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The Selenium Content of Scottish Soil and Food Products

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Introduction

This report presents the findings of a pilot study to examine the relationships between the selenium content of Scottish soils and foodstuffs produced in Scotland, commissioned by the Food Standards Agency Scotland (FSAS) between 2008 and 2009.

Selenium (Se) is a trace element, essential for human health, and a key component of many physiological and metabolic processes, including immune function. The Scientific Advisory Committee on Nutrition (SACN) is currently scoping the literature concerning Se and health, which will subsequently inform their position on this issue. The FSAS is awaiting SACN to complete their work before conclusions on the health consequences of current Se intakes can be made.

In line with the rest of the UK, there is evidence that dietary Se intakes in Scotland have fallen in recent years, due to changes in the sourcing of bread-making wheat, *i.e.,* using European wheat, rather than that grown on the Se-rich soils of North America.

There is no national soil-Se geochemistry database for Scotland, capable of identifying areas of relatively high or low soil-Se concentrations. However, the Scottish environment is thought to be lacking in Se, relative to other parts of the UK (due to the country's underlying geology and particular climatic conditions). Dietary-Se intakes may, therefore, be compromised by the current move towards the consumption of locally-produced foods.

Therefore, this project was designed to establish the links between the underlying geology and the concentrations of Se, both in the overlying soils and in the foods produced on them. Although only a feasibility study, it is hoped that this information will assist with the development of informed food-policy in Scotland.

Aims

The main aims of the project were to assess:

- whether existing information on Scottish geology, could be used to predict the Se status of overlying soils.

- whether differences in soil-Se status were reflected in the Se concentration of the foodstuffs produced on them.

- whether existing geological information, relating specifically to Scotland, could be used to predict the Se status of foodstuffs produced on the overlying Scottish soils.

- which factors, *e.g.,* soil pH, soil organic matter content (LOI), plant type/variety, had the most significant impact on the agricultural utilisation of soil-Se

- the suitability of the project design (including farmer surveys) for use in similar investigations in the future, but on a wider-scale.

- whether the Se concentrations of locally-produced foods were in line with those produced in other regions of the UK/world.

Findings

The key findings of the project were:

- it was generally possible to predict 'high' and 'low' total soil-Se concentrations, based on soil parent material geological information, maps, *etc*. However, the difference in soil-Se concentrations between the two soil types was small (0.48 and 0.37 mg kg^{-1}, average values, respectively).

- the majority of soil samples (90%) could be classed as being Se-deficient, irrespective of which predicted soil-Se area they were from.

- the concentrations of Se in calabrese (broccoli), wheat, beef and grass (from the beef farms) were statistically higher in the predicted 'high' Se regions than in comparable commodities grown in 'low' regions. The concentrations of Se in milk, grass (from the milk farms) and potatoes showed a similar trend, but were not statistically different.

- wheat and between-farm potato sample Se concentrations were significantly correlated with the total soil-Se concentration.

- the concentrations of Se in beef were, on average, marginally lower than those reported in the 2006 UK Total Diet Survey.

- the concentrations of Se in calabrese, potatoes and both winter and summer milk samples were comparable to those reported in the 2006 UK Total Diet Survey.

- the concentrations of Se in wheat were comparable to data reported previously for Scottish wheat, but were lower than measured in samples from other parts of the UK.

- both calabrese and wheat Se concentrations were significantly lower (10x and 20x, respectively) than reported for similar commodities originating from North America and potato Se concentrations were lower (5x) than potatoes imported into the UK.

- there were no significant differences in the Se concentrations measured in the milk samples from the two Se settings. However, Se concentrations in winter milk were greater than in summer milk.

In addition, the study demonstrated that 'high' and 'low' water-soluble soil-Se concentrations could not, as expected, be predicted based on geological information alone. Soil pH did not influence the relative concentrations of water-soluble soil-Se measured in the two predicted soil types, whereas the amount of soil organic material did.

Statistical analysis of the 'power' calculation used, when establishing the sampling plan, showed that the assumptions and approach were correct, and could be applied to any future study. If a commodity, such as wheat or calabrese were chosen in a future study, fewer replicates could be taken (3 for wheat, or 5 for calabrese).

The accuracy of the soil-Se status high and low areas definition could be improved in the future by refinement of the prediction using the soil-Se information gained from the present study. A national soil-Se geochemical database will become available for Scotland within the next two years and, although at very low resolution (1 sample per 20 km grid), this will also help to predict the Se status of Scottish soils in the future.

Conclusions

- the results go some way to support the use of geological information on soil parent materials for the prediction of Se-favourable agricultural production. However, other factors, such as soil organic material and commodity type, require further consideration.

- the results demonstrated that the concentrations of Se in the soil were, in general, reflected in the concentrations measured in the resulting plant material. However, the uptake of Se into plants and animals is affected by many complex environmental and physiological processes, and care must be taken if attempting to estimate how particular plants/animals may respond in a predicted high or low-Se location.

- overall, given the limitations of the size of the sample set, the data would suggest that consumption of the locally-produced commodities studied here (particularly beef and wheat) might result in lower dietary-Se intakes than could be expected if consuming similar foods produced in other areas of the UK.

Recommendations

1. Given the low concentrations of Se found in the soils - hence low to average Se concentrations in the foodstuffs reported in this study - further investigations to more fully characterise the Se concentrations of Scottish soils and agricultural produce are warranted.

2. Studies should be considered which assess the impact of consuming locally-produced foodstuffs on the dietary intakes and Se status of people in Scotland.

3. If the results of recommendations 1 & 2 confirm the findings of this preliminary study, *i.e.,* that consumption of locally-produced foods has a negative impact on the Se status of the Scottish population, ways of addressing this should be considered, and might include the following:
   - application of Se fertilisers
   - advice to farmers on the utilisation of Se-accumulating foodstuff varieties
   - advice on the use of Se-rich feeds/supplements to augment animal production

1. Introduction

1.1. Selenium and Health

Selenium (Se) is essential to human and animal health, in trace amounts, but can be harmful in excess. Selenium has a very narrow range between dietary deficiency and excess; the Lower Reference Nutrient Intake (LRNI) is set at 40 µg day^{-1} (DOH, 1991), and a safe upper level of intake set at 450 µg day^{-1} (EVM, 2003).

Dietary Se intake is important because the element plays a key role in a number of metabolic processes, including; antioxidant systems, thyroid hormone metabolism, immune function and reproduction. In animals and humans, Se forms a vital constituent of the biologically important enzyme glutathione peroxidase (GSH-Px) and, to date, approximately 25 essential selenoproteins have been identified (Rayman, 2005; Rayman, 2008).

