremely important in this respect. Given that the concentrations of Zn, Ca, Mg and K in fish range from 32-254, 900-22630, 620-1720 and 1184-1328 mg kg^{-1}, respectively (Ferguson *et al.,* 1989) and mean *per capita* consumption of fish is 14 g d^{-1} (FAO, 2011), this dietary component would contribute an intake of 0.45–3.56 mg K d^{-1}, 12.6–316.8 mg Zn d^{-1}, 8.7–24.1 mg Ca d^{-1} and 16.6–18.6 mg Mg d^{-1}. With their Zn concentration of 9–33 mg kg^{-1} (Ferguson *et al.,* 1989) and mean *per capita* consumption of 49 g d^{-1} (FAO, 2011), legumes would contribute 0.44–1.62 mg Zn d^{-1}. These data suggest that Zn intake in Malawi may be sufficient but the intake of Ca and K is likely to be lower than RDA or RNI. However, although, Zn intake could be regarded as adequate, there are reports that sub-optimal intake of this element are prevalent in Malawi as 36% of women have low plasma concentrations of Zn and 46% have low hair concentrations, with 60% of Zn intake coming from cereals (Gibson and Huddle, 1998). This could be due to factors affecting the availability of Zn in the human body; for example, as phytate inhibits absorption of Zn in humans, a high dietary intake of phytate may induce Zn deficiency (Turnlund et al., 1984). Iron intake values are within the RDA of 8-18 mg d^{-1} (Table 6.1) but Fe is one of the micronutrients considered to be deficient in the human diet in Malawi and this could be the problem of availability in the body. The Fe intake is higher than the mean Fe intake in Sweden of 9.2 mg d^{-1} (Becker et al., 2011).

Mean Mn intake values of 2.5, 3.7 and 2.6 mg d^{-1} respectively have been reported for France, Sweden and Belgium (Biego et al., 1998). Intake of Mn can therefore be considered to likely be adequate in Malawi. Much higher values of Co were reported for wheat grain in France, which ranged from 0.002-0.005 mg kg^{-1} (Biego et al., 1998) and although there is no current RDA for Co, estimated mean intakes of 26 µg d^{-1} have been reported in France (Barberá et al., 1993), while a value of 11 µg d^{-1} was reported for Sweden (Becker et al., 2011). Mean Ni intake values of 231, 199 and 82 µg d^{-1} have been reported in France, Denmark and Sweden respectively (Biego et al., 1998). Nickel concentration in cereal products in Sweden ranged between 0.014-0.18 mg kg^{-1} with a mean intake of 100 µg d^{-1} (Becker et al., 2011). Intake of Ni from maize in Malawi is therefore adequate but there is a need to be alert for any significant intake of Ni from other food

sources as this could pose a threat to human health. The Mo intake value has exceeded the RDA of 45 µg d^{-1} but is within the RNI set in the UK of 50-400 µg d^{-1} (Table 6.1). Estimated mean Mo intake in France and Sweden was 275 and 150 µg d^{-1} respectively (Biego et al., 1998); Mo intake in Malawi is therefore within the range reported for other countries (Biego et al., 1998)

A mean Cr concentration of 0.31±0.11 has been reported for rice grain in Iran, with a range of 0.13-0.56 mg kg^{-1} (Zazouli et al., 2006). Chromium concentrations in cereals and pulses in Greece exceeded 0.1 mg kg^{-1}, while the mean concentration in cereal products in Sweden was 0.021±0.007 mg kg^{-1} (Becker et al., 2011). Other reported Cr intakes are ranging 13-48 and 80-107 µg d^{-1} in the USA and UK respectively (NAS, 1980, Smart and Sherlock, 1985). Grain Cr concentration ranged from 0.004-0.366 mg kg^{-1} with a mean of 0.037 mg kg^{-1} but the Cr data seem to indicate some degree of contamination from dust because most of the samples were very low except two samples which gave high values. Estimated dietary intake of Cr in Malawi was therefore lower than the relevant RDA in the USA (Table 6.1) and RNI in the UK (NAS, 1980, Smart and Sherlock, 1985), but is within the Cr intake reported in USA.

Cadmium intakes of 35-50 µg d^{-1} and 4-84 µg Cd d^{-1} were reported in Japan and in European community countries in non-polluted areas, but could be >400 µg d^{-1} in contaminated areas (ECC, 1978, Frieberg and Elinder, 1988). Mean intakes of 27, 19 and 12 µg Cd d^{-1} respectively were reported for France, Denmark and Sweden (Biego et al., 1998). Cadmium is regarded the most serious trace metal contaminant of soil and, as an accumulative toxin, even low concentrations could pose a threat resulting from regular and continuous intake. As the maximum permissible concentration (MPC) for rice is 0.1 mg Cd kg^{-1} (Adams et al., 2004), the Cd concentrations in maize grain would appear to be safe. Reported Cd concentrations for wheat grain ranged from <0.002-0.21 mg kg^{-1} dry weight in the USA (Wolnik et al., 1983) and from 0.024-0.41 mg kg^{-1} in the Netherlands (Wiersma et al., 1986); the Cd concentrations reported for both countries exceed the MPC of 0.1 mg kg^{-1} for some of samples analysed. By contrast, the mean Cd

concentration of 0.024±0.003 mg kg^{-1} for cereal products in Sweden was well below the MPC (Becker et al., 2011). However, although Cd concentration in maize grain and dietary intake in Malawi are low and could be considered safe, there is a need to keep monitoring the situation in the country.

It is interesting to note that, although soil pH varied between sites (Chapter 3), this did not affect uptake of Zn, Fe and Mn by maize, perhaps because the roots were able to create favourable soil pH conditions in the rhizosphere by exuding organic acids which solubilised these elements for plant uptake. It has been reported that Fe-deficient grasses and cereals release organic acids, thereby solubilising ferric compounds and increasing their bioavailability for uptake by roots (Römheld, 1987). Organic acids released by roots may also solubilise other micronutrients such as Mn, Cu and Zn (Treeby et al., 1989), while the root exudates of graminaceous species are effective in mobilising Zn in calcareous soils (Marschner, 1993). However, some graminaceous plants use a different strategy to acquire Fe in which exudation of mugineic acids solubilises and chelates sparingly soluble inorganic iron; because of its higher chelation affinity for Fe than Ca and Mg, the uptake of Fe is not affected even in high pH soils (Ma and Nomoto, 1996). Marschner (1993) reported that rhizosphere acidification in high pH soils by ammonium nitrogen was effective in enhancing Zn mobilisation, and that plants responded to Zn deficiency by rhizosphere acidification through increased excretion of organic acids and chelation, thereby increasing Zn bioavailability (Marschner, 1993). The range of concentrations of Ca, Mg, K, Cu, Mn, Zn and Fe in maize in Pakistan (Shar *et al.,* 2011) were similar to the present study except that Ca, Mg and Fe concentrations were higher in Malawi.

Food diversity in Malawi is narrow and mainly based on maize, which provides over 50% of the average calorie intake; the remainder comes mainly from cassava, sweet potatoes, rice, legumes and vegetables (FAO, 2011). Consumption of animal products is low in rural areas and, although fish may be the most commonly consumed animal protein, consumption is low in areas away from the lakes. Maize may therefore be considered as the

major nutrient carrier for the population in the Sub-Saharan Africa, particularly in Malawi.

6.5 Conclusions

The incidence of intake deficiency for various mineral nutrients varies within Malawi. National data published in 1989 showed severe endemic iodine deficiency disorder in Chitipa, Karonga, Rumphi, Mzimba, Lilongwe, Mchinji, Dedza and Ntcheu (Network for Sustained Elimination of Iodine Deficiency, 2011). The prevalence of HIV and AIDS also varied across the country (World Bank, 2007) and a similar trend might also occur for other diseases. The results of the present study suggest that these trends may reflect variation in the concentrations of mineral nutrients in food across the country. There is therefore an urgent need for a detailed dietary survey in which all types of food consumed throughout the country would be collected and analysed for all nutrients to establish the nature and extent of the variation in daily intake.

It is evident that food crops such as maize must be regarded as a carrier of essential nutrients and, Malawi will aim to increase production in the future, it should also explore how to increase nutrient content to address diet nutrient deficiencies in the national population. Nutrient concentrations in maize grain and estimated dietary intake are influenced by soil conditions and other factors such as rainfall. However, a complete evaluation of dietary intake in Malawi and other SSA countries would require a nationwide dietary surveys in which all foods consumed within typical households were analysed to provide robust data regarding the national intake of essential dietary elements.

Chapter 7: General Discussion and Conclusions

7.1 Soil and maize survey throughout Malawi

Selenium is an essential element in the human diet but suboptimal intake may arise where food choices are narrow. Suboptimal dietary intake of Se is widespread in Malawi. In the present study, the median Se concentration in maize grain was 0.019 mg kg^{-1} (range 0.005-0.533), giving an intake of 6.7 µg Se $person^{-1}$ d^{-1} from maize flour based on national consumption patterns (Table 3.2). Maize grain Se concentration was up to 10-fold higher in crops grown on soils with naturally high pH values (Eutric Vertisols; pH > 7) (Fig. 3.1). Under alkaline conditions, Se becomes considerably more available to plants due to the greater solubility of $Se^{(IV)}$ species and oxidation to $Se^{(VI)}$ which is only weakly adsorbed in soil. The survey of soil and maize grain throughout Malawi (Chapter 3) showed that total Se concentration in soil, which ranged from 0.0521 to 0.6195 mg kg^{-1}, provided a poor index of Se availability. By contrast, there was some evidence that both Se extractable with KH_2PO_4 (range 0.0013 to 0.0158 mg kg^{-1}) and soil pH affected plant uptake and grain Se concentration (Fig. 3.1 and 3.2). Nevertheless, only a broad correlation between soil properties and grain-Se content was seen across the full range of soils studied. The data were quite polarised between the acidic soils which predominate over most of Malawi and the comparatively rare calcareous Eutric Vertisols.

Results showed that grain Se concentration of greater than 0.15 mg Se kg^{-1} was achieved when KH_2PO_4-extractable Se concentration in soil >0.01 mg kg^{-1} and soil pH value >6.5 (Fig. 3.2). The latter requirement was more important, in fact grain Se concentrations were high when grown in soils with a high pH even though total soil Se concentrations were lower than at other, more acidic, sites (Fig. 3.1). The results in Chapter 3 have indicated that mean dietary Se intakes of 39.8 and 24.4 µg Se $person^{-1}$ d^{-1} were estimated for Mangochi and Zomba Districts, respectively, with Se intake from all non-maize sources being 22.4 and 15.5 µg Se $person^{-1}$ d^{-1}, respectively (Table 3.1). A suboptimal dietary intake of 20-30 µg Se person^{-}

[1] d^{-1} is therefore widespread in Malawi, based on spatial integration of Se concentrations in maize grain and soil surveys collected from 88 field sites, representing 10 primary soil types and >75% of the national land area (Table 3.2). However, the survey also showed that alkaline Vertisols in the Shire Valley Agricultural Development Division had high KH_2PO_4-extractable soil Se and maize grain Se concentrations (0.17-0.53 mg kg^{-1}); this would be sufficient to achieve adequate Se intake within the local population if people were dependent on home-produced food.

These findings are in agreement with several previous reports that inorganic Se is present as selenite under acidic soil conditions, which is strongly sorbed to Fe oxides and hydroxides, resulting in low grain Se concentrations (Elrashid et al., 1987, Duc et al., 2003, Duc et al., 2006). Several spectroscopic studies, using Extended X-ray Absorption Fine Structure, EXAFS) have shown that selenite (Se^{IV}) forms strong inner-sphere surface complexes on iron oxides such as goethite and haematite and isomorphically substitutes for phosphate within apatite (Hayes *et al.,* 1987; Duc*et al.,* 2003). By contrast, although there is some evidence that selenate (Se^{VI}) *may* form an inner sphere complex on Goethite at low pH; this is transformed to an outer sphere complex at high pH (Peak and Sparks, 2002). It is more generally reported that selenate is weakly bonded to oxides, probably as a non-specifically sorbed outer sphere complex (Hayes *et al.,* 1987). For both Se^{IV} and Se^{VI}, strength of sorption decreases as soil pH increases (eg Figure 7.1), leading to an increase in soil Se solubility and grain Se concentration (Duc et al., 2006).

Thus Se availability is affected both by soil pH and redox potential, as well as the presence of competing anions and mineralogy (Elrashid, 1987). Under conditions of low-medium redox potential and low-neutral pH, selenite is the dominant form of inorganic Se and is relatively insoluble in soil and hence not readily available for uptake by plants (Fig. 7.2). However, at high pH (pH > pKa_2 for selenite) and high redox potential, (i) the sorption of selenite decreases and (ii) selenate becomes the stable form of inorganic Se (Figs 7.1 and 7.2). Both factors together appear to explain

the much greater bioavailability of Se seen between Ngabu and other sites (Figs. 3.1, 7.2).

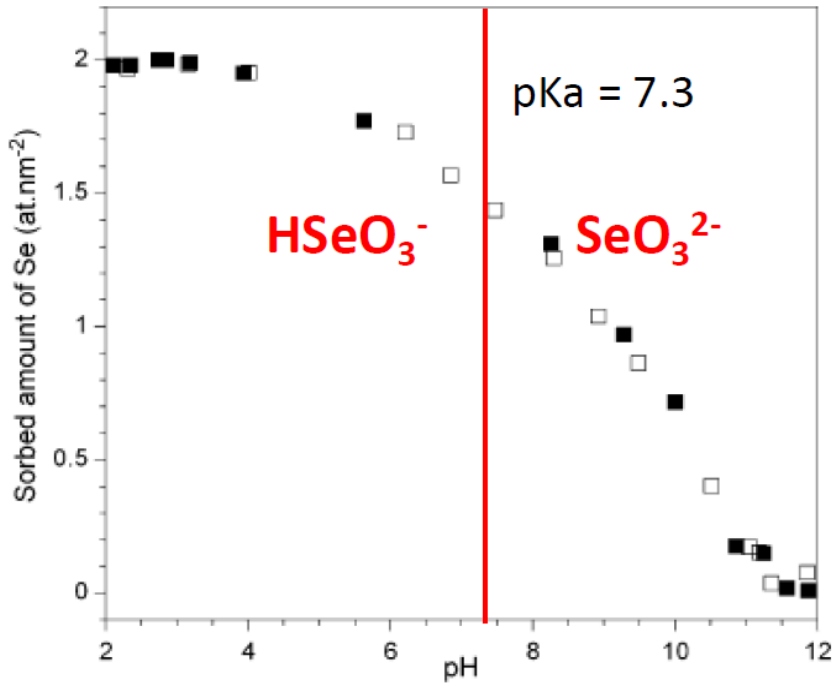

Figure 7.1. Relationship between the amount of sorbed selenite and soil pH ([Se] = 4×10^{-4} M) for two ionic strengths: $NaNO_3$ 0.01 M (open symbols) and 0.1 M (closed symbols) (Source: Duc *et al.,* 2006).

The maps in Figs 3.1 and 3.4 showing the variation in grain Se concentration throughout Malawi confirm that dietary intake of Se is low in most areas (Table 3.2). However, when the results were extrapolated on the basis of soil type, it is apparent that some areas in Salima and Karonga are likely to have higher grain Se concentrations (Fig. 3.4). These areas are dominated by calcareous vertisols, as in Ngabu (Fig. 3.4).

This survey is among the first of its type in Sub-Saharan Africa (SSA) and the results may provide valuable guidance on Se intake in the region. For example, the relationship between grain Se and soil pH and KH_2PO_4-extractable Se could be used more widely to identify areas of high and low grain Se concentrations in SSA. Most soils in the region are highly weathered and have a low soil pH, which will result in low grain Se concentrations due to the predominance of selenite and strong adsorption by Fe oxides. The grain Se data also could provide useful, and more direct,

information for maize in SSA and beyond. As there is little information on grain Se concentration in maize grown in SSA, the information generated is likely to trigger interest in research on Se within the region. As maize is the major staple food crop in SSA, the extensive database obtained in the present study will help to reveal the extent of deficient dietary intake of Se.