Due to the complementary role of Se and Vitamin E, practically all Se deficiency diseases in animals are concordant with vitamin E deficiency. Selenium is necessary for growth and fertility, with clinical signs of deficiency including; reduced appetite, growth and reproductive fertility and muscle weakness. These disorders are generally described as white muscle disease (WMD) (Levander, 1986; Fordyce, 2005 and Fordyce, 2007).

In humans, no clear-cut pathological health effect resulting from Se deficiency alone has been identified. However, the element has been implicated in a number of conditions including; reproductive disorders, impaired immune system function, heart disease (Keshan Disease is a cardiomyopathy reported from China), osteoarthritic disorders (Kashin-Beck Disease is reported from China and Russia), muscular dystrophy, muscular sclerosis and cancer (Levander, 1986, WHO, 1987; WHO, 1996). There is currently much interest in the role of Se deficiency in emerging viral diseases such as HIV-Aids and avian flu. Viral mutagenicity has been proven to occur in Se-deficient conditions and many of these emerging diseases emanate from Se-deficient parts of the world (Fordyce, 2007).

Selenium toxicity disorders known as selenosis can occur at high dietary intakes of Se (Fordyce, 2005 and Fordyce, 2007) but these are not discussed in detail here, as Se toxicity is not likely to be an issue in Scotland. However it should be noted that there is emerging evidence (from trials into the potential benefits of Se in prostate cancer in the United States) of a possible link between moderately high (200 µg day^{-1}) intakes of Se and increased risk of Type 2 diabetes (Stranges *et al.*, 2007; Lippman *et al.*, 2009).

In most cases, food forms the main source of Se for humans because concentrations of the element in water and air are generally low. The concentration of Se in foodstuffs depends primarily upon the Se concentration of the soil on which the food was grown or reared (Fleming, 1980; WHO, 1987; Fordyce, 2005). Hence, there is an importance in understanding the relationships between environmental exposure and health.

1.2. Environmental Sources of Se

1.2.1. Selenium in Rocks

Selenium is a naturally occurring metalloid element that is found in all natural materials on Earth including rocks, soils, water, air and plant and animal tissues. Selenium concentrations in most rock types are generally low. Sedimentary rocks contain more of the element than igneous rocks; however, concentrations in most limestones and sandstones rarely exceed 0.05 mg kg^{-1}. Selenium is often associated with the clay fraction in sediments, and is found in greater concentrations in shales (0.06 mg kg^{-1}) than limestones or sandstones. Very high concentrations (up to 300 mg kg^{-1}) have also been reported in some phosphatic rocks. Coals and other organic-rich deposits can be enriched in the element relative to other rock types, typically ranging from 1 to 20 mg kg^{-1}. However, values of over 600 mg kg^{-1} have been reported in some black shales and exceptionally high concentrations of 6000 mg kg^{-1} in Se-rich coals in China. In addition, Se is often found as a minor component of sulphide mineral deposits (Jacobs; 1989; Neal, 1995; Fordyce, 2005; Fordyce, 2007). Therefore, the distribution of Se in the geological environment is highly variable, reflecting the properties of different rock types (Fordyce *et al*., 2008).

1.2.2. Selenium in Soils

In most soils, geology has a fundamental effect on the distribution of Se, as rocks and superficial deposits form the parent materials from which soils are derived *via* the weathering process. The Se concentration of most soils is very low 0.01 to 2 mg kg^{-1} (world mean = 0.4 mg kg^{-1}) but high concentrations of up to 1200 mg kg^{-1} have been reported in some seleniferous areas (Fleming, 1980; Jacobs; 1989; Neal, 1995; Fordyce *et al*., 2008). Selenium is also cycled from the oceans to soils *via* atmospheric deposition (Haygarth, 1994).

In addition to these natural sources, anthropogenic activity can also be important. Selenium is widely used in a number of industries, as a pigment in glass and ceramic manufacture; as the light-sensitive photoconductor layer in photocopiers; as an antioxidant in inks and oils and as an anti-fungal agent in pharmaceuticals, and can therefore be released to the environment during these processes (WHO, 1987; Neal, 1995). Selenium also enters the atmosphere as a result of fossil fuel combustion, and is eventually deposited onto soils. Indeed there is evidence that the Se concentration of the UK environment has fallen as a result of the move away from coal use following the Clean Air Act in the 1960s (Haygarth, 1994). Selenium is also released inadvertently to the environment from the

agricultural use of phosphate fertilisers; from the application of sewage sludge and manure to land and from the use of Se-containing fungicides (Neal, 1995).

Table 1 summarises many of the literature references described above, and the Se concentration values quoted in them.

Total Soil-Se mg kg^{-1}	**Count**	**Min**	**Max**	**Med**	**Mean**	**Reference**
World Soils (non seleniferous)		0.01	2.00		0.40	Fordyce (2005)
World Deficient					< 0.60	Fordyce (2005)
England/Wales (General)		0.01	4.70			Thornton *et al*. (1983)
New Zealand (General)		0.10	4.00			Oldfield (1999)
USA (General)		0.10	4.30			Jacobs (1989)
Finland (General)		0.10	8.30	0.146		Reimann and Caritat (1998)
China (General)		0.02	3.81			Tan (1989)
Northern Ireland	6937	0.10	7.80	0.70	0.80	GSNI (In Prep)
Eastern England	19562	0.00	20.10	0.30	0.40	BGS (2009)
Scotland	10	0.02	0.36			Ure *et al*. (1979)
Scotland (Aberdeenshire)	4	0.55	0.76			MacLeod *et al*. (1996)
Scotland Glasgow rural soils	241	0.10	6.60	0.90	1.00	Fordyce *et al*. (2009)
Water-soluble Soil-Se µg kg^{-1}	**Count**	**Min**	**Max**	**Med**	**Mean**	**Reference**
England/Wales (General)		50	390			Fordyce (2005)
India Se Deficient		19	66			Fordyce (2005)
China Se Deficient	100	0.03	5			Fordyce (2005)
China (General)	354	0.6	109.4	6.4	4	Tan *et al*. (2002)
Grass Total Se µg kg^{-1}	**Count**	**Min**	**Max**	**Med**	**Mean**	**Reference**
USA grass		10	40			Jacobs (1989)
Chile grass (dry weight)		30	40			Contreras *et al*. (2005)
Germany grass (dry weight)					25	Gierus *et al*. (2003)
Finland grass/silage (dry weight)	56	3	83		28	Eurola *et al*. (2003)

Table 1. Soil and grass Se concentrations reported from around the world

Organic matter has a propensity to bind Se in the soil, hence peats and other organic-rich soil types can be relatively enriched in the element as, in addition to terrestrial sources, they are particularly sensitive to atmospheric sources. However, in some cases, this Se may be so strongly bound that it is not available for plant uptake (Fordyce, 2005).