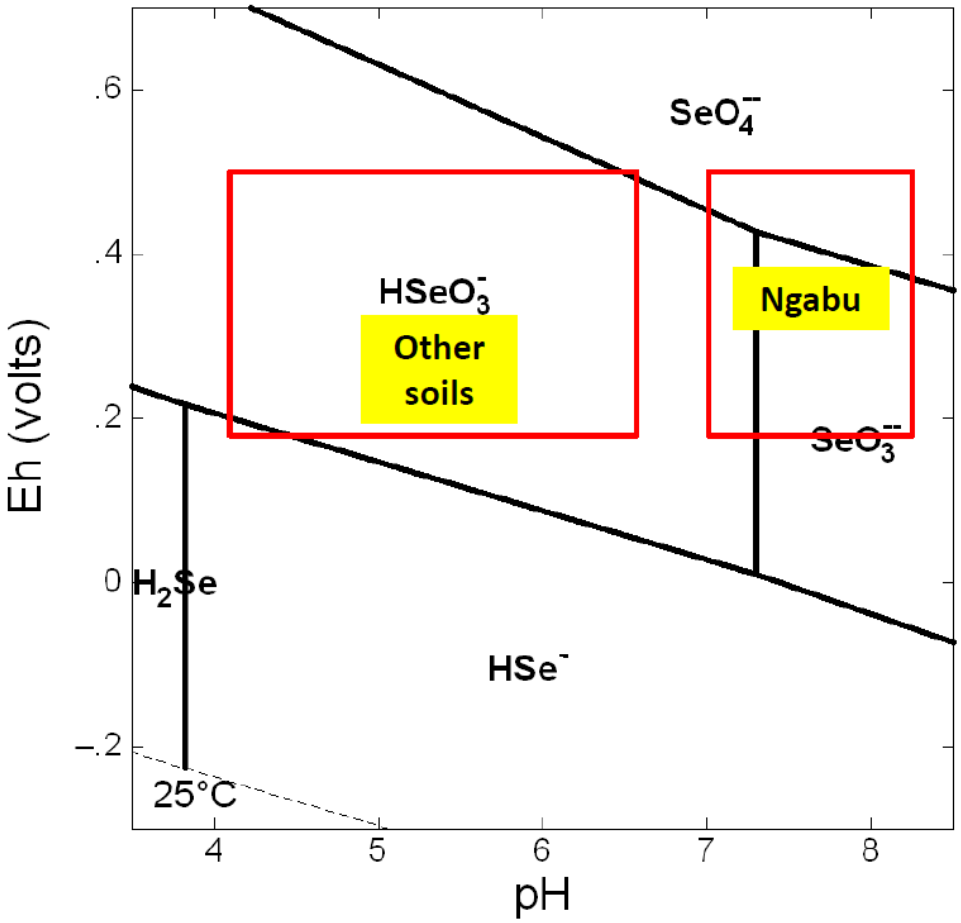

Figure 7.2. Diagram summarising selenium species stability at 25 °C, 1 bar pressure and *Ionic strength*=0 for a dissolved selenium activity of 10^{-10} mol L^{-1} (adapted from (Séby et al., 2001).

Although the survey reported in Chapter 3 provided valuable information on soil and grain Se concentration throughout Malawi, further work is required to cover all soil types and agro-ecological zones in the country. There is therefore a need to conduct a well structured survey of all soils and major food crops to identify Se-deficient and adequate areas and quantify national dietary Se intakes. As SSA is heavily affected by the prevalence of HIV/AIDs, surveys of soil and food crops of this type are urgently needed to determine the extent of Se deficiencies as effective strategies to eliminate

Se deficiency in the region may help to mitigate the impact of HIV and AIDs (Foster, 2003).

7.2 Agronomic biofortification of maize with Se

The results presented in Chapter 3 showing that Malawi experiences widespread suboptimal Se intake were substantiated by low blood plasma Se concentrations, which ranged from 0.5 to 0.7 µmol L^{-1} and represented 73-95% of the population. This observation indicates that Se deficiency is endemic in Malawi (van Lettow *et al.*, 2003, van Lettow *et al.*, 2004). It has been proposed that plasma/serum Se levels >70 µg L^{-1} (>0.89 µmol L^{-1}) represent an adequate level above which no further increase in glutathione peroxidase (GPx) activity to Se supplementation occurred (Neve, 1995). In rural Malawi, blood plasma Se concentration was <0.89 µmol L^{-1}, the critical value below which Se is regarded as being deficient (Fairweather-Tait et al., 2011). In Finland, implementation of agronomic biofortification of food crops with Se resulted in an increase in serum Se concentration (Alfthan, 2005); Fig. 7.3).

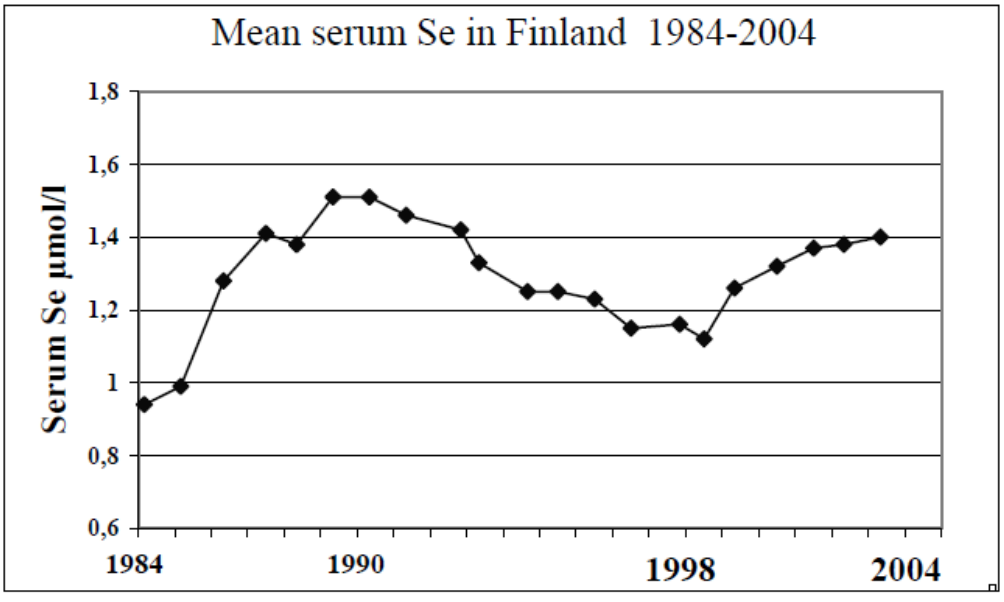

Figure 7.3. Mean annual serum Se concentration in healthy Finns for twenty years of Se fertilisation in Finland, 1984-2004 (source: Alfthan, 2005).

In view of the survey, results showing that suboptimal dietary Se intake are endemic in rural Malawi due to the low levels of bioavailable Se in most soils and limited soil-to-crop transfer, the potential for biofortifying maize with Se using fertilisers was evaluated. Crop responses to three forms of selenate-Se containing fertiliser were examined; Se treatments included a liquid drench of $Na_2SeO_{4(aq)}$ (0-100 g Se ha^{-1}), a compound NPK+Se fertiliser (0-6 g Se ha^{-1}), or calcium ammonium nitrate (CAN+Se; 0-20 g Se ha^{-1}) (Chapter 4).

The response of grain and stover Se concentration to all forms of Se, and application rates, was approximately linear (R^2>0.90 for 27 of the 30 experimental units (Table 4.6; Figs. 4.6-4.9 & 4.11). Mean whole-grain concentration increased by 19.7, 20.7 and 14.8 µg Se kg^{-1} grain for each gram of Se applied as $Na_2SeO_{4(aq)}$, NPK+Se or CAN+Se, respectively (Fig. 4.12). The results support several previous reports of increased Se concentration in food crops in response to Se application. For example, addition of Se in fertiliser applied to crops in Finland increased Se concentration in 125 indigenous food items; most notably, the Se concentration of wheat bread was increased 10-fold from 0.03 to 0.35 mg Se kg^{-1} DW (Eurola et al., 1991). In Norway, application of 6.5 g Se ha^{-1} using calcium nitrate enriched with 25 mg Se kg^{-1} increased Se concentration in wheat grain to the desired level (Tveitnes et al., 1995), while in Australia, selenium applications ranging from 4 to 120 g Se ha^{-1} progressively increased grain Se concentration by up to 133-fold when sprayed onto soil when the crops were sown, and by up to 20-fold when applied after flowering (Lyons et al., 2005b). In the United Kingdom, application of Na_2SeO_4 solution as a single, high volume drench significantly increased Se concentration in wheat, by 0.0167 mg kg^{-1} DW for straw and 0.026 mg kg^{-1} DW for grain for each g Se ha^{-1} applied (Broadley et al., 2010). Finland has now achieved safe and sufficient dietary Se levels and the addition of selenate to NPK fertilisers has proved an effective method of increasing the selenium status of the national population (Eurola, 2005, Aro et al., 1995).

Grain and stover yields were unaffected by Se application (Tables 4.3-4.5). Whole-crop recovery of Se ranged from 3 to 45% (Table 4.8), demonstrating the existence of significant variation in recovery between sites. Selenium recovery of <5% and 10% has been reported previously (Stephen *et al.,* 1989; Lyons *et al.,* 2004) and recoveries of 20% and ≤35 % have been reported for wheat (Curtin *et al.,* 2008; Broadley *et al.,* 2010). As crop uptake of selenate-Se declines within weeks of application (Gissel-Nielsen and Bisbjerg, 1970), a greater understanding of the fate of Se in the soil-crop system is required to optimise Se-use efficiency.

A significant interaction between the rate of Se application and the grain processing procedure employed was detected ($P<0.005$) as the processing procedure significantly affected the Se concentration of flour at 50 g Se ha^{-1} of application (50 g Se ha^{-1}; $P<0.005$), but not at lower application rates (Fig. 4.13). There was no evidence of any significant loss of Se from maize flour during traditional milling at the lower rates of Se application. This finding is important in Malawi where maize is processed to very fine flour by fermentation; the lack of an effect of processing on Se concentration would mean that Se intake will not be affected by processing in the event that agronomic biofortification is adopted in Malawi.

The use of granular fertiliser to biofortify maize with Se is feasible in Malawi where subsidies currently make fertiliser accessible to the majority of the rural population. At present, a basal dressing of 200 kg ha^{-1} NPK fertiliser and a top dressing of 100 kg ha^{-1}urea are applied to maize. The most appropriate approach would be for farmers to apply urea enriched with Se as a top dressing to their maize crops as the results obtained show that top dressings are superior to basal dressings in increasing grain Se concentration (Fig. 4.10). Urea would therefore have to be enriched with 0.005% of Se to provide 5 g Se ha^{-1}, whereas NPK will have to be enriched with Se concentration of 0.0025% to apply 5 g Se ha^{-1}. Increased Se intake in Malawi would have far reaching benefits for the health of its population.

The present study has shown that application of 5 g Se ha^{-1} to maize crops would increase average dietary Se intake in Malawi by 26.3-36.6 µg Se

person^{-1} d^{-1}. Such levels would increase dietary Se intake to the accepted reference values of 50-70 µg Se person^{-1} d^{-1} (Fairweather-Tait et al., 2011). The risk of overdose, based on current upper safe intake limits of 400 µg Se person^{-1} d^{-1} (Department of Health, 1991; Institute of Medicine, 2000) appears be minimal at these application levels, even for individuals with diverse diets. However, any public health intervention involving widespread agronomic biofortification with Se would clearly require careful monitoring to ensure beneficial health outcomes. Whilst it is widely accepted that Se intake <30 µg Se d^{-1} is suboptimal for most adults, there remain considerable gaps in our knowledge of the relationships between Se intake, plasma Se concentrations and selenoenzyme activities, and definitive health outcomes (e.g. immune functioning), especially among individuals of very low-Se status in SSA. This situation must now be addressed, via controlled intervention experiments, as a matter of urgency.

7.3 Fate of applied Se in maize cropping systems

The fate of applied Se and the residual effects of Se application have been of great concern due their importance in the biofortification of food crops to improve human health and the fear of causing environmental pollution. Isotopic labelling studies with ^{74}Se were conducted at two sites in Malawi, Mbawa and Chitedze, using maize as a test crop to study the fate of applied Se. Residual effects were assessed in the following year by planting another maize crop on the plots fertilised using liquid sodium selenate in the previous year. The analyses of ^{74}Se concentrations in the grain and stover were successful and recovery of applied Se (10 g Se ha^{-1}) was 0.65 g and 1.08 g Se ha^{-1} at Chitedze and Mbawa, respectively, representing 6.5% and 10.8% of the applied Se (Fig. 5.3b). A Se recovery of 10% has previously been reported for wheat (Lyons et al., 2004), although a recovery of 20-35% has also been obtained for the same species (Broadley et al., 2010). Whole plant recovery of Se ranged from 3 to 45 % in the present study, suggesting that recovery varied greatly between sites due to differences in soil and environmental factors, as discussed in Chapter 4. Fertiliser-derived Se (^{74}Se-labelled) constituted 71% and 82% of plant-Se recovery at

Chitedze and Mbawa, respectively (Fig 5.3a, c). This was broadly in line with expectation from the response trend seen in the biofortification trials for an equivalent application of 10 g ha^{-1}.

The residual effects of Se application in grain Se concentration (µg kg^{-1}) were linear and the coefficients of determinations (R^2) were 0.9070 and 0.9404 at Chitedze and Mbawa, respectively but the rate of increase was higher at Mbawa than Chitedze (Figs. 5.5a, c). When the grain Se concentration was converted to grain Se uptake (g ha^{-1} per g applied Se ha^{-1}, the differences in grain Se uptake between the two sites disappeared (Figs. 5.5b, d). The proportions of grain Se of the applied Se were 0.0023 and 0.0019 g ha^{-1} per g Se applied ha^{-1} for Chitedze and Mbawa, respectively (Figs. 5.6b, d), giving the recovery of 0.23 and 0.19 % of the applied Se, respectively. When the soil Se concentration was converted to gravimetric units (g ha^{-1}) as the grain Se uptake for the two sites, it gave consistent values with the grain Se uptake. The proportion of KH_2PO_4 extractable Se of the applied Se of Chitedze and Mbawa were 0.0347 and 0.0476 (Figs. 5.4c, e) representing 3.47 and 4.76% recovery of the residual applied Se. The results show that the KH_2PO_4 extracted more Se than 10 fold of the residual Se than that was recovered by the maize crop. 'Available' inorganic Se measured by KH_2PO_4 extraction at harvest and uptake by a subsequent maize crop the following year both clearly demonstrated residual effects of Se application. Attempts were made in Finland to monitor the effects of Se application on residual Se over a period of several years, but a measureable residue could not be confirmed; this was attributed to the use of a strong extractant, Aqua regia, so that small Se residues were not measureable against the comparatively large background soil Se solubilised (Yli-Halla, 2005). The positive residual effect of Se application observed using either KH_2PO_4 extraction or measurements of grain Se concentration reported in Chapter 5 has provided results of significance to researchers who wish to monitor the effects of Se application on the subsequent crops or the environment.

7.4 Concentration of macro- and micronutrients in maize grain and dietary intake based on *per capita* maize consumption in Malawi

As maize is a major staple food crop in SSA, and Malawi in particular, its contribution to human mineral nutrition is potentially of great importance. In addition to determining Se concentration in maize grain, the concentrations of several macro- and micronutrient elements including Ca, Mg, K, Zn, Cu, Fe, Mn, Cr, Ni, Co, Cd and Mo were also measured. These data were used to estimate dietary intake for minerals throughout Malawi, where over 97% of the population grow maize for food. It is of paramount importance that the nations of SSA are aware of the quantity of mineral nutrients contributed by maize as this is crucial to adequate provision of essential nutrients to the human diet. Dietary intake values were based on current data for *per capita* consumption maize in Malawi (FAO, 2011). The results revealed that the population of Malawi may be exposed to severe Ca deficiency as its concentration was very low in maize, with the result that the computed intake was also very low. Potassium, Cu and Zn were also low, although other dietary sources might mitigate the limited dietary intake of these elements from maize. However, the results indicate that maize is a good source of Mg, Mn, Fe and Mo, for which dietary intake was within adequate ranges. It may also be useful to monitor the dietary intake of Ni, Co, Cr and Cd by the population as the results revealed that the intake of Ni from maize alone was adequate and might even exceed its RDA, while Mo exceeded its RDA and therefore requires careful monitoring of the dietary intake of this element to ensure the safety of the population. Increased intakes of Co, Cr and Cd could have adverse health effects because they are highly toxic and are cumulative toxicants(Barberá et al., 1993, Mudgal et al., 2010).

7.5 Conclusions

Countries in SSA, including Malawi, are currently experiencing increasing incidences of diabetes, asthma, high blood pressure and heart disease,

cancer and HIV/AIDS. There is evidence that the incidence of certain non-communicable diseases, such as diabetes and hypertension, is increasing rapidly in parts of SSA. Other diseases such as asthma and epilepsy are common but poorly managed (Unwin et al., 1999). The incidence of hypertension is increasing rapidly and ranges from 3% in rural areas to >30% in some urban settings. The incidence of diabetes mirrors that of hypertension, ranging from <1% in some rural areas to >20% in some populations and racial groupings in urban settings. The predominant type is Type 2 diabetes, which accounted for >80% of all cases in some reports and tends to develop later in life (Mufunda et al., 2006). Values of 32.9% for hypertension and 5.6% for diabetes have been reported and chronic non-communicable diseases are becoming increasingly important causes of morbidity and mortality in adults in developing countries (WHO, 2009). Of the infectious diseases, which currently account for 60% of deaths, HIV and AIDS were the major causes of mortality in Malawi (Bowie, 2006). Deficiencies of *Vitamin*s and micronutrients such as Zn, I, Fe and, more recently, Se have been shown to increase the incidence or progression of the above-mentioned diseases(Bowie, 2006, WHO, 2009). In particular, Se deficiency has been strongly linked to increased progression of HIV and AIDS and mortality resulting from these diseases, while increased intake of Se has been reported to improve the health of HIV and AIDS patients (Foster, 2003, Gupta et al., 2009, Combs, 2001, Burbano et al., 2002, Rayman, 2002). An estimated 22.5 million people were living with HIV in Sub-Saharan Africa at the end of 2009, including 2.3 million children, and 1.3 million people died from AIDS. At the end of 2009, Malawi had 920,000 people living with HIV, with an adult prevalence of 11% and 51,000 deaths from AIDS (National Aids Commission, 2009).

Any increase in dietary Se in Malawi, and more widely in SSA, is therefore likely to have a major impact in mitigating the effects of HIV, AIDS and other diseases. This would improve public health and quality of life, reduce expenditure on health services and increase productivity and economic development within the region. Agronomic biofortification of maize with Se in Malawi is feasible through existing Farm Input Subsidy schemes, if deemed to be economically and politically acceptable. However, any public

health intervention involving widespread agronomic biofortification with Se would clearly require careful monitoring to ensure beneficial health outcomes. Whilst it is widely accepted that intake of Se is suboptimal for adults in Malawi, there remain considerable gaps in our knowledge concerning the relationships between Se intake, plasma Se concentration and selenoenzyme activity and definitive health outcomes (e.g. immune functioning), especially among individuals with very low-Se status in SSA. This situation must be addressed immediately *via* controlled intervention experiments, as a matter of urgency. Malawi will also need to improve its national analytical services in order to monitor the contribution of any biofortification programme on human nutrition and health.

7.6 Some thoughts on proposed future work

The data obtained from the studies reported in this thesis clearly suggest other important areas of research that could provide useful information to enhace Se intake in Malawi and SSA in general and the following are some of the future research areas:

7.6.1 Se intake, status and human health as affected by consumption of biofortified maize

There is an urgent need to conduct research on the effect of consuming biofortified maize on Se intake, status and human health. This could be followed by determination of Nutrient Required Intake of Se in Malawi based on the maximal expression of glutathione peroxidase activities.

7.6.2 Effect liming low pH soils on Se availability for plant uptake

The Se studies have also shown that Se availability is mainly controlled by soil pH and the higher the soil pH the higher the availability of Se for plant uptake. It would be interesting to evaluate the effect of liming acid soils in Malawi on Se availability for plant uptake. Malawi has enormous limestone

deposits that could be used to lime the acid soils should the results show promising of increasing Se concentration in food crops.

7.6.3 Effect of Se application in Se concentration of food crops grown in different cropping systems

Smallholder farmers in Sub-Saharan Africa practice different cropping systems such intercropping, agroforestry, zero and minimum tillage and studies on the effect of Se application in these cropping systems in Se concentration of the food produced would be very useful.

7.6.4 Effect of Se addition in livestock feed in Se deposition in meat, milk and eggs.

The investigation on the effect of adding Se in livestock feed particularly for small ruminants on Se concentration in meat and milk and for poultry on Se concentration in meat and eggs would be required as this would provide diversified sources of Se for human nutrition in Malawi.

7.6.5 Identification of local sources of Se

Identification of local sources of Se need to be investigated as Malawi has coal, rock phosphate and limestone deposits, which are known to be associated with high Se concentration. Copper mining in Zambia has been on-going for decades and it is likely that Se sourced from this activity could be used within the region to biofortify crops with Se.

7.6.6 Conducting structured survey on soil, food crops and diets across the country.

The results of the survey on soil and maize grain need to be extended to cover all soil types, all food crops and all diets of the country so that Se concentration of soil, food crops and diets of the people are accurately documented and then accurate Se intakes across the country are known in the country. This would provide strong foundation of future research in Malawi.

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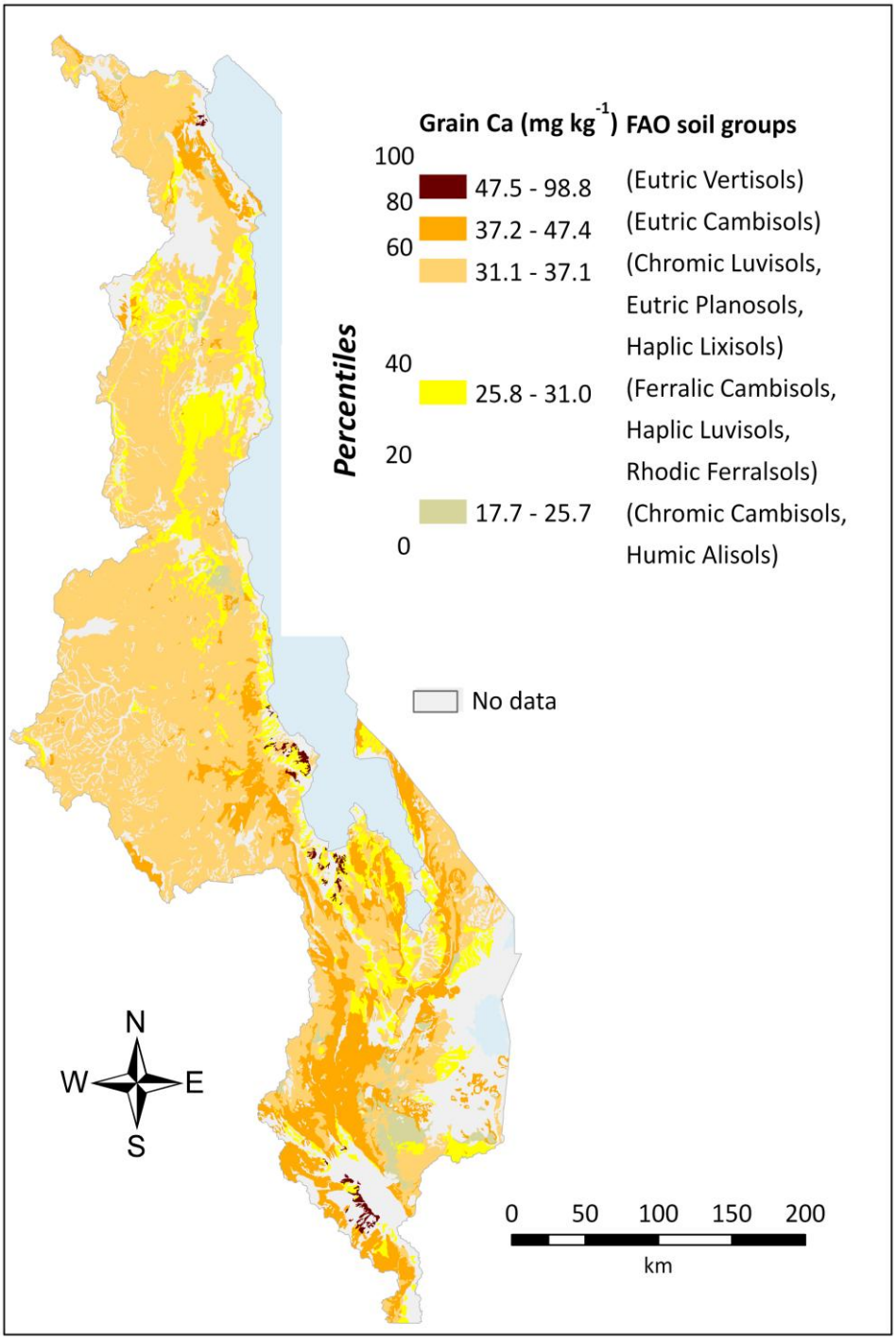
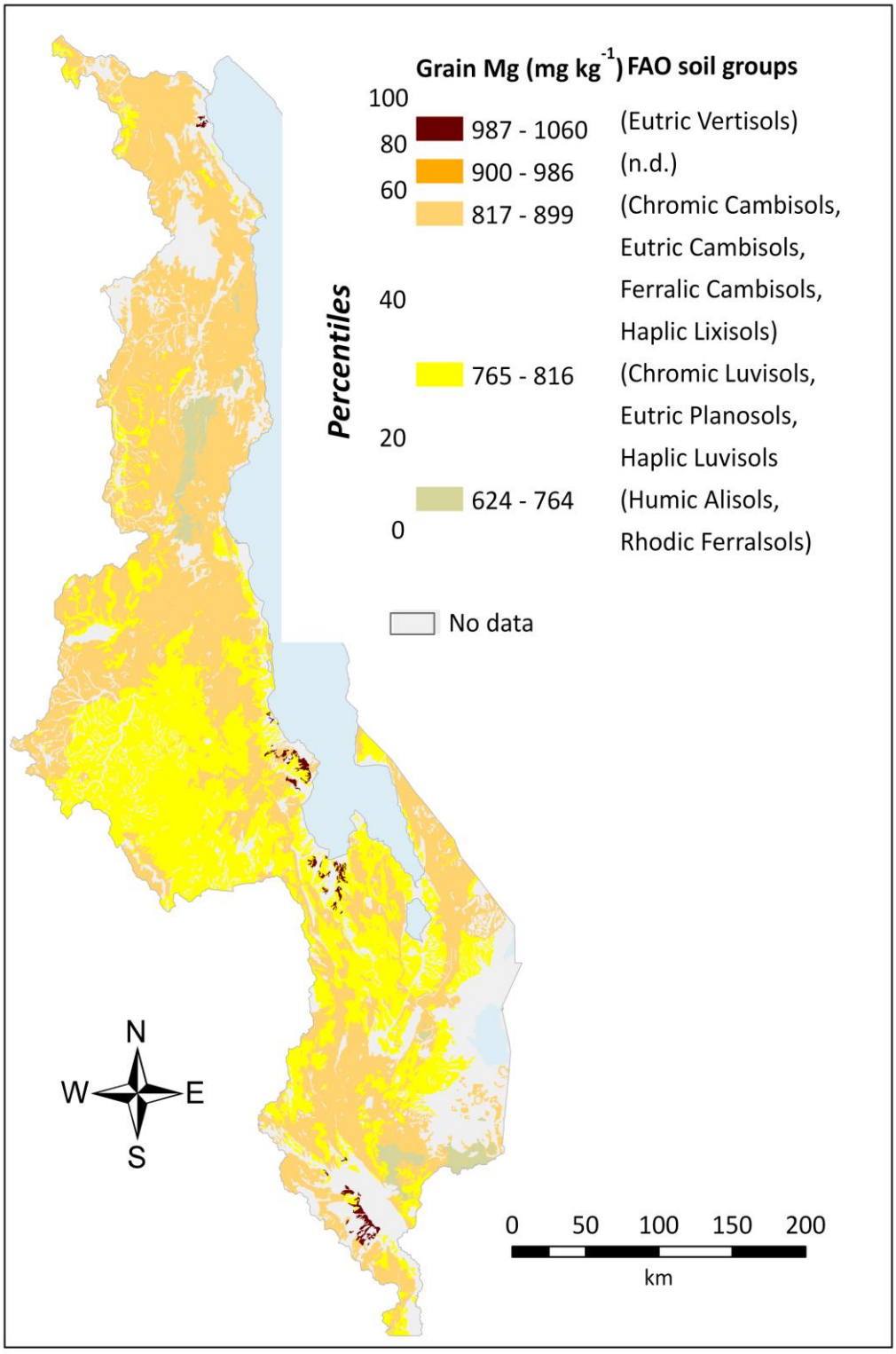
APPENDICES

Appendix 1. Maize grain nutrient concentrations (Ca, Mg, K, Fe, Zn, Cu, Mo, Co, Ni) across Malawi based on soil types.