1.2.3. Bioavailability of Soil-Se to Plants and Animals

In addition to the total concentration of the element in the soil, the uptake of Se into plants and animals is strongly controlled by the physico-chemical properties of the soil, which in turn determine the chemical form of Se and how readily it is absorbed by plants and animals. This is known as the bioavailability of the element (Jacobs, 1989).

Selenium bioavailability in soils is a function of the underlying geology, soil pH, redox conditions, amounts of organic matter, competing ionic species such as sulphate, microbial activity, soil texture, compaction and mineralogy, soil temperature, level of rainfall during the growing season and

irrigation. High soil organic matter, Fe-oxyhydroxide and clay mineral content (all of which can adsorb or bind Se to the soil) can inhibit the uptake of Se into plants and animals. Selenium in the form of selenate is more mobile, soluble and less-well adsorbed than selenite. Therefore, oxidising, alkaline conditions that favour the formation of selenate improve bioavailability, whereas selenite formed under reducing acid conditions is less bioavailable (Fleming, 1980; Jacobs; 1989; Neal, 1995; Fordyce, 2005).

It is estimated that perhaps 50% of the Se in some soils may be in the form of organo-Se compounds; however, few have been isolated and identified. Selenomethionine has been extracted from soils and is two to four times more bioavailable to plants than inorganic selenite, whereas selenocysteine is less bioavailable than selenomethionine (Jacobs; 1989; Neal, 1995).

Anthropological inputs, such as the use of fertilisers, have been reported as having a deleterious effect on the uptake of selenium from soils into crops and animals. This is due to the presence of competing ions such as sulphate and phosphate in the fertiliser that can inhibit Se uptake or the increased growth of plants as a result of fertiliser application, which can 'dilute' the Se concentration in the resultant greater volume of plant material (Fleming; 1980; Jacobs; 1989; Neal 1995).

Based on investigations into relationships between environmental and food-crop Se concentrations, and Se deficiency/toxicity effects in humans; Chinese scientists have suggested deficiency and toxicity thresholds for the element in soils (Table 2). However, it should be noted that the difference in ranges between these categories is extremely narrow. On this basis, soils marginal or deficient in total Se could be defined as containing < 0.175 mg kg^{-1} of the element (Tan, 1989). More generally, total soil-Se concentrations between 0 – 0.6 mg kg^{-1} are considered deficient as these are the concentrations found in regions where Se-deficient livestock are commonplace such as New Zealand, Denmark and the Atlantic Region of Canada (Fordyce, 2005).

Category	Total Se in Top Soil mg kg^{-1}	Water-soluble Se in Top Soil µg kg^{-1}
Deficient	< 0.125	< 3
Marginal	0.125 – 0.175	3 - 6
Moderate	0.175 – 0.400	6 - 8
High	> 0.400	> 8
Excessive	> 3	> 20

From Tan (1989)

Table 2. Thresholds for Se in soils

1.2.4. Selenium in Plants and Animals

In addition to the complex relationships governing Se uptake from soil; different species of plants and animals also assimilate Se to varying degrees. Evidence for whether Se is essential for plant and crop health is equivocal, but plants can be divided into three groups: Se-accumulators, Se-indicators (or

secondary Se-accumulators) and non-accumulators. Non-accumulators rarely assimilate more than 100 mg kg^{-1} Se (dry weight), whereas Se-accumulators can contain up to 40 000 mg kg^{-1} Se (dry weight) when grown in seleniferous environments (Jacobs, 1989; Neal 1995). The only Se-accumulator plant regularly used as a food source is the tree *Bertholletia excelsa*, which produces Brazil nuts. These nuts represent the richest source of dietary Se available to the UK public. However, some common crop species are secondary Se-accumulators; for example, Brassica species (rapeseed, calabrese (broccoli), cabbage and Allium species (garlic, onions, leeks and wild leeks). Cereal crops such as wheat, oats, rye and barley are non-accumulators (WHO, 1987; Broadley et al., 2006). Forage crops containing < 40 µg kg^{-1} Se are generally associated with deficiency in grazing animals (Levander, 1986).

Different parts of plants and animals also contain variable amounts of Se. The distribution of Se in the various parts of plants depends on the species, phase of development and physiological condition. In Se-accumulators, Se is concentrated in young leaves during the early vegetative stage of growth but, during the reproductive stage, it is found at much higher concentrations in the seeds. In non-accumulator cereal crops, the grain and roots often contain similar amounts of the element whereas concentrations in the stems and leaves are lower (Jacobs, 1989; Rayman, 2008).

In animals, Se tends to concentrate in the liver, kidneys and to a lesser extent, in the muscle. The Se status of animals also depends on factors such as age, physiological status and inputs by man such as the use of medications or mineral supplements (Levander, 1986).

Therefore, the Se composition of any given foodstuff is the culmination of many complex factors. Hence, concentrations are highly variable but, in general, organ meats, seafood and red meat contain more Se than most cereal products or fruit and vegetables (Tables 3 and 4).

1.3. Population Se Intake and Status in the UK

Information on Se intakes in the UK comes from the UK Total Diet Survey (TDS). Although the TDS does not constitute robust trend data, overall it suggests a fall in Se intakes in the UK, from a mean of 60 µg day^{-1} in the 1970s, to 30-40 µg day^{-1} in recent years (FSA, 2009) (Table 5). Exposure estimates of 48 – 58 µg day^{-1} from the 2006 TDS were slightly higher than those between 1994 and 2000, but were still low compared to the recommended reference nutrient intakes (RNIs) (60 – 75 µg day^{-1}).

The findings of the most recent 2006 TDS (FSA, 2009), indicated that Se concentrations ranged from below the limit of detection in fruits, beverages, vegetables, sugar and preservatives, dairy products and oils and fats, to 770 µg kg^{-1} in offal (Table 6). Selenium concentrations in most food groups were slightly higher than those reported in the 2000 TDS, and the concentration in offal was nearly twice the value reported in the previous survey (460 µg kg^{-1}) (FSA, 2009).

The overall decline in Se intakes is attributed to a number of factors including; reduced dietary intakes of cereals (bread), red meat and offal; changes in the sourcing of bread-making wheat from North America (which contains high concentrations of Se) to wheat from the European Union; changing farming practices *i.e.,* increased use of sulphur fertilisers (which compete for uptake, with the chemically-similar Se) and breeding for higher grain yield per plant. Other factors, such as the lower atmospheric deposition of Se from coal combustion, are also reported to have an impact on soil-Se concentrations (Rayman, 2008; Broadley *et al*., 2006; Macpherson *et al*., 1997; Ysart *et al*., 2000).

Although concentrations in cereals are very low, because these food types constitute a significant portion of the UK diet, the miscellaneous cereals group (16%) along with the meat products group (15%) make the greatest contribution to population dietary exposure (Table 6). Rich sources of Se included Brazil nuts, fish and offal, and the main sources of Se in the UK diet were breads, cereals, fish, poultry and meat (FSA, 2009).

As bread is an important dietary source of Se, additional information is given regarding the background to this particular issue: Macpherson *et al*. (1997) reported that until the mid-1970s; 50% of UK bread-making wheat was imported from North America, from Canada in particular. However, by 1995, imports of Canadian wheat had fallen to 10% of the 1970 level. Canadian and American wheat tends to contain more Se, as it is grown over black shale rock types on the prairies, which are rich in the element. In contrast, wheat from the EU is lower in Se as Se-rich rock types and soils are less common; and acid-neutral soils are typical over much of Northern Europe (Fordyce, 2005).

In line with the fall in Se intakes, observed through the TDS, there are data that also suggest a decline in Se status. A study conducted by Macpherson et al. (1997) between 1985 and 1994 reported a fall in plasma Se concentrations in Scotland from 1.5 to 0.9 µmol L^{-1}. The National Diet and Nutrition Survey (NDNS), carried out in 2000/2001, contains the most recent population level data on Se status. The NDNS recorded a mean plasma Se concentration of 1.11 µmol L^{-1} in UK men and 1.10 µmol L^{-1} in UK women (Table 7) (Ruston *et al*. 2004). In addition, the results demonstrated that the Se-status of low-income populations was lower than that of the general population (Table 7).

Regarding the relationship between Se and health; the position of the Scientific Advisory Committee on Nutrition (SACN) is that they are scoping the literature, which will subsequently inform their position on this issue. The FSAS is aware that there is some evidence linking Se to various health outcomes, but the findings have been inconsistent. The FSAS is awaiting the SACN to complete their work before conclusions on the health consequences of current Se intakes can be made.

1.4. Selenium Status in Scotland

Although there is no existing national soil-Se geochemical dataset for Scotland, it is recognised that the Se status of Scottish soils is likely to be low due to their acid nature and geological parent

materials (Fordyce *et al*, 2008). Indeed, historically, WMD in animals has been reported in several areas of Scotland, due to the generally low-Se status of the Scottish environment.

In a study of trace element deficiencies in animals across Scotland, SARI (1982) reported that 80% of cattle not given Se supplements had blood-Se concentrations considered to be deficient (< 0.64 µmol L^{-1} (50 µg L^{-1})). However, only 5% of herds displayed clinical signs of Se deficiency. This disparity between Se-deficient status and overt clinical symptoms of disease is very common in trace element relationships in animals. Often the effects of deficiency are sub-clinical, and the main outcome of Se supplementation is to improve productivity across a herd rather than to combat a high prevalence of overt clinical symptoms. These studies also showed that 78% of Se deficiency cases (258 cases) in cattle occurred in the Aberdeenshire and Dumfries areas, as well as the greatest number of sheep classified as Se-deficient (< 0.64 µmol L^{-1} (50 µg L^{-1}) blood Se). Please note: when viewing these statistics, they also reflect the fact that Aberdeenshire and Dumfries are the main animal rearing areas of Scotland (so where most animals are located). The figures will also be influenced by the likely distribution of Se in the Scottish environment, *i.e.,* concentrations will tend to be higher in the Central Belt and parts of Fife, because of the geology and soil types, than Dumfries and Aberdeenshire, which are areas that are likely to be low in Se.

Adams *et al*. (2002) presented evidence that the Se concentration of Scottish wheat (< 40 µg kg^{-1}) was lower than other parts of the UK, such as the major wheat producing region of East Anglia (40 to > 60 µg kg^{-1}) (Table 4). Macpherson *et al*, 1997 reported that, as part of the general trend in wheat sourcing in the UK, Scotland experienced a rise in EU imports and a drop in Canadian imports (Table 8). In part, as a result of the difference in Se concentrations between flour and resultant bread products from these two sources, concentrations of Se in the Scottish diet fell by 50% between 1974 and 1994 from 60 to 32 µg day^{-1} (Table 9). This fall was reflected in the Se status of the Scottish population, which dropped by 42%, from 1.50 µmol L^{-1} in 1985 to 0.86 µmol L^{-1} in 1994 (Table 9). Data from the most recent NDNS (Ruston *et al*., 2004) also suggested that the Se status of the Scottish population (women 1.07 µmol L^{-1} and men 1.09 µmol L^{-1} blood plasma Se) was marginally lower than the rest of the UK (Table 7).

If the low-Se status of the Scottish soils is reflected in the foodstuffs produced on them; then the current move to procure locally-produced foods may potentially impact further on the Se status of the Scottish population (Scottish Government, 2009). Therefore, as an initial step towards obtaining a fuller understanding of the situation prevalent in Scotland, the FSAS sponsored this feasibility project.

1.5. Selenium in Scottish Soils and Food Products Study

The hypothesis was that, by understanding the relationship between the underlying geology (using geological maps, *etc.*), the overlying soil and the foodstuffs grown on it, information could be gained regarding;

- **concentrations of Se in the Scottish environment;**
- **whether Se-supplementation of soils or foods was appropriate;**
- **which dietary-relevant foodstuffs best utilise the available Se, *etc*.**

The main aims of the project were to assess:

- whether existing information on Scottish geology, could be used to predict the Se status of overlying soils.
- whether differences in soil-Se status were reflected in the Se concentration of the foodstuffs produced on them.
- whether existing geological information, relating specifically to Scotland, could be used to predict the Se status of foodstuffs produced on the overlying soils.
- which factors, *e.g.,* soil pH, soil organic matter content (LOI), plant type/variety, had the most significant impact on the agricultural utilisation of soil-Se
- the suitability of the project design (including farmer surveys) for use in similar investigations in the future, but on a wider-scale.
- whether the Se concentrations of locally-produced foods were in line with those produced in other regions of the UK/world.

The British Geological Survey (BGS) was responsible for identifying geographical regions where high and low-Se soils were most likely to be found, based on the assumption that there was a fundamental relationship between the underlying geology and the overlying soil. The Food and Environment Research Agency (Fera) undertook the identification of a range of food commodities known to be widely produced in Scotland. The Science and Advice for Scottish Agriculture (SASA) then combined the two sets of information, and attempted to identify whether the chosen commodities were grown within both of the predicted high/low-Se settings. Finally, all three contributors were responsible for constructing either the sampling protocols (BGS and Fera) or on-farm questionnaires (SASA). SASA

carried out the soil and food sampling for the project, the soil samples were analysed by the BGS laboratories and the food samples by Fera.

The findings of this feasibility study are presented in this report. All tables and figures referred to in the main body of the report are listed in Appendix 1. In addition, a number of key tables and figures are embedded in the main body of the text of this report, for ease of reference, and to better illustrate the results.