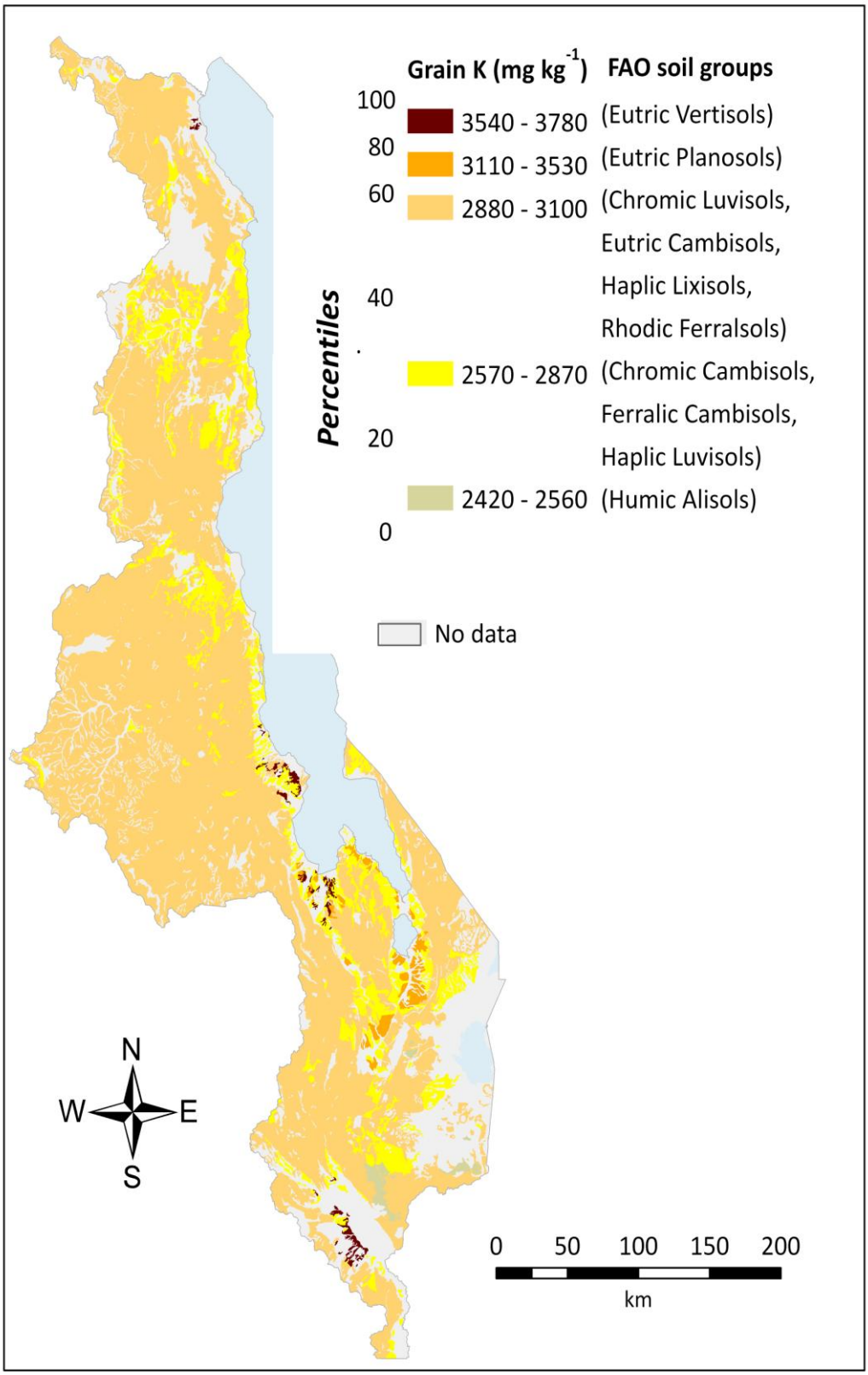
(a) Maize grain Ca concentration



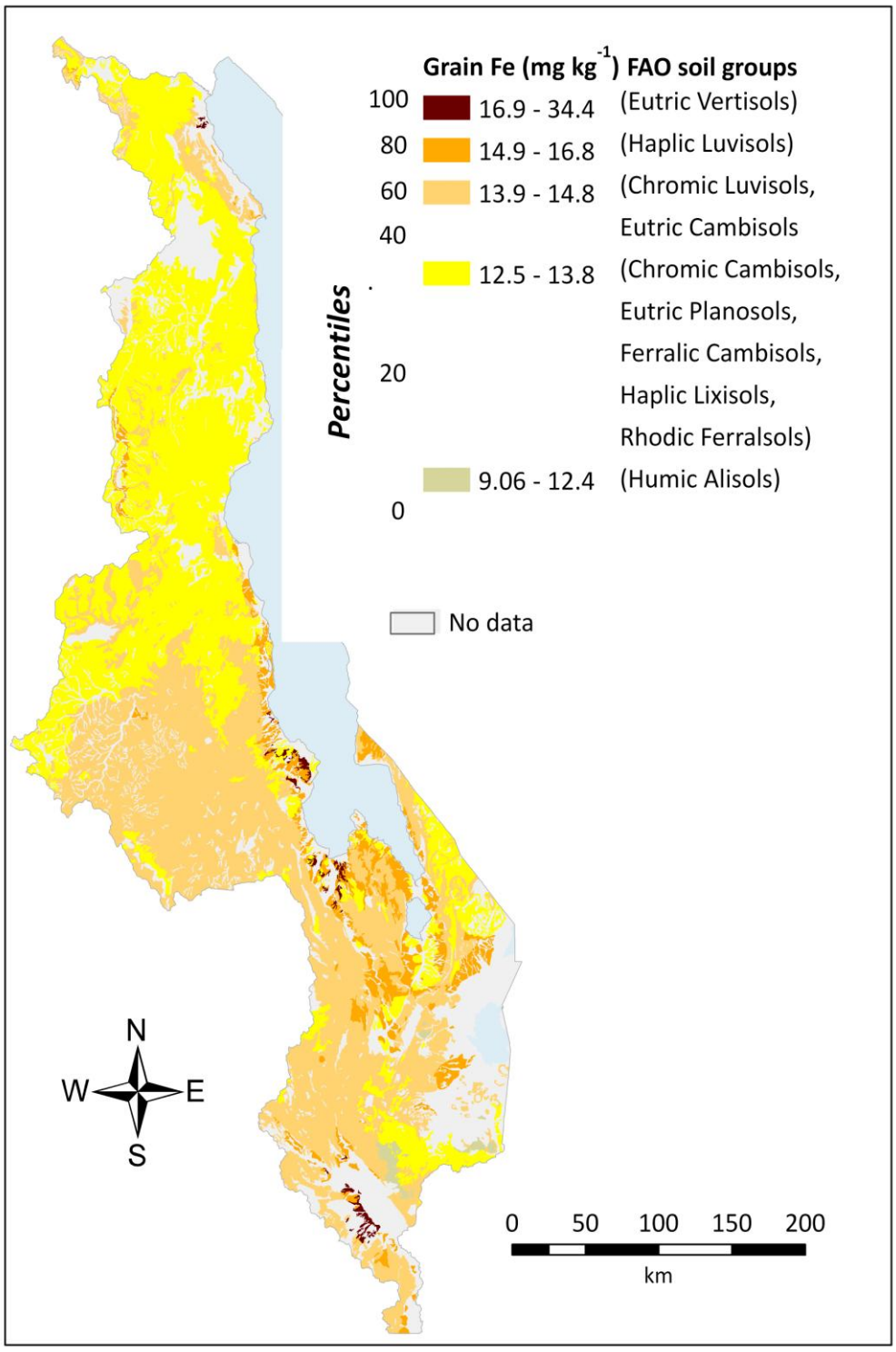
(b) Maize grain Mg concentration



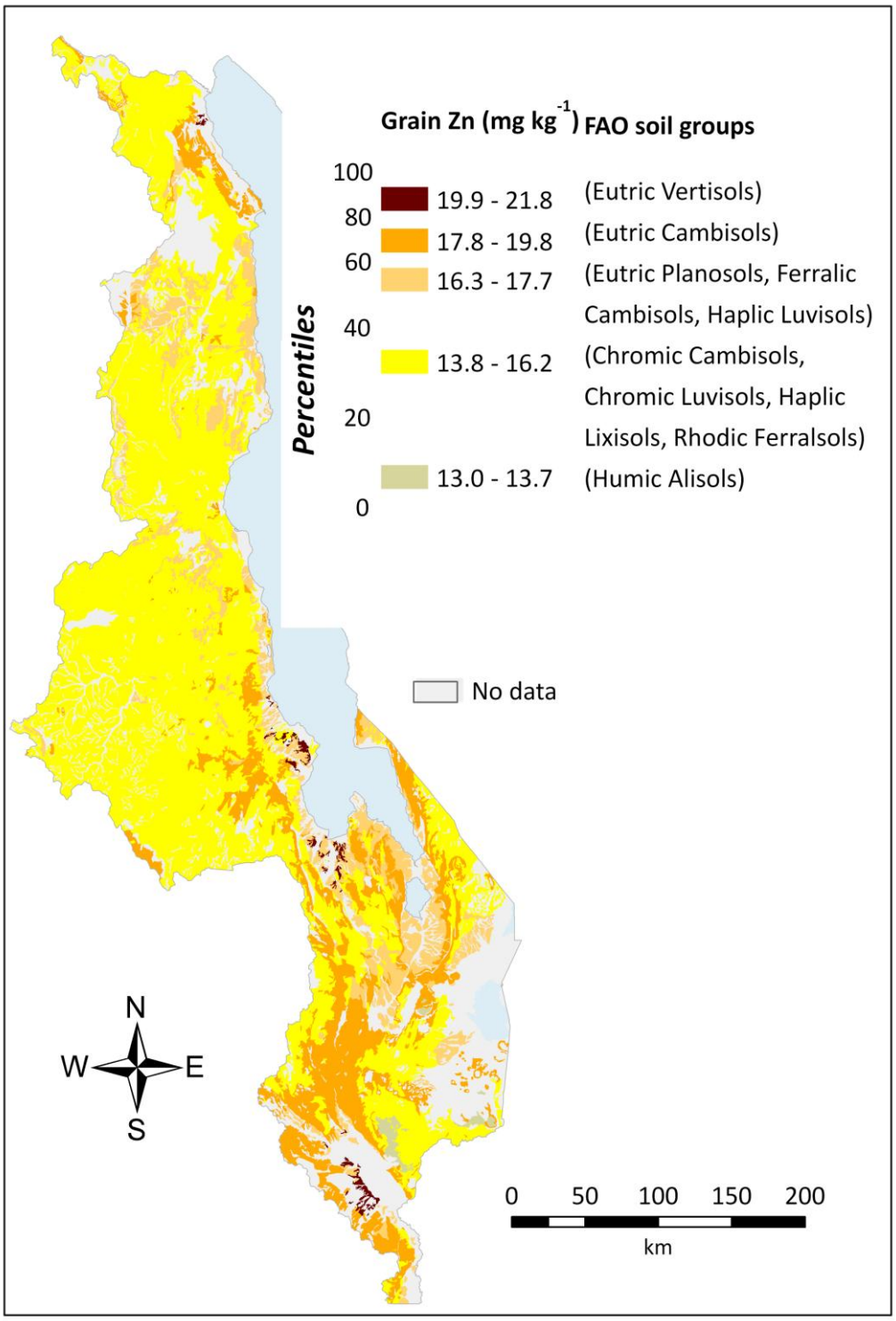
(c) Maize grain K concentration



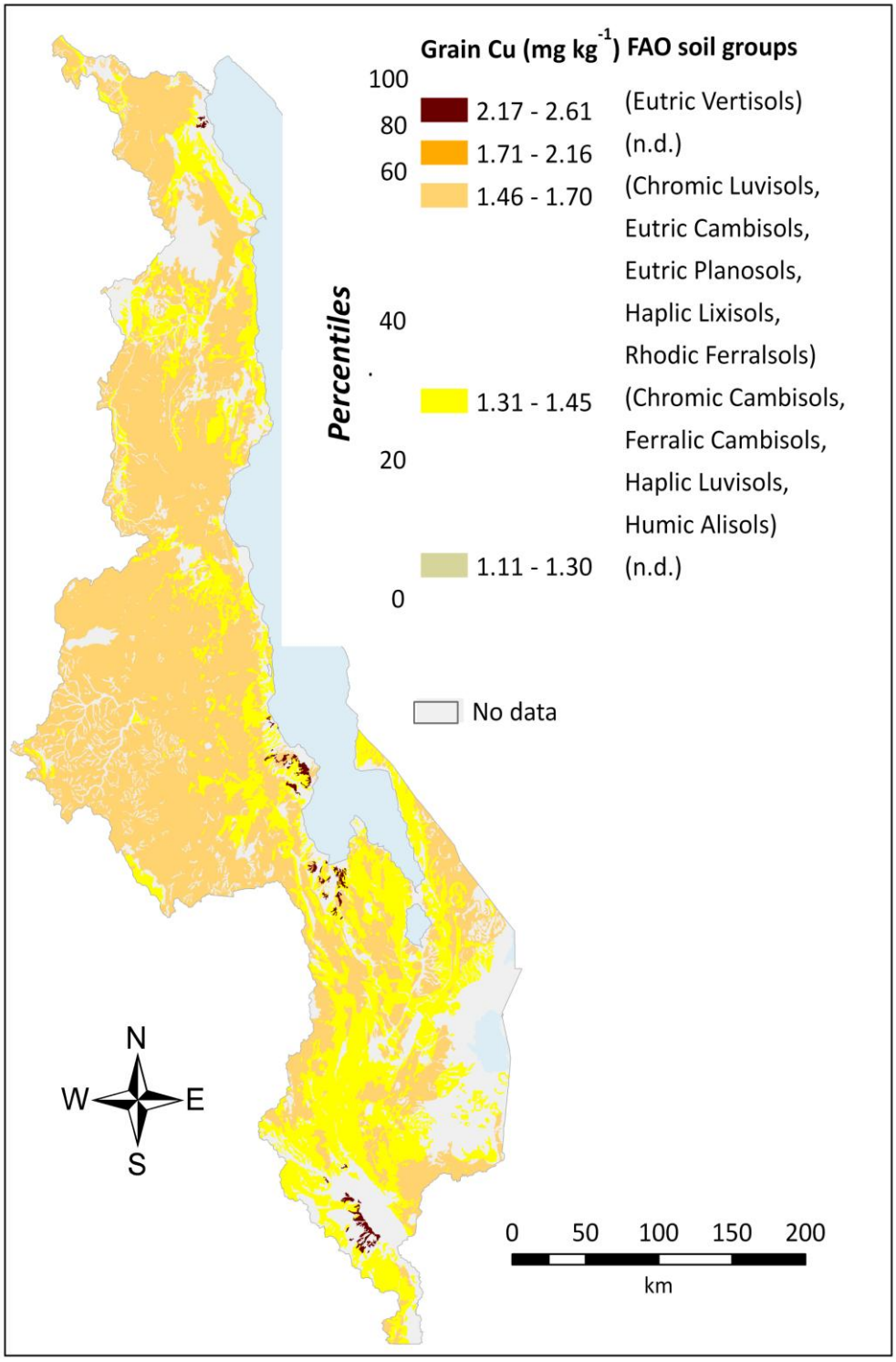
(d) Maize grain Fe concentration



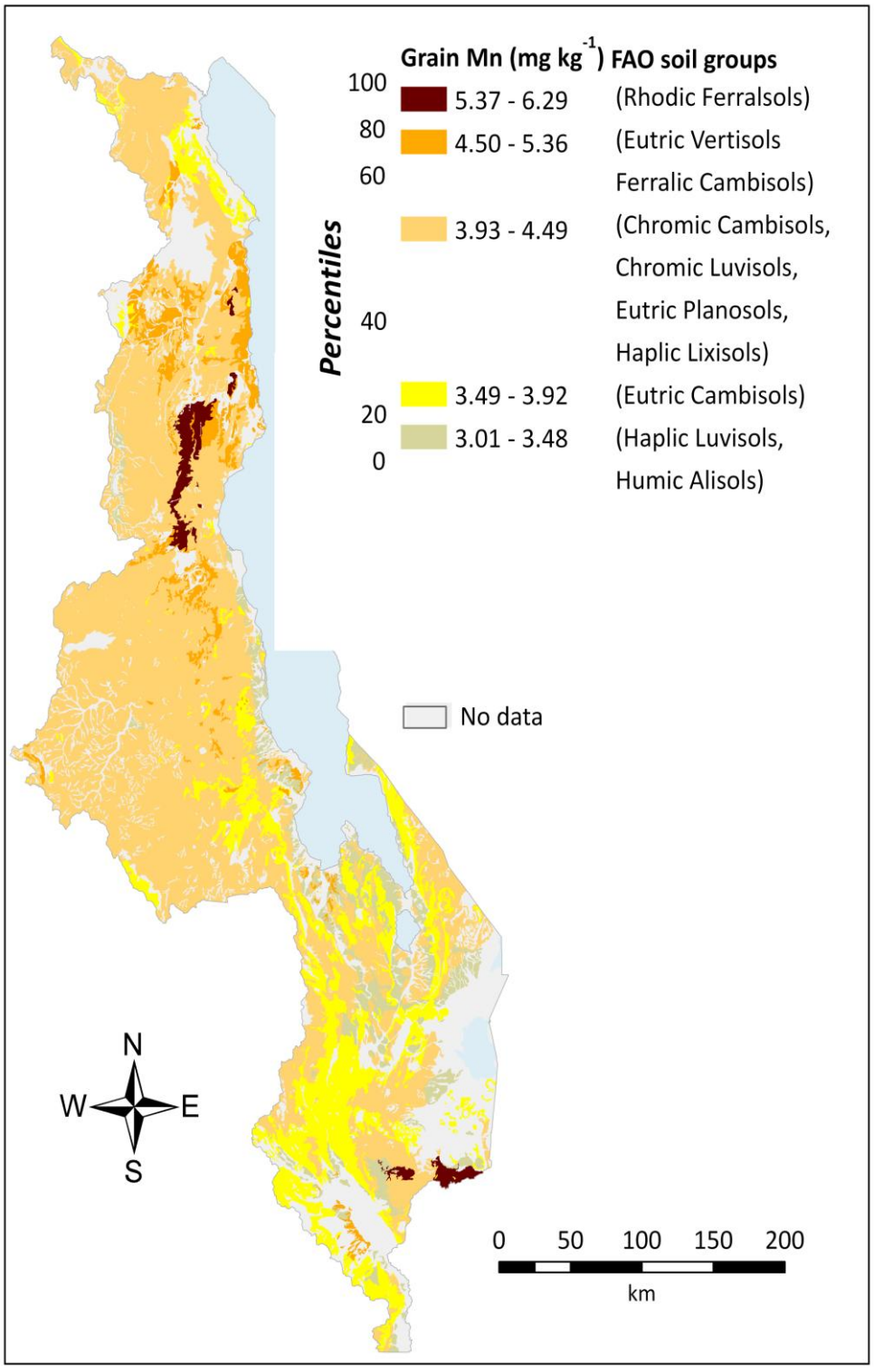
(e) Maize grain Zn concentration



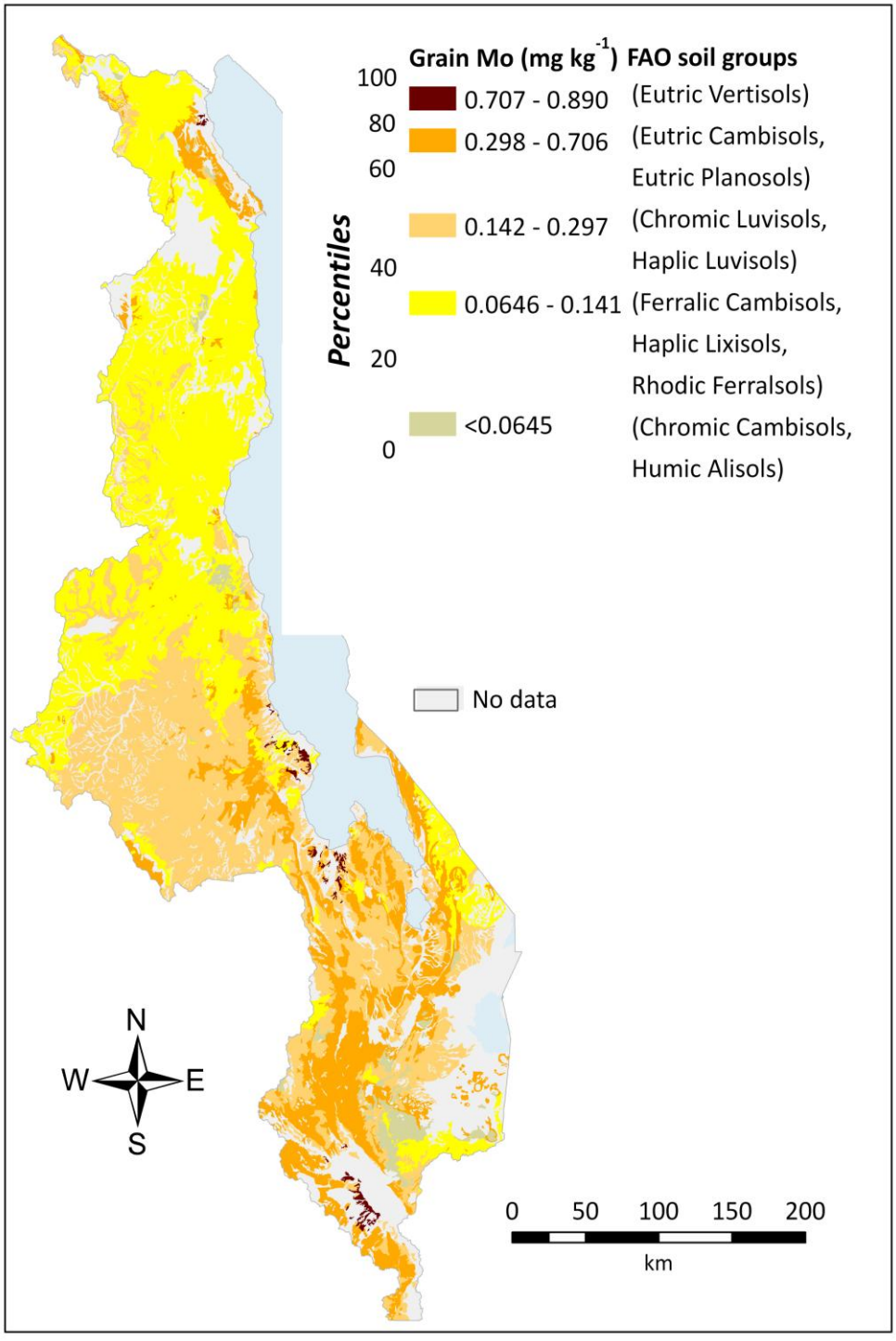
(f) Maize grain Cu concentration