2. Methods

2.1. Project Design

2.1.1. Sampling Design

At the outset of this project, very little data were available regarding the Se concentration in either Scottish soils or the commodities grown on them. Therefore, a number of assumptions/decisions were made, in order to devise a robust, but cost-effective sampling plan;

1. in consultation with the FSA Scotland, it was agreed that at least five food commodities should be investigated, and that they should meet the following criteria;

- staple or regularly-consumed foods, in Scotland
- regularly grown, in Scotland
- relatively widespread production, to cover both high and low soil-Se settings
- reasonable amounts of background information regarding concentrations measured in produce from Scotland and other production areas
- direct spatial relationship to soil *e.g.,* wheat, brassicas, and potatoes, or indirect (but traceable) spatial relationship between the soil and a higher trophic level *e.g.,* beef and milk *via* grass

2. to minimise variability, replicate samples for a specific commodity should come from a single farm (a separate experiment was performed to look at between-farm variability).

3. using data obtained whilst performing the many FSA-funded and commercial surveys that Fera had been involved in, over the last 20 years, an estimation of analytical parameters was made, *e.g.,* within-sample and between-sample variability, instrumental variance, *etc.* This information was then used by Fera's Statistics Team to calculate the number of replicate samples required, if given performance criteria were to be met, *i.e.,* a 'power' of 80% and a statistical significance level of 5%.

Figure 1 is a graphical representation of the outcome of the calculation, from which the number of replicate samples required was extrapolated, allowing analytical variation and soil-Se differences (*i.e.,* x-fold difference between 'high' and 'low' soil-Se concentrations) to be considered when agreeing an appropriate sampling plan.

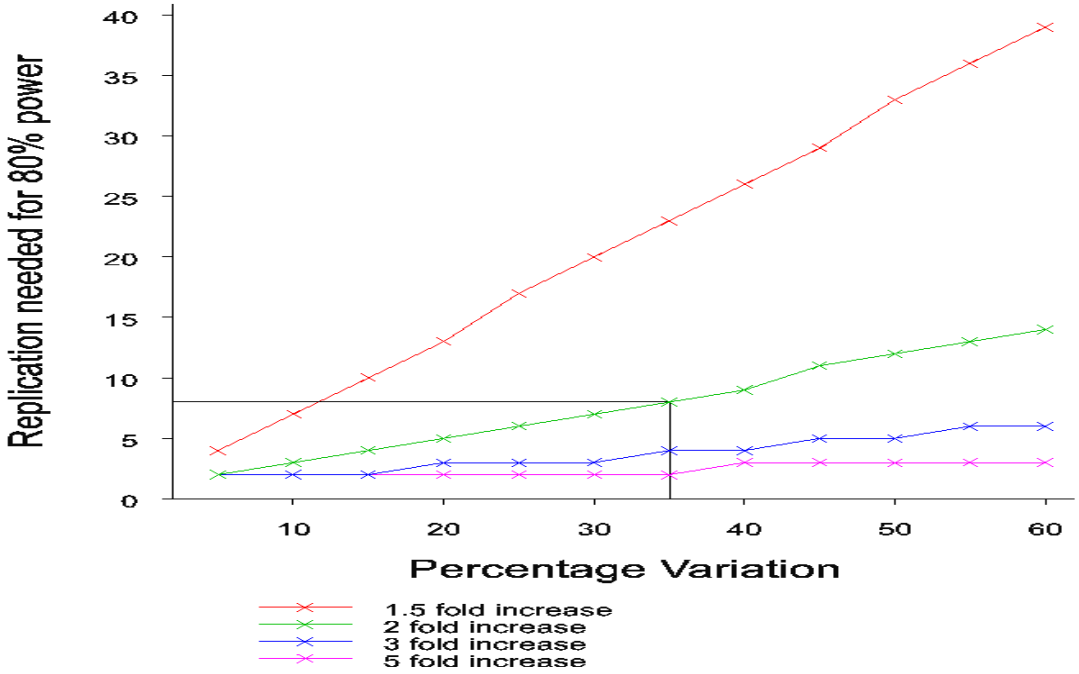

Figure 1. Number of samples required to achieve 80% statistical power per commodity for the two soil types.

Replicate Sample Number Calculation

The number of replicates required in the main part of the project was calculated assuming a range of concentration differences between the low and the high soil-Se samples; *i.e.,* from five-fold (*e.g.* 0.5 mg kg^{-1} Se for low and 2.5 mg kg^{-1} Se for high samples) down to 1.5-fold (*e.g.* 0.5 mg kg^{-1} Se for low and 0.75 mg kg^{-1} Se for high samples). The variance was assumed to range from a minimum of 5% (representing the analytical/sample variation) to 60% (incorporating possible farm/site and commodity-variety variation).

From Figure 1, it can be seen that, to detect a two-fold difference in Se concentrations in food from high and low soil-Se areas, with 35% variation in the overall precision, eight samples were required for each respective commodity type, per farm. As a larger difference in Se concentrations in food between high and low soil-Se areas would require a smaller number of samples to be taken, per farm, it was anticipated that eight sample replicates would be sufficient to accommodate the worst case scenario.

Between-farm Replicate Sample Number Calculation

To give a better indication of the range of Se concentrations in soils and foodstuffs across a greater geographic area of Scotland; one of the commodities (potatoes) was collected from a number of farms, in both high and low predicted soil-Se regions, along with representative samples of the soil in which they were grown. Potatoes were chosen for this aspect of the project as they were easy to collect; widely grown across Scotland; and were deemed likely to be consumed locally.

The design of the between-farm variation sampling protocol was based on the statistical power calculation outlined in Figure 1. This indicated that a dataset size of 18 was required to accurately assess between-farm variability. Therefore, additional sets of potatoes, and the soils on which they were grown, were collected from a further 17 farms (in both the high and the low soil-Se areas 17 x 2 = 34 samples).

The soil and potato samples were collected from the same field, with the soils being taken at the base of the potato plants. In each field, eight sub-samples of both the soil and the potatoes were taken and homogenised to form a single composite sample of each matrix, from each farm. The sampling strategy is outlined in Table 10.

Within-farm Variability Dataset:

High (H) Selenium Soils	**Low (L) Selenium Soils**
Farm-1 (H): Wheat (x 8) + soil (x 8)	Farm-6 (L): Wheat (x 8) + soil (x 8)
Farm-2 (H): Potato (x 8) + soil (x 8)	Farm-7 (L): Potato (x 8) + soil (x 8)
Farm-3 (H): Calabrese (x 8) + soil (x 8)	Farm-8 (L): Calabrese (x 8) + soil (x 8)
Farm-4 (H): Milk (summer x 8) + soil (x 8) + grass (x 8) + milk (winter x 8)	Farm-9 (L): Milk (summer x 8) + soil (x 8) + grass (x 8) + milk (winter x 8)
Farm-5 (H): Beef (x 8) + soil (x 8) + grass (x 8)	Farm-10 (L): Beef (x 8) + soil (x 8) + grass (x 8)

Between-farm Variability Dataset:

High (H) Selenium Soils	**Low (L) Selenium Soils**
17 Farms (L): Potato (x 1 composite of 8 sub-samples per farm) + soil (x 1 composite of 8 sub-samples per farm)	17 Farms (L): Potato (x 1 composite of 8 sub-samples per farm) + soil (x 1 composite of 8 sub-samples per farm)

Table 10. Sampling design adopted for the project.

2.1.2. Definition of Low and High-Se Areas Across Scotland

As there was limited information available regarding the Se status of Scottish soils (due to; 1; poor spatial extent of existing geochemical surveys, and 2; a lack of comparability between the analytical techniques used to obtain the survey data), it was decided to incorporate geological and geochemical

information into a scheme, devised by Fordyce *et al*. (2008) for this project, to predict likely soil-Se concentrations. However, as many factors, not just underlying geology, will also affect the concentration of Se in an overlying soil, the prediction of high/low-Se settings was subject to significant levels of uncertainty. It should also be emphasised that the terms 'low' and 'high' refer to concentrations relative to each other, rather than the absolute concentration of Se in the soil, as most agricultural soils in Scotland were predicted to contain very little Se.

The areas of interest for the present study were defined as follows:

Step 1 The main agricultural producing area of Scotland was selected for the study using a geographic information system (GIS) based on Arc9.2® software (Figure 2). The far north of Scotland was excluded for the purposes of this study, as agricultural production of food is limited in this area.

Step 2 A map of parish boundaries in Scotland, provided by SASA, was incorporated into the GIS. The parish boundary information was required to identify farms, as these are located by 'parish' in the Agricultural Census Database. Approximately 700 parishes were included in the study area for the project.

Step 3 The bedrock geology for this area was incorporated into the GIS from the BGS digital geological map of Scotland at a scale of 1: 50,000 (BGS DiGMapGB-50®). This is the most detailed national geological dataset held by BGS, and was selected for this study to provide as much information as possible to aid the identification of individual farms in the correct Se setting.

Step 4 As a result of Step 3, approximately 2000 different rock types were identified in the study area. Using geological and geochemical knowledge, these were classified as relatively low or high-Se soil parent material type, according to the broad scheme outlined in Table 11.

Step 5 The resultant map of predicted 'low' and 'high' Se soils (Figure 2) was combined in the GIS with the map of parish boundaries for Scotland.

Step 6 Using the GIS, the aerial extent of predicted low and high-Se soils in each parish was calculated and expressed as a percentage.

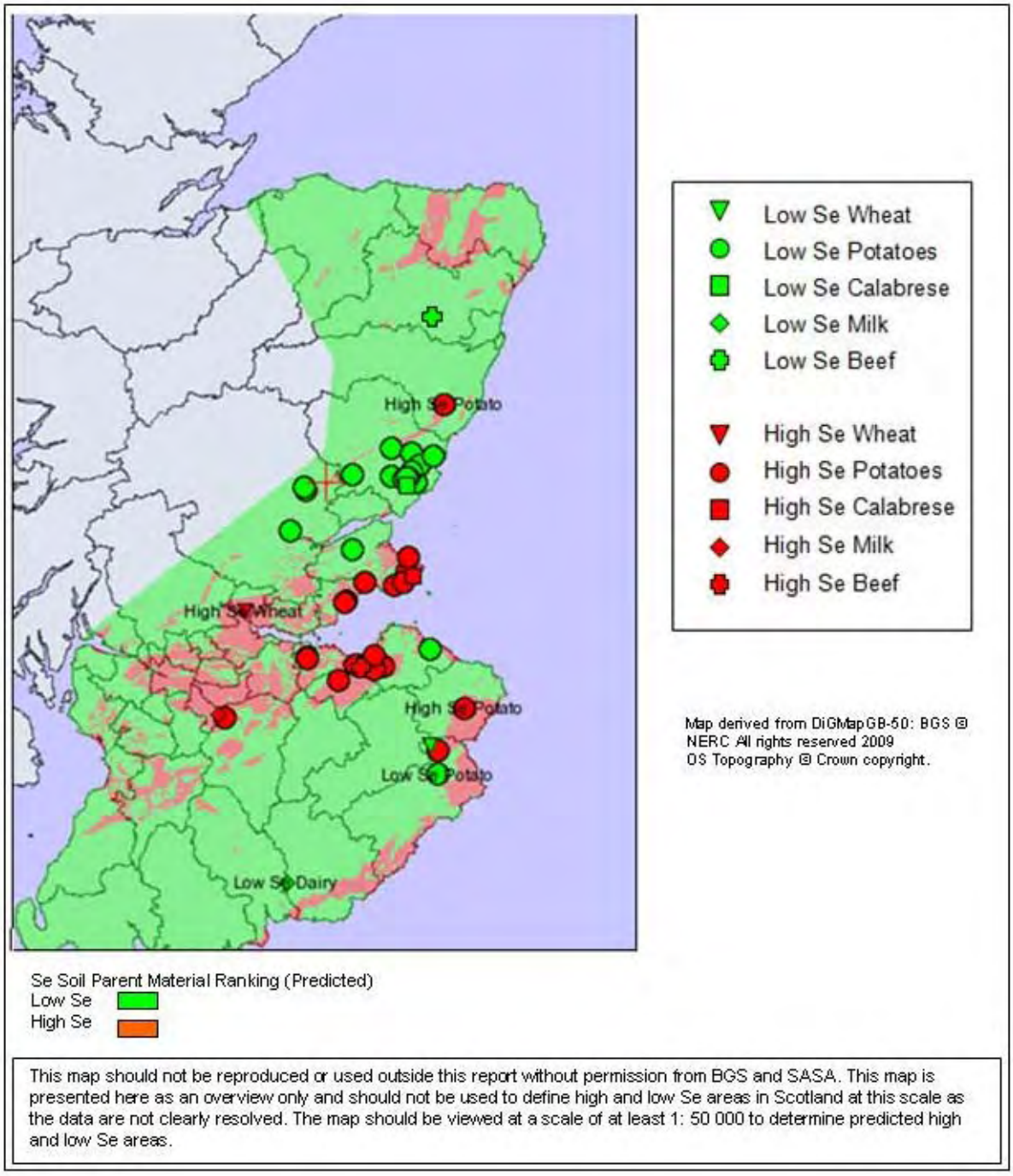

Figure 2. Map of predicted high and low-Se soils in the main agricultural growing areas of Scotland based on bedrock geology and sample sites for the present study.