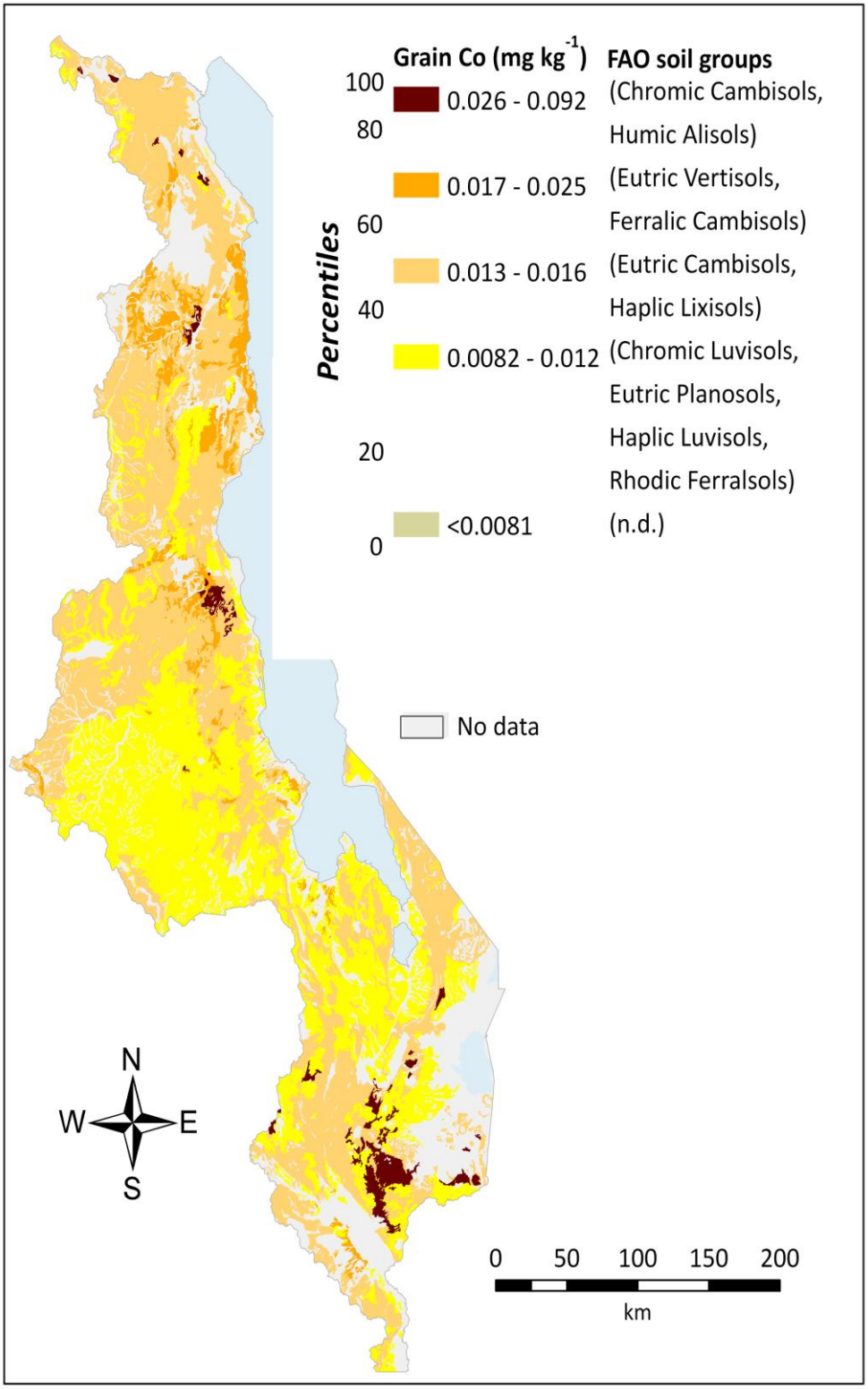
(g) Maize grain Mn concentration



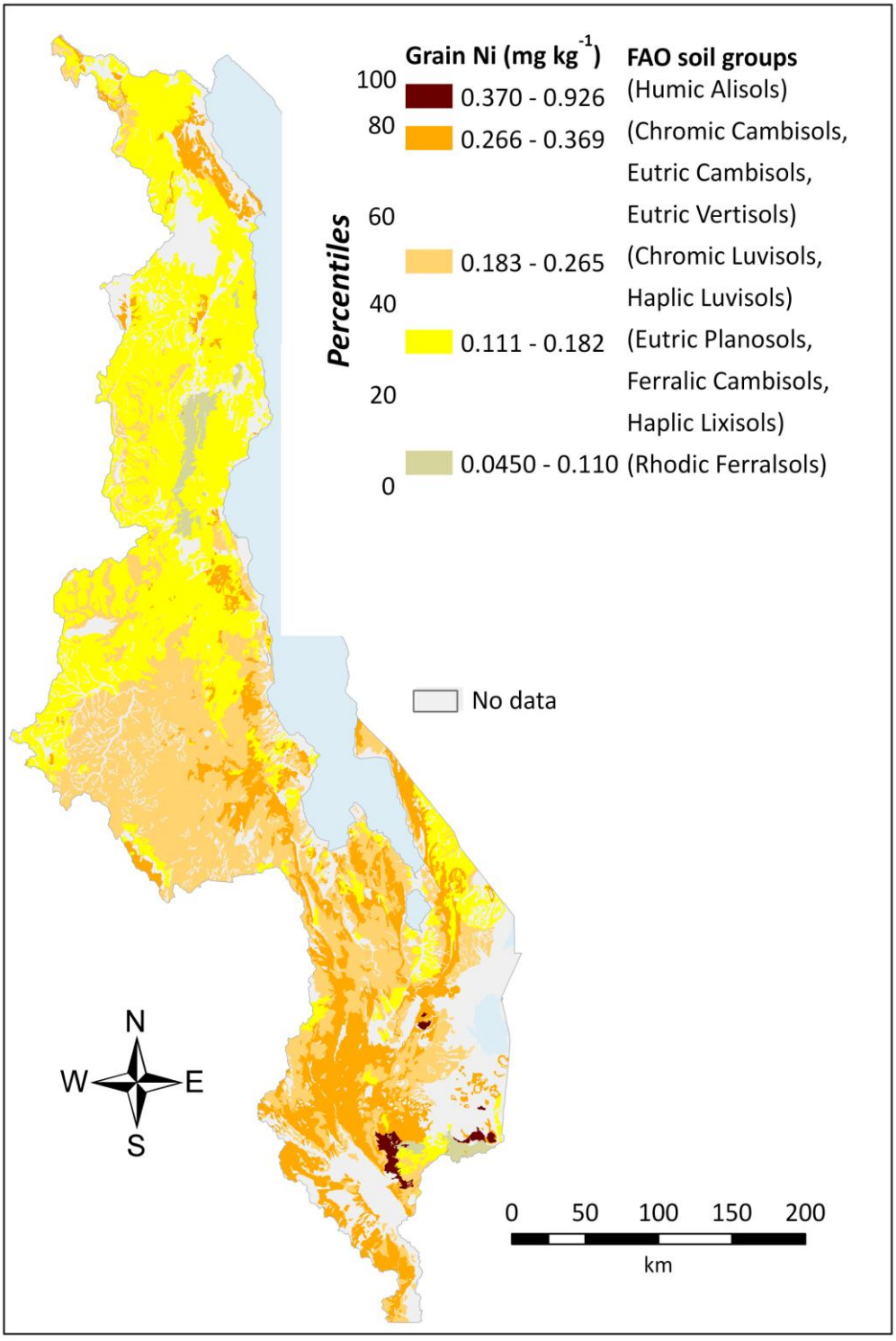
(h) Maize grain Mo concentration



(i) Maize grain Co concentration

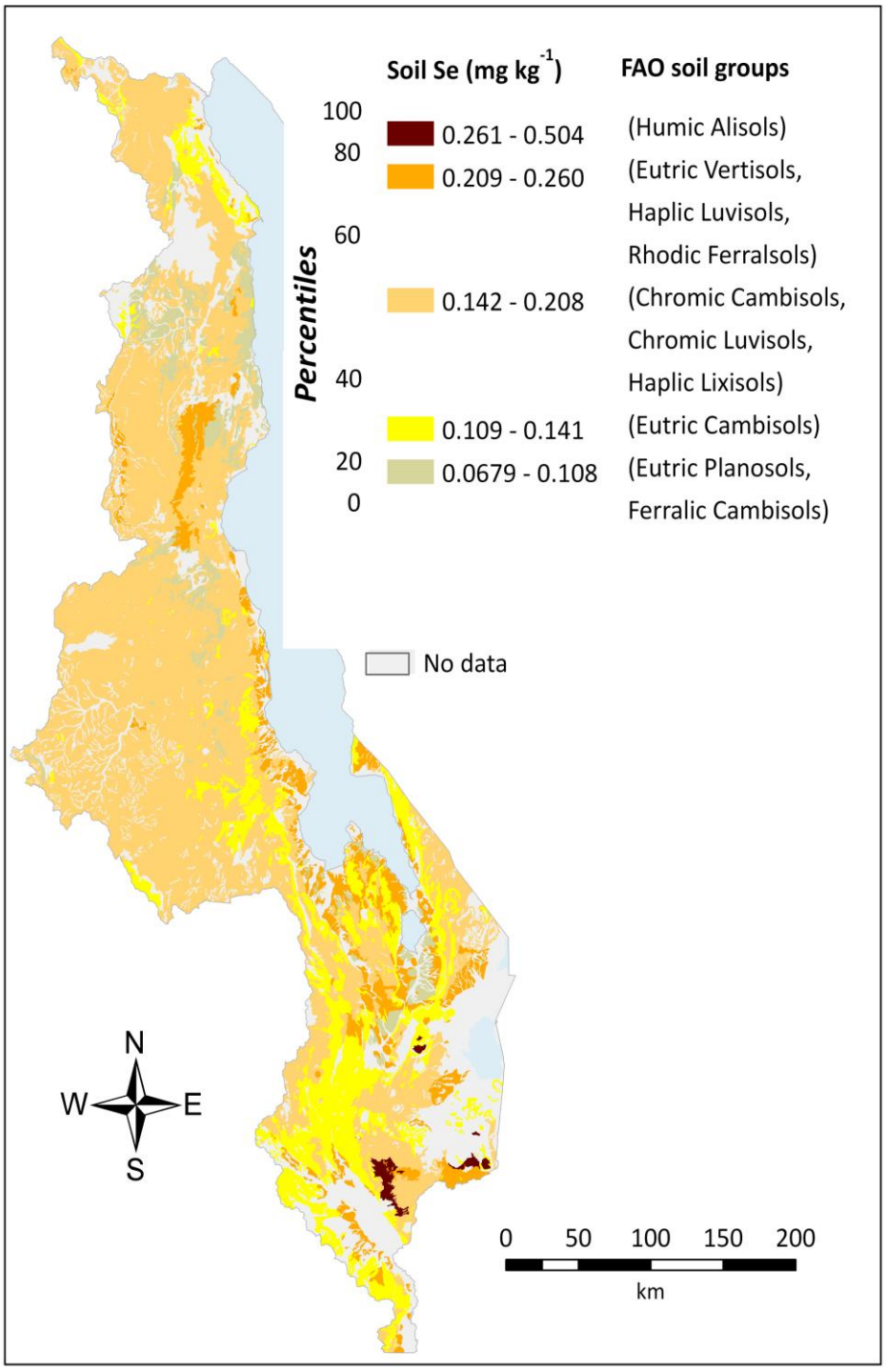


(j) Maize grain Ni concentration

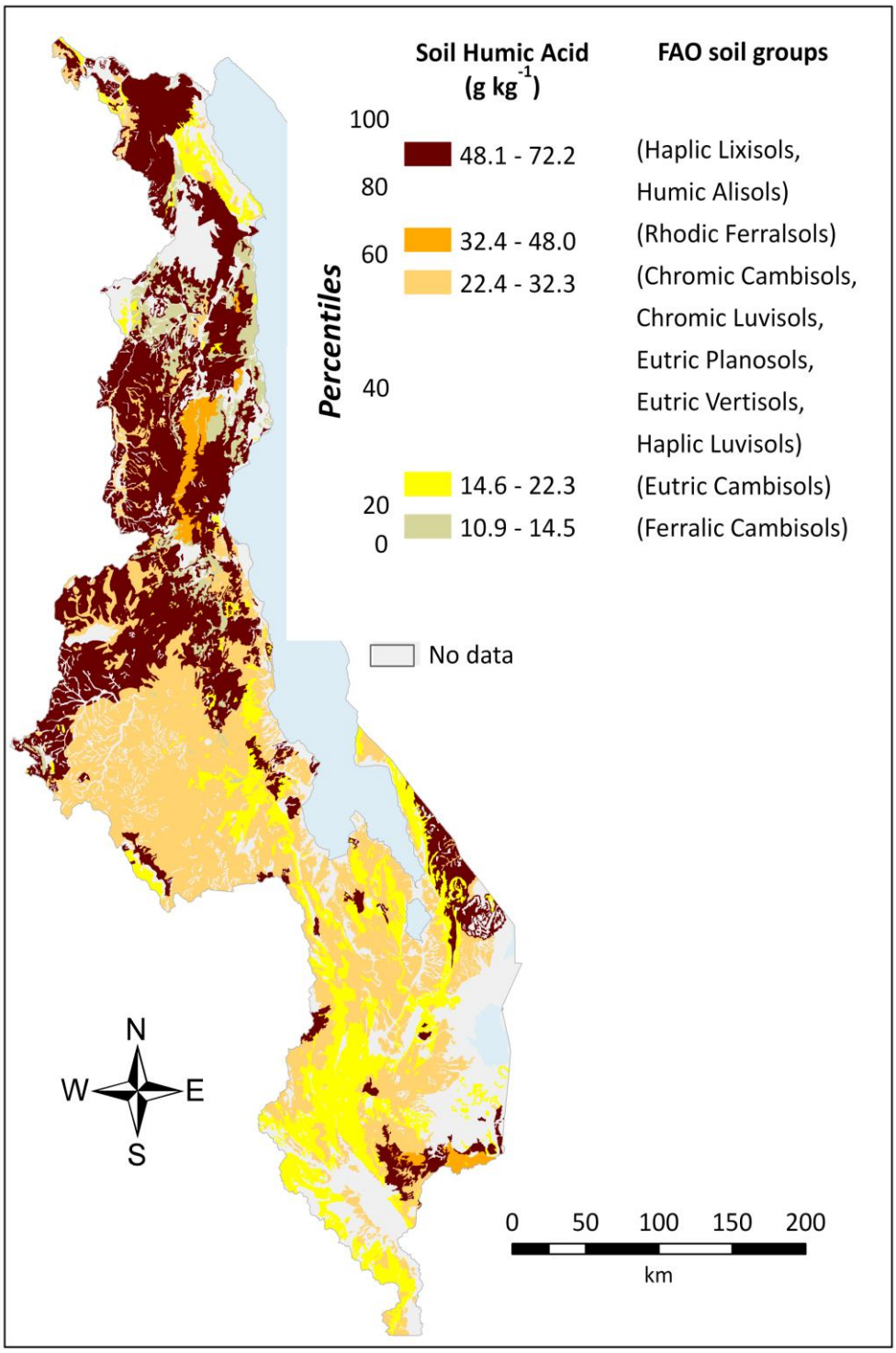


Appendix 2. Soil nutrient concentrations (Se, Ca, Mg, K, Fe, Zn, I, Cu, Mo, Co, Ni) and humic acid concentration across Malawi, based on soil types.