2.1.3. Selection of Farms for the Study

Using the definitions of predicted low and high soil-Se parishes in Scotland, farms growing the commodities of interest to the study were identified from the Agricultural Census Database. Priority was given to parishes that had either predominately low or high-Se soils to ensure that the farms lay within the correct Se setting for the project.

The aim of the recruitment process was to select crop varieties and animal breeds that were representative of Scottish agriculture, and were cultivated or bred for human consumption. It was ensured that the same varieties or breeds of each of the commodities were available from those recruited in the high and low-Se areas. This approach reduced sample variability associated with breed or varietal characteristics. Figure 2 shows a map of the sampling sites and predicted high and low-Se soils. The types of farms and animal breeds and crop varieties selected for the present study are outlined in the following sections of this report.

2.1.4. Selection of Commodities for the Study

To examine the relationships between the soil and the food products, and to keep other variables to a minimum; the varieties of crop; crop maturity; breed of animal; age of animal and cut of beef collected from the cattle were standardised. Additionally, all the soil, grass and crop samples were collected from the same field on each farm. The advantages of taking this approach were that the within-farm and between-variety variability were minimised. For the crop and grass samples, the soils were taken at the base of the plants collected for the study so that soil-plant relationships could be assessed directly.

The commodities chosen for this project were calabrese (broccoli), potato, wheat, beef and milk (summer and winter). Grass samples were also collected from the pasture grazed by the cattle from which the summer milk and beef samples were obtained in both the high and low-Se settings.

A) Calabrese

The variety of calabrese selected was Parthenon. This variety produces heavy domed heads with an average weight of 400-600 g. Parthenon has the advantage of keeping very good colour and quality in erratic weather conditions, which makes it particularly suitable for UK climatic conditions.

B) Potato

The Maris Piper potato was selected as it is the most common potato variety grown for human consumption in Scotland, accounting for ca 18% of the Scottish crop in 2007 (SASA, unpublished data). The next most popular variety, Saxon, (accounting for ca 14%) had to be included in the between-farm variability study, in order to create a sufficiently large sample set.

C) Wheat

The variety of winter wheat selected for the study was Consort. This is primarily a biscuit-making wheat, and was the second most commonly grown variety in Scotland in the 2006 Pesticide Usage Survey, accounting for 26% of the winter wheat surveyed (Struthers, 2007). Consort is a slow developing variety that is specifically recommended for use in Northern Britain. This variety has an average protein content of 11.8% and a mean 1000 grain weight of 50.7 g (HGCA, 2008).

D) Beef

It is likely that derived meat products such as mince, pies, sausages *etc*. are more commonly consumed in the Scottish diet than beef steak. However, early in the project design stage, it was realised that it would be almost impossible to trace derived meat products back to the farms on which the animals had been reared, due to mixing of products from different sources at the abattoir stage. Therefore, lean fillet steak was selected as the cut of beef for the survey to minimise differences in Se concentration between fat and muscle in the meat cut. The breed of cattle selected was Aberdeen Angus. The farmers selected used the Scotbeef abattoir: Scotbeef Ltd, Longleys Farm, Bridge Of Allan, Stirling, which is one of the main abattoirs supplying meat to the Scottish market.

E) Milk

Both of the dairy farms selected for the study had similarly sized herds of Friesian/Holstein-cross cows (113 and 136 dairy cows in the low and high-Se settings respectively). Over 50% of UK dairy herds consist of Friesian/Holstein-cross cattle (Defra, 2007).

Samples of milk were collected in summer and winter (August and December respectively) from the two dairy farms. This was done so as to check for seasonal variations in milk Se concentrations, due to the cows having been fed on different sources of feed, *i.e.,* grass and silage/concentrates in summer and winter, respectively.

2.1.5. Main Study Design (Within-farm Variability Sampling)

Replicate samples (8) of the soil, the five food commodities, and the grass fodder (for beef and milk sampling) were taken from individual farms, providing the project with a quantitative assessment of the within-farm variability for each matrix type, in each soil-Se setting.

2.1.6. Food Samples and Farms Selected for Between-farm Variability Sampling

Between-farm variability was assessed by collecting single potato and soil samples from 17 farms, in each soil-Se setting. This was done so as to provide an initial indication regarding the potential range of Se concentrations across a greater geographic area of Scotland.

It was found that, in order to get sufficient farms in the correct Se settings, two varieties of potato, Maris Piper and Saxon, had to be selected for the between-farm variability sampling. Eight farms growing Maris Piper and nine farms growing Saxon were selected in both the high and low-Se areas. Both varieties selected produce similarly shaped tubers, thereby minimising potential variability due to differing skin/flesh ratios.

The details of the farmers recruited for the project were reported to the FSAS (SASA, 2008).

2.2. Sample Collection

The soil, grass and food products required for the study were sampled according to the protocols outlined in Appendix 2 (soil) and Appendix 3 (grass and food samples) of this report.

The British National Grid (BNG) co-ordinates of each soil/crop sampling site were recorded using a global positioning system (GPS). Observations on sampling depth, soil colour, texture, presence of contamination, land use, weather and other features required for data interpretation were recorded on a sampling sheet (Appendix 4). Information on crop variety, animal breed, agricultural practices, fertiliser use, commodity destinations and farm details were also recorded on a standardised sampling questionnaire (Appendix 5).

Information gathered in the field, on sampling sheets and questionnaires, was entered into an Excel® database for the project, and was provided to the FSAS.

2.2.1. Soil Sampling

Samples of top soil (0.05 - 0.20 m depth; taking 0 - 0.05 m as the litter layer) were collected for the present study, as this is the rooting zone for crops and, in the case of beef and milk production, the grass on which the cattle graze. Samples were collected using a Dutch hand-held auger into Kraft® paper bags resulting in ~ 0.5 kg of soil from each sampling site. Soils on farm L02 (low-Se beef farm) were only 0.15 m deep; therefore samples were collected 0.05 - 0.15 m. Note: Two sets of soil samples were collected, one for the within-farm variability component of the project, and the other for the between-farm variability component.

Within-farm Sites

At each farm, eight individual soil samples were taken from as wide a sampling pattern as possible, within the field from which the foodstuff of interest was also sampled (Appendix 2). Each of the soil samples was a composite of five auger-flights (or head). In the case of the wheat, calabrese and potato samples, the five auger-flights full of soil were collected from the base of the plant sampled for the study. In the case of dairy and beef farms, each of the eight soils was collected from the field from which the grass was sampled. Each soil sample comprised five auger-flights taken from the centre and corners of a 2 x 2 m square. On dairy farms, the soil samples were collected from the field grazed by the cattle in the previous 24 hours prior to summer milk sampling to capture the rapid turnaround of Se from grass intake into cows' milk. On beef farms, the soil samples were collected from the field grazed prior to cattle slaughter, within 0 – 3 months of slaughter and not beyond 6 months from slaughter (Appendix 2).