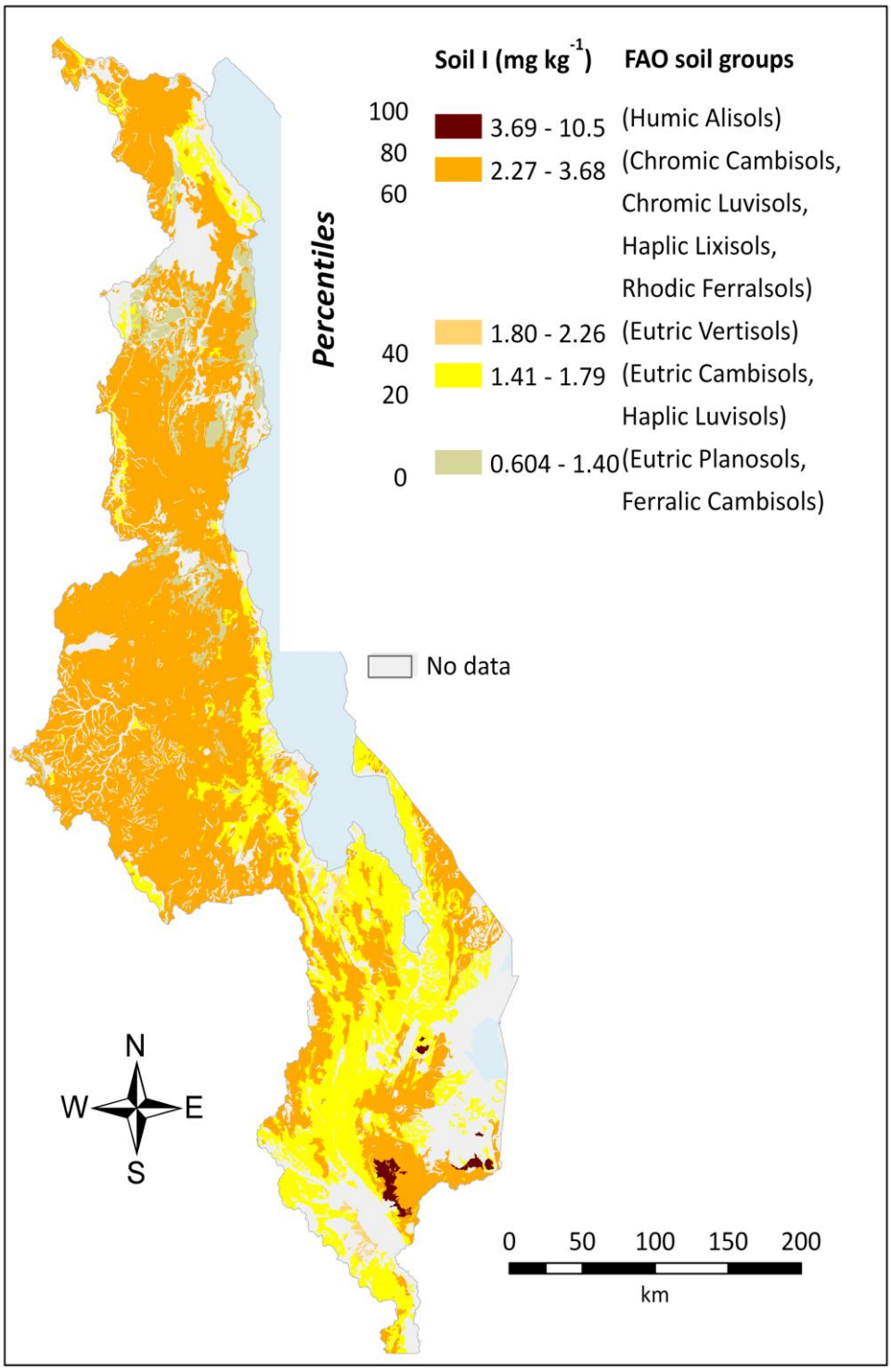
(a) Total soil Se concentration



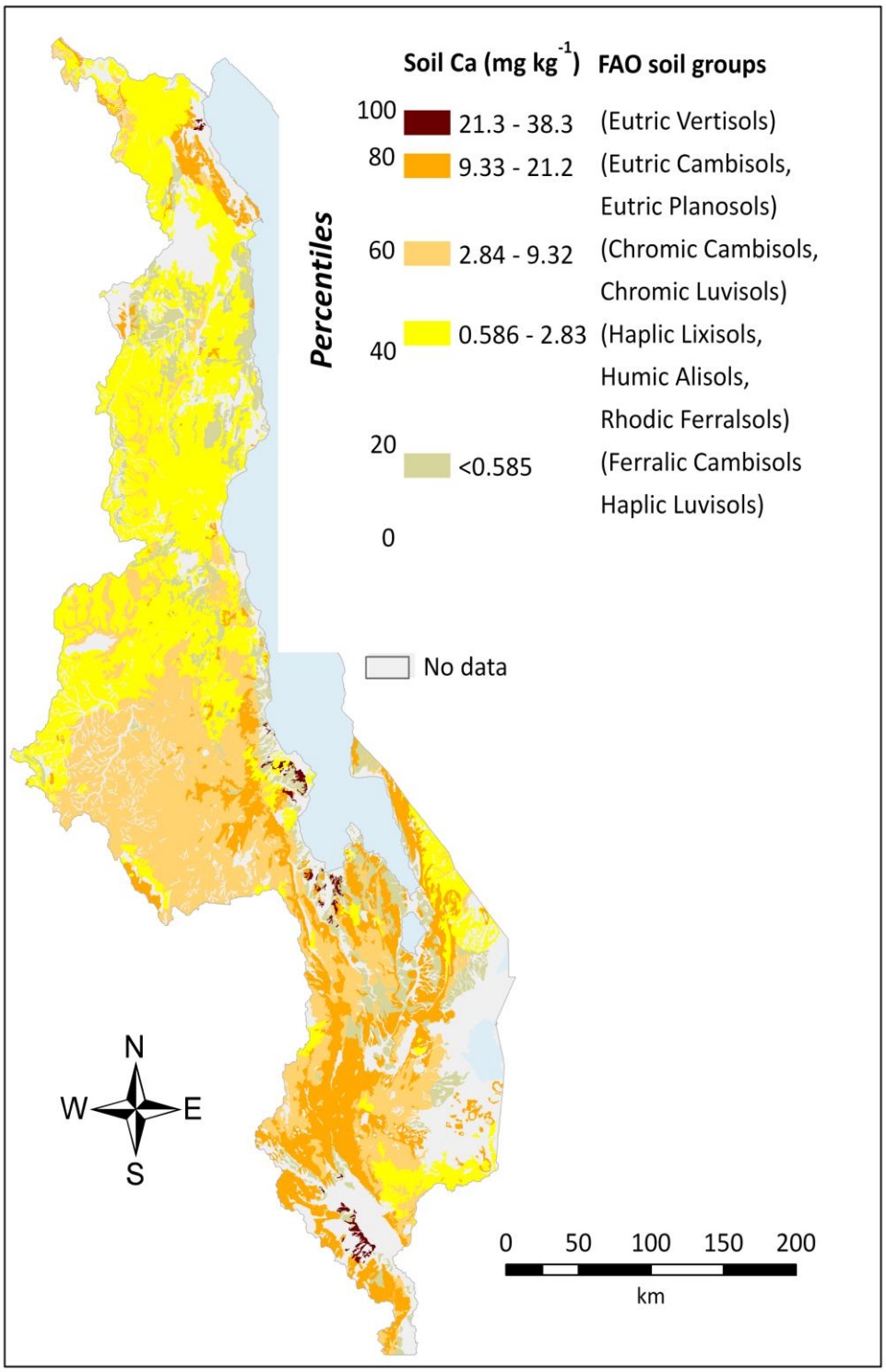
(b) Soil humic acid concentration



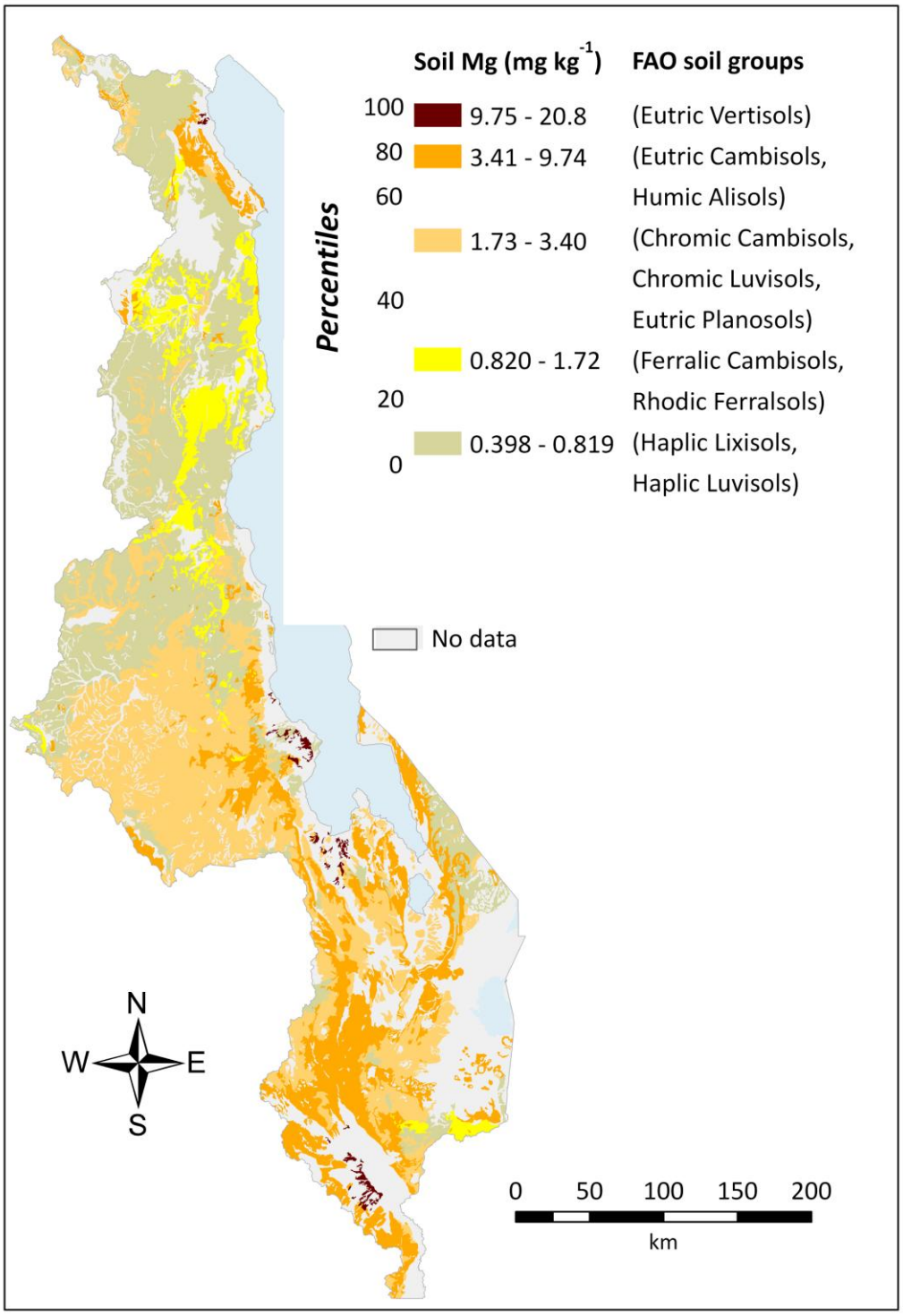
(c) Soil I concentration (TMAH extractable)



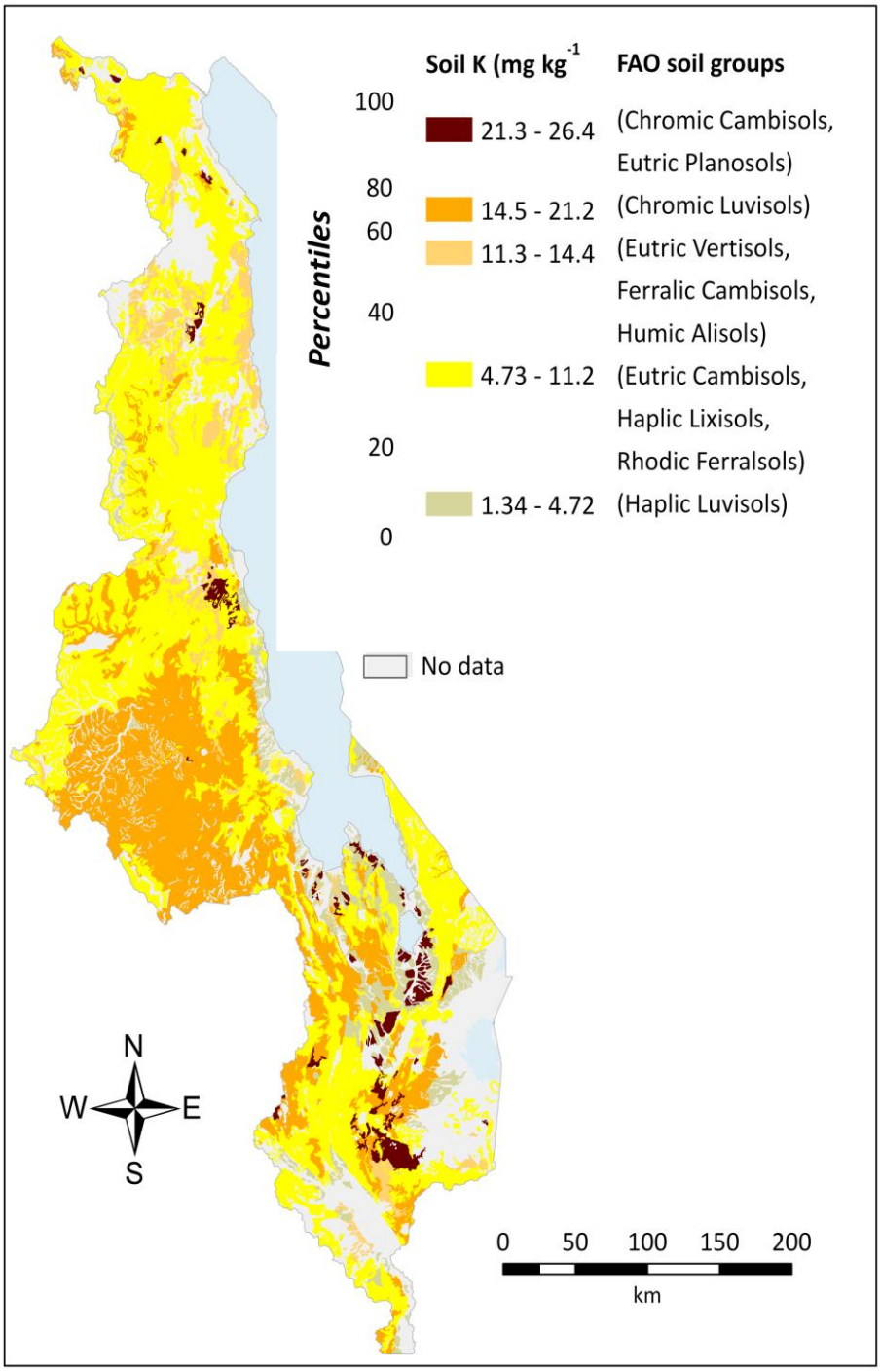
(d) Total soil Ca concentration



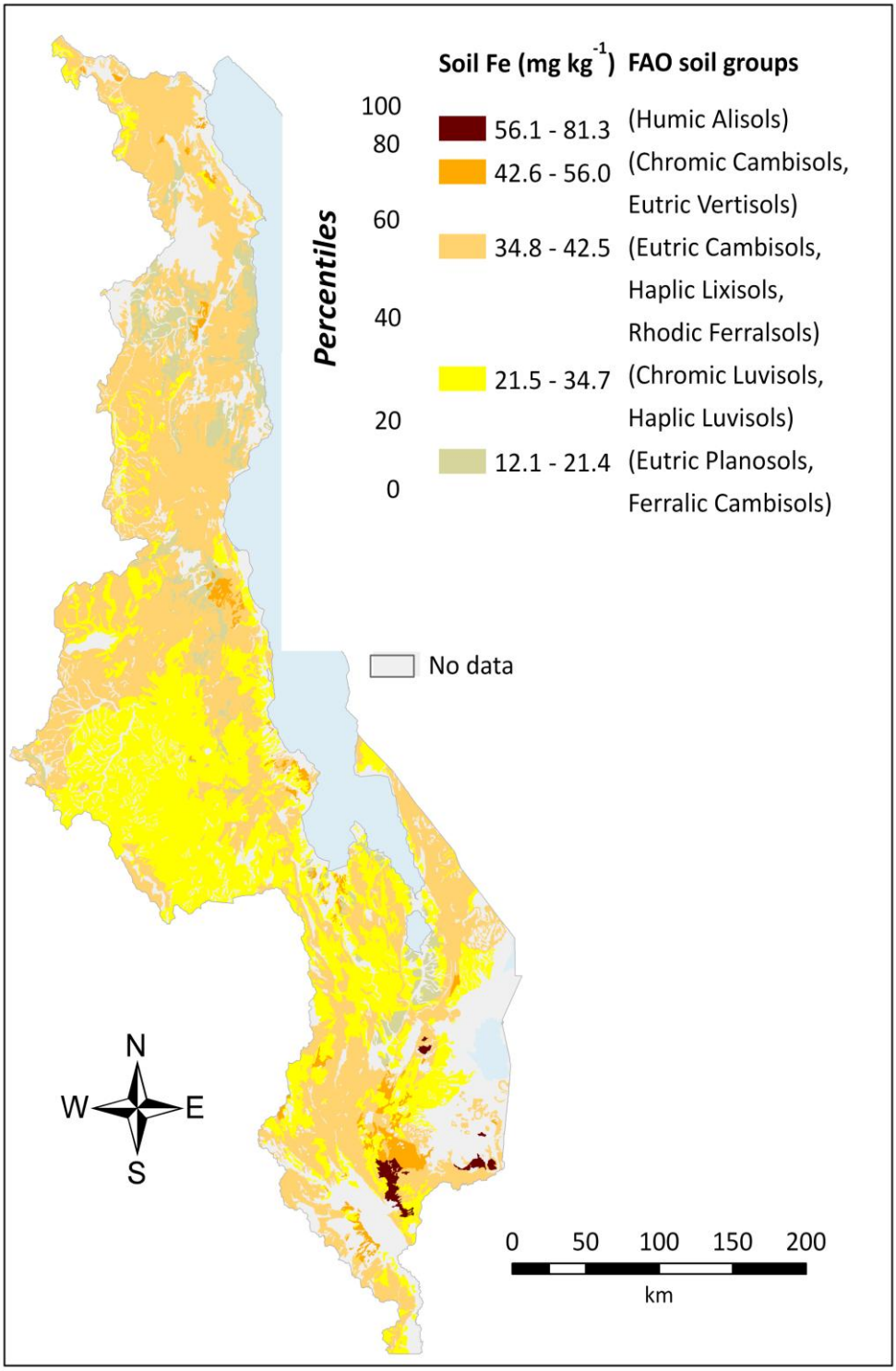
(e) Total soil Mg concentration



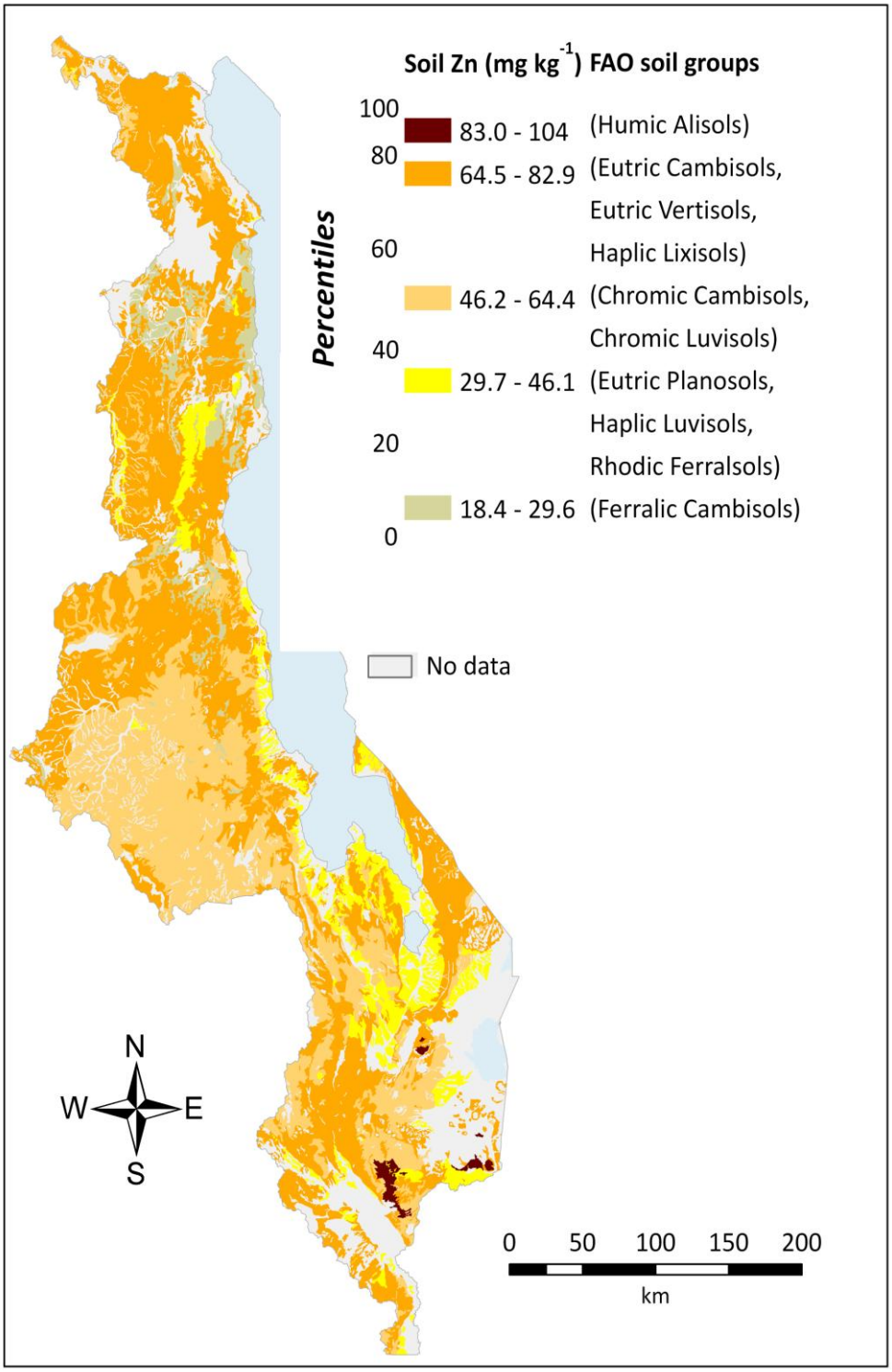
(f) Total soil K concentration



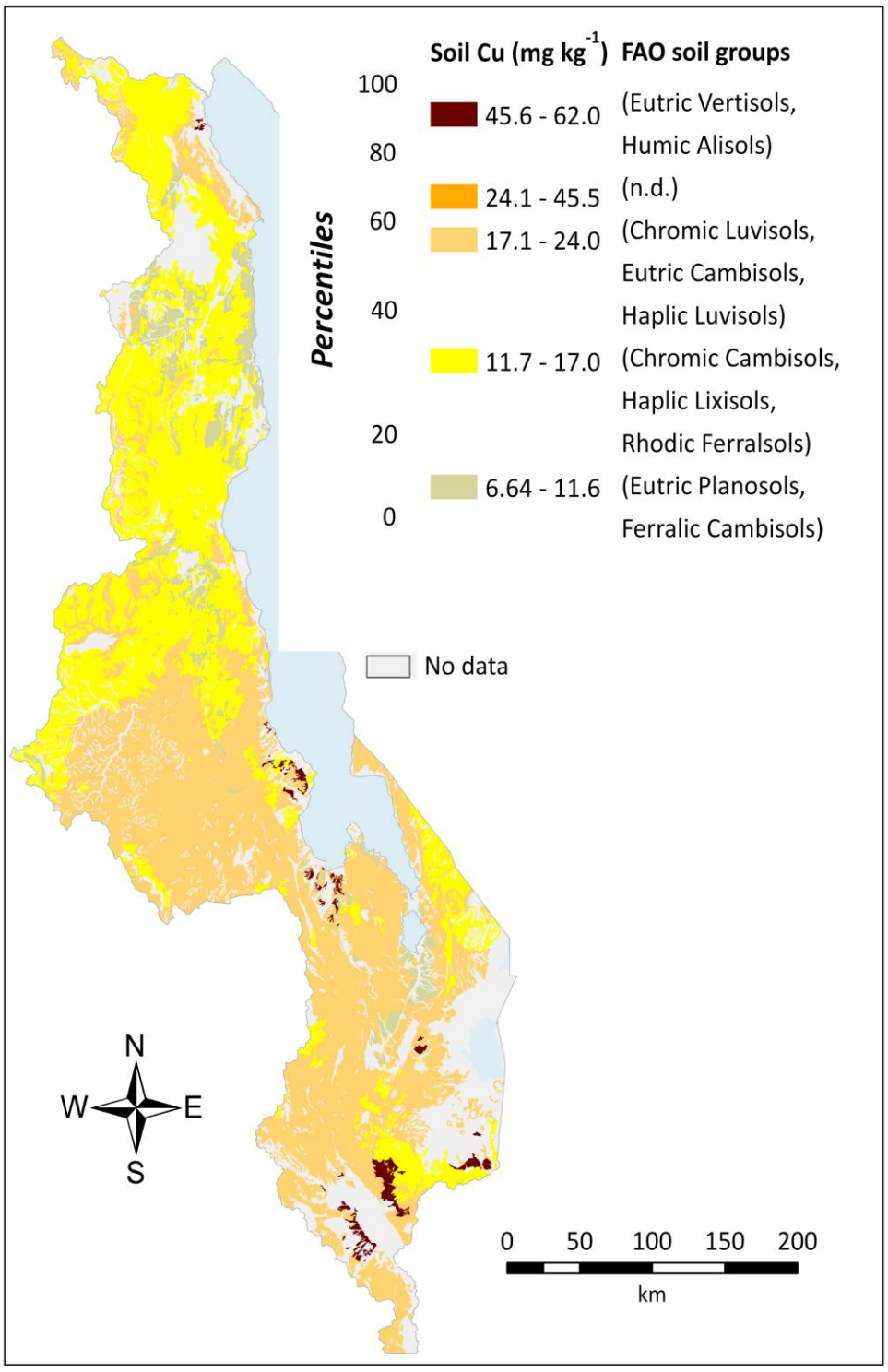
(g) Total soil Fe concentration



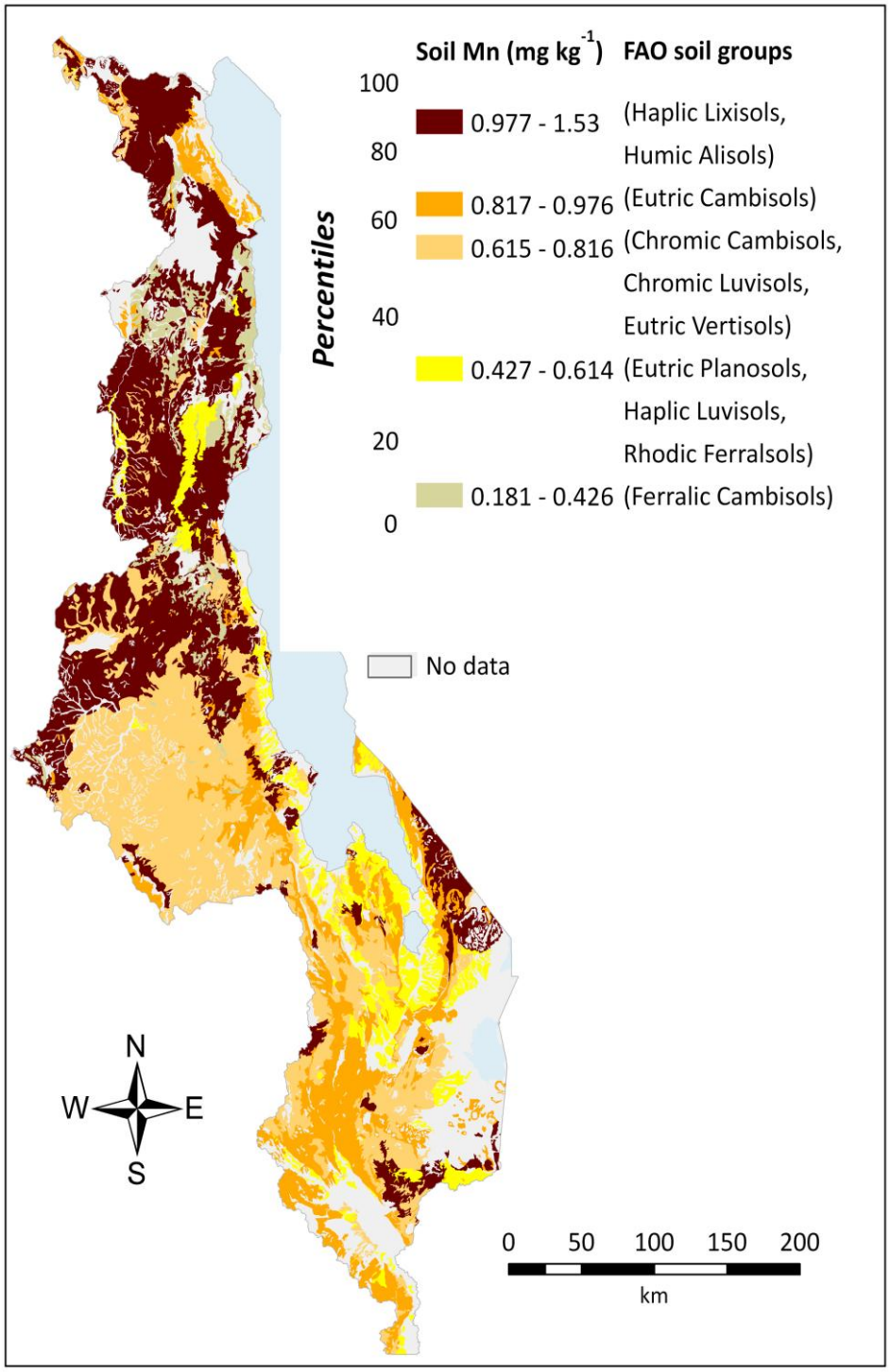
(h) Total soil Zn concentration



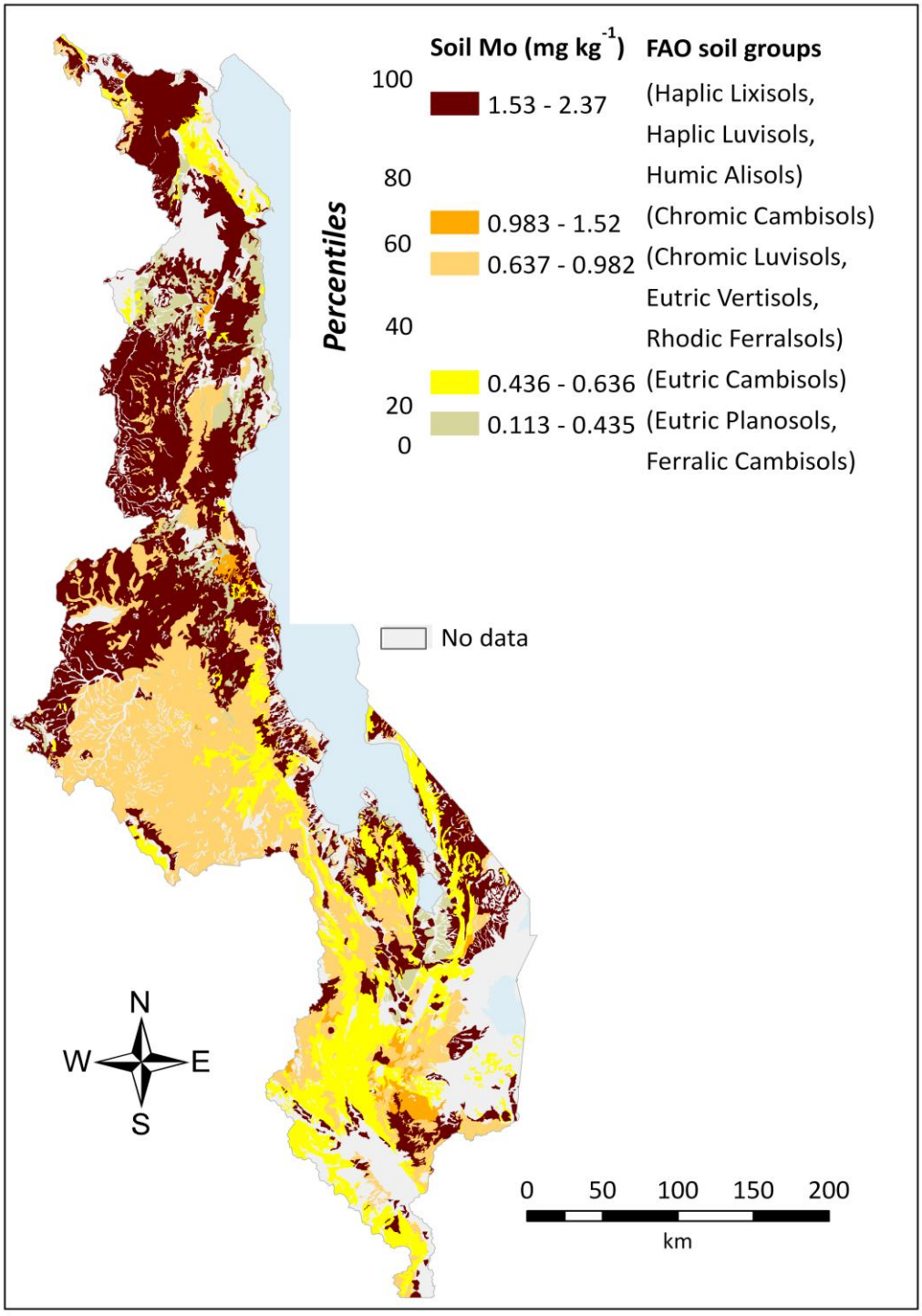
(i) Total soil Cu concentration



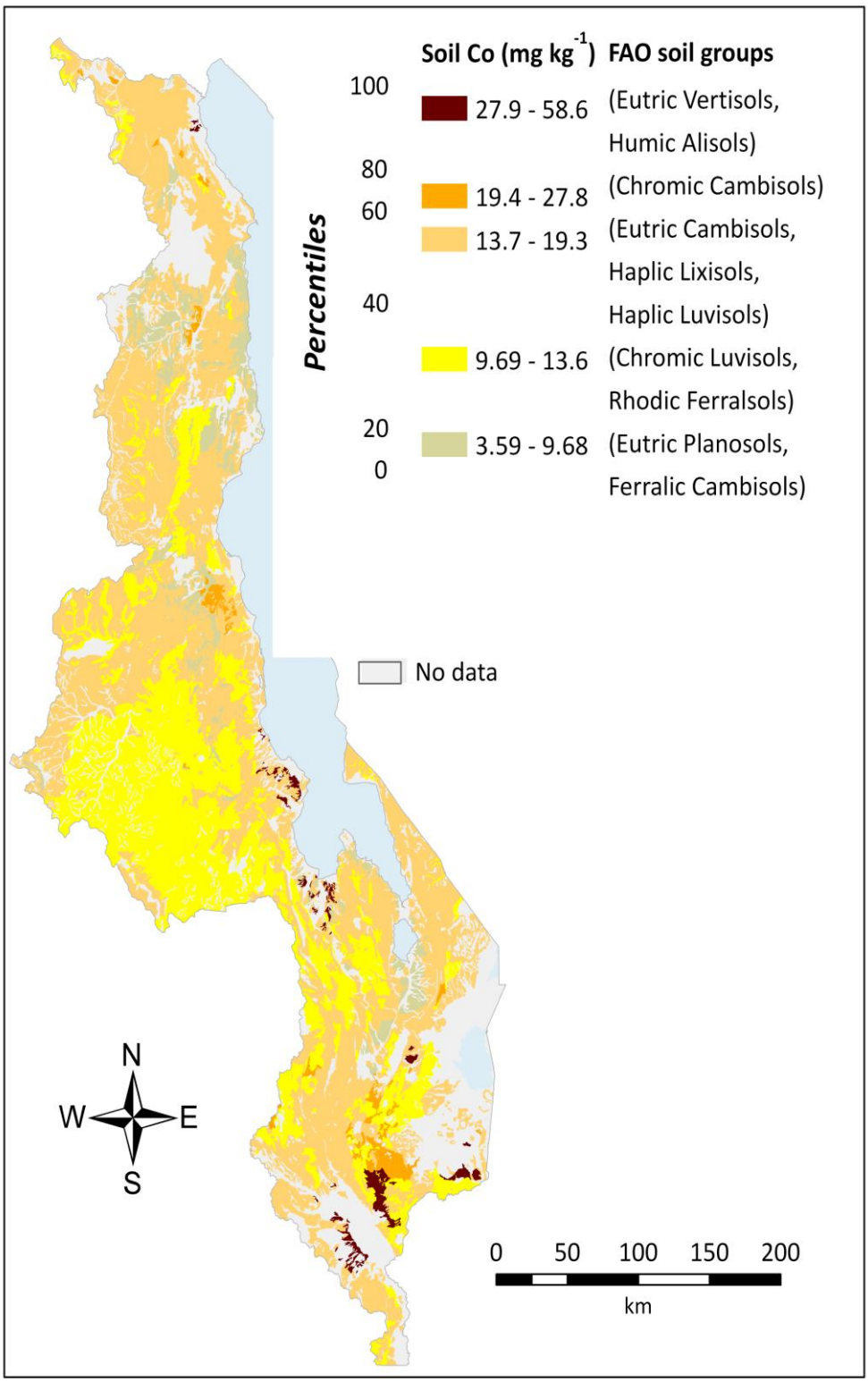
(j) Total soil Mn concentration



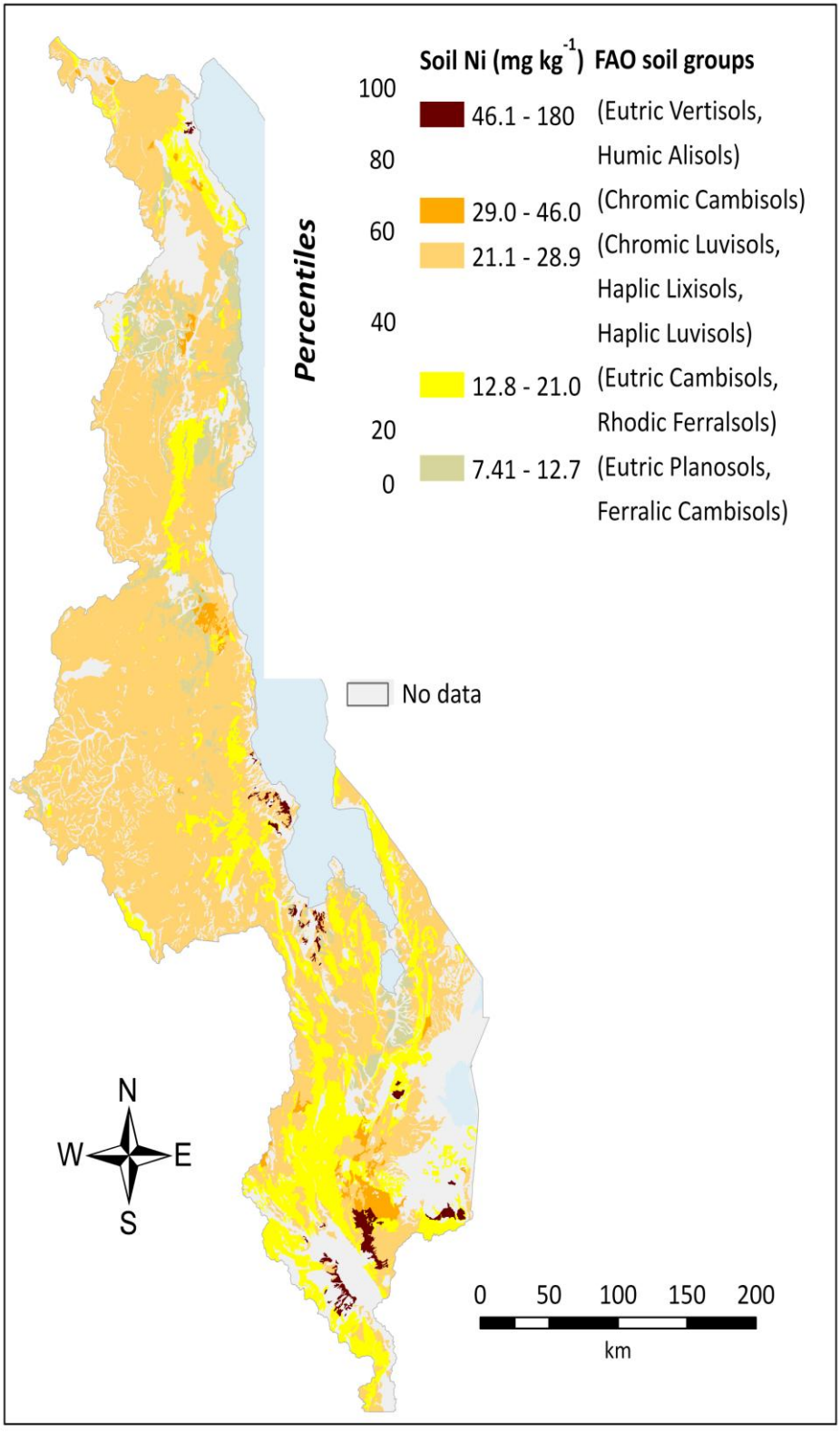
(k) Total soil Mo concentration



(l) Total soil Co concentration



(m) Total soil Ni contration



Appendix 3: Certificate of Analysis of sodium selenate used in the study

Product Name	Sodium Selenate	Na_2SeO_4
Items	**Specification**	**Results**
Na_2SeO_4	≥98%	98.7%
Se	≥41.0%	41.3%
Pb	≤ 20ppm	7ppm
As	≤5ppm	3ppm
Fe	≤10ppm	2ppm
Cu	≤10ppm	3ppm
Cd	≤5ppm	3ppm
Hg	≤1ppm	0.2ppm
Ni	≤10ppm	5ppm
Te	≤100ppm	20ppm
Moisture	≤0.5%	0.3%
Selenite	≤0.5%	0.4%
Insoluble Residue	≤0.1%	0.05%

Appendix 4: Soils Hydrofluric Acid Digestion using Block Digester procedures

1. Weigh 0.2 g sample into block digester tubes.
2. Add 4 ml HNO_3, swirl and leave for 30 mins.
3. Place in block digester and turn on Programme 1.
4. Leave overnight.
5. Add 2 ml HNO_3.
6. Add 1 ml $HClO_4$.
7. Place in block digester and turn on Programme 2.
8. Leave overnight
9. Add 2.5 ml HF
10. Turn on block digester Programme 3. Monitor the digestion regularly during the day.
11. Add 2.5 ml HNO_3 and 2.5 ml MilliQ water then leave at 50^{o}C for 1 hour.
12. Turn off block.
13. Remove from hot block and cool completely.
14. Make up to 50 ml with MilliQ water in plastic volumetric flasks.

For non-organic soils you can omit steps 2, 3 and 4

HF DIGESTION WITH BLOCK DIGESTER: PROGRAMMES

Program 1	**Program 2**	**Program 3**
STEP: 30 ^{0}C	STEP: 80 ^{0}C	STEP: 120 ^{0}C
DWELL: 0.5 hr	DWELL: 8hrs	DWELL: 1 hr
STEP: 50 ^{0}C	STEP: 100 ^{0}C	STEP: 140 ^{0}C
DWELL: 1hr	DWELL: 2hrs	DWELL: 3hrs
STEP: 80 ^{0}C	END	STEP: 160 ^{0}C
DWELL: 14hrs		DWELL: 4hrs
STEP: 30 ^{0}C		STEP: 50 ^{0}C
END		END

Appendix 5: Thermo-Fisher X-SeriesII Inductively Coupled Plasma Mass Spectrometer (ICPMS) applications

The inductively coupled plasma mass spectrometer (ICPMS) was used for one of three (multi-element) analytical applications:

1. Trace-element analysis at the ppb (µg L^{-1}) level; detector operating in pulse-counting mode but with a mass-dependent cross calibration factor to convert analogue signals.
2. Major element (Ca, Mg, K, Na) analysis at the ppm (mg L^{-1}) level; detector operating in analogue mode only.
3. Determination of isotope ratios for individual elements; detector operating in pulse-counting mode only.

ICPMS facility was installed including a Thermo-Fisher X-Series II instrument with 'collision cell technology - energy discrimination' (CCTED) capability and a Cetac ASX-520 autosampler (Plate 1). The ICP and autosampler are controlled by 'Plasmalab' software.

For proper use for analyses, the following aspects were considered

1. Preparation of samples for analysis: acceptable matrices and analyte concentration ranges, filtration procedures, preparation of calibration standards, internal standards, 'tune solution' and wash solution.
2. Preparation of the instrument for analysis: maintaining and setting up the ICP and autosampler, performance checking and tuning procedures.
3. Setting up your experiment in the Plasmalab software: choice of operating conditions, acquisition parameters, choice of analyte isotopes, preparing a sample list, running an experiment, processing your data.

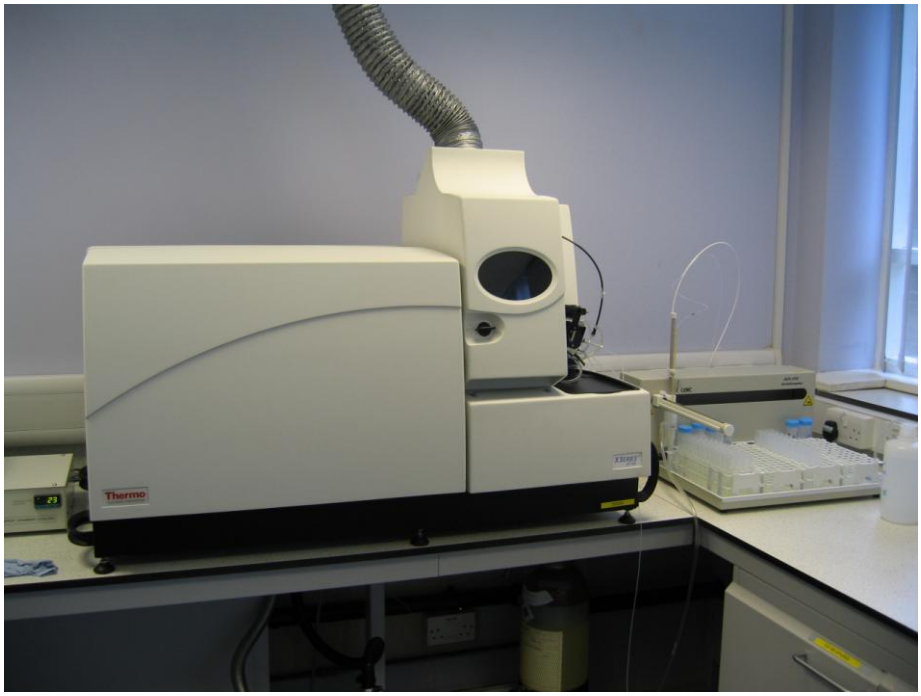


Plate 1. Thermo-Fisher X-Series II ICPMS

Typical operating conditions for CCT mode X Series ICPMS Operating Conditions: Se analysis

Forward power = 1404 W

Nebuliser (Carrier gas) = 0.82 L min^{-1}

Extraction lens = -129.4 V

Lens 1 = -729 V

Lens 2 = -46.3 V

Lens 3 = -195.3 V

Focus = -8.0 V

D1 = -40.8 V

D2 = --110 V

DA = -25.1 V

Hexapole bias = -18.0 V

Pole bias = -14.0

Reaction cell gas = 4.00 mL min^{-1} of 7% H_2 in He

Quadrupole dwell times 78Se = 500 ms

^{45} Sc, ^{103}Rh, ^{103}Ir = 20 ms

Appendix 6: Photos showing maize field crops of agronomic biofortification experiments at Makoka (top), Chitedze (middle) and Ngabu (lower).

Appendix 7: Publications

Chilimba, A.D.C., Young, S.D., Black, C.R., Rogerson, K.B., Ander, E.L., Watts, M., Lammel, J., Broadley, M.R. 2011. Maize grain and soil surveys reveal suboptimal dietary selenium intake is widespread in Malawi. Scientific Reports, 1, 72; DOI:10.1038/srep00072

Chilimba, A.D.C., Young, S.D., Black, C.R., Meacham, M.C., Lammel, J., Broadley, M.R. 2012. Agronomic biofortification of maize with selenium (Se) in Malawi. *Field Crops Research, 125:118-128***.**

Chilimba, A.D.C., Black, C.R., Lammel, J., Meacham, M.C., Young, S.D., Broadley, M.R**.** 2009. Agronomic biofortification of maize (*Zea mays* L.) with selenium in Malawi. In: Banuelos GS, Lin Z-Q, Yin X eds. *Selenium deficiency toxicity and biofortification for human health*. pp 77-78. Hefei, China: University of Science and Technology of China Press.

Chilimba, A.D.C. Young, S.D., Black, C.R. Meacham, M.C., Lammel, J., Broadley, M.R. The fate of applied Se in a maize cropping system in Malawi. China: *University of Science and Technology of China Press.* In press.