Between-farm Sites

At each of the between-farm potato farms, one composite soil and one composite potato sample were collected from the field designated for sampling. Each composite comprised an auger-flight of soil was

collected from the base of each of the eight potato plants selected for study. The soil from the eight auger-flights was then thoroughly homogenised on a plastic sheet, and a composite soil sample collected in a Kraft® paper bag (Appendix 2).

Once collected, the soils were air dried at temperatures of <30^{o}C, prior to transportation to the laboratory for analysis.

2.2.2. Food Sampling

For the crop samples, wheat, calabrese and potatoes, mature samples were collected from each farm just prior to commercial harvest, according to the protocols outlined in Appendix 3.

Calabrese

A single head of calabrese was taken, and any inedible parts removed. Soil splash was rinsed of with tap water, and then carefully dried with a clean paper towel. Calabrese samples were selected so as to be of a similar size to each other, and to those from the other Se-setting farm.

Wheat

Ears of wheat from a single plant were collected, chaff removed, and the grain dried (if necessary) by leaving it on a dust-free surface for 24 hours. The grain from a single plant constituted a sample.

Potatoes

A single tuber was taken, per plant, and constituted a sample in the within-farm part of the study. In the between-farm section of the project, eight tubers were collected from each farm, but then combined to make one bulk sample, which constituted the sample from that farm. Any adhering soil was washed off, and the tuber dried prior to storage. Only tubers of the same size (45-65 mm diameter) were collected, in order to reduce potential Se concentration differences arising from different skin-to-flesh ratios. The characteristics of the two potato varieties are outlined by SASA (2008).

Beef

A single cut of fillet beef (weight approx: 150-200 g) from a cow, constituted a single sample. Tissue samples from cattle that had grazed on the sampled fields were taken by personnel from the Scotbeef abattoir. Cattle were 20 to 24 months old at slaughter. The fillet tail, *i.e.,* the end of the fillet steak region of the carcass, was taken from each animal. Fillet steak was selected because it is a lean cut of meat; thereby reducing the variability in Se due to differences in fat content between samples. Carcasses were hung for approximately 10-14 days following slaughter, before being butchered and samples taken. The samples were collected on the day they were taken and were labelled with the animal's unique ID number. This ensured that the cuts of beef selected for Se analysis could be traced back to the soil samples selected for Se analysis.

Milk

Milk samples of 60-100 ml were taken, directly, from individual cows, after rinsing the teat. Summer and winter milk samples were taken in August and in December respectively.

Grass

Samples were collected from the same sections of 2 x 2 m square as the soil samples, by clipping the grass with clean scissors. All soil contamination was shaken off the vegetation before being placed in the sample bag. Grass samples were collected from dairy pasture at the same time as summer milk collection. Grass from beef pasture sites was collected in early autumn, prior to cattle slaughter.

Following sampling, wheat and potatoes were stored at ambient store temperature (<25^{o}C), calabrese and grass samples were refrigerated, and beef and milk samples were frozen until transportation to Fera for analysis. All wheat and calabrese samples were received by the laboratory within 4 days of sampling; grass and potato samples were received within 5 days; milk within 10 days; and beef samples within 7 weeks of sampling. Experience gained during the performance of similar surveys showed that, as long as the samples were stored appropriately, the total Se concentration would not vary over time.

2.3. Laboratory Analysis

2.3.1. Soil Analysis

The 114 soil samples collected for the present study were analysed at the BGS laboratories for total Se, water-soluble soil-Se, soil pH and loss-on-ignition (LOI - as a measure of organic matter content) according to the protocols detailed in Appendix 6 of this report.

Total Se is defined as being the measure of the Se concentration of the soil, extractable into aqua regia (3 HCl + 1 HNO_3). However, as outlined in Chapter 1 of this report; the uptake of Se from the soil into plants and animals is determined by complex relationships between a soil's physico-chemical properties. Therefore, the water-soluble soil-Se concentration was also determined, as a measure of the most mobile portion of Se in the soil. As this portion is likely to be the most readily available, or 'bioavailable', for plant uptake, it is often used as an indicator of Se bioavailability in soil science. However, it should be noted that it may not reflect the whole bioavailable Se concentration of the soil. Soil pH and LOI also exert a fundamental control on the amount and bioavailability of Se in soil. Hence these parameters were also included in this study's analytical suite.

Total soil-Se was determined by digesting milled soil material in an aqua regia solution followed by analysis by inductively coupled plasma mass spectrometry (ICP-MS) (Appendix 6).

The water-soluble soil-Se concentration was determined by shaking milled soil material in deionised water, followed by analysis using ICP-MS (Appendix 6).

Soil pH was determined by shaking 10 g of < 2 mm soil material in a calcium chloride solution ($CaCl_2.2H_2O$) and analysis by pH electrode. This method of pH determination generally gives lower results (0.5 pH units) than water-based methods (Rowell, 1994).

Soil LOI was measured by heating milled soil material in a furnace at 450 °C for four hours. The change in weight (g) of the samples before and after heating was determined as the LOI. LOI is an approximate measure of the organic matter content of the soil. LOI is a better measure of the organic matter of sandy soils than clay soils as clays can lose structural water during heating (Rowell, 1994).

Soil Data Quality Control

Data precision and accuracy were assured by inclusion of sample replicates; analytical duplicates; international Certified Reference Materials (CRMs) for total and water-soluble soil-Se analysis, and BGS in-house quality control standards (for soil pH and LOI determinations) in the analytical runs according to the methods outlined in Appendix 6 of this report.

2.3.2. Food Analysis

The 162 food commodity samples collected for the present study were analysed at Fera for total Se, according to the protocols detailed in Appendix 7 of this report. Following thorough homogenisation of the samples, an aliquot (0.5 g to 3 g, depending on expected water content, *i.e.,* solids/liquids) was digested in concentrated nitric acid, using a high temperature, high pressure, microwave digestion system. After quantitative transfer, the samples were analysed by ICP-MS. Data were reported as fresh weight.

Food Data Quality Control

Data precision and accuracy were assured by inclusion of sample replicates; Certified Reference Materials (CRMs), reagent blanks and spikes, according to the methods outlined in Appendix 7 of this report.

2.4. Analytical Data Statistical Processing

Prior to statistical processing, the analytical data for total soil-Se, water-soluble soil-Se, pH and LOI, and the total Se concentration of grass, calabrese, potatoes, wheat, beef and milk, were compiled into two datasets. The within-farm dataset comprised eight samples of soil and associated food commodities collected in both high and low soil-Se settings (16 samples in total) (Table 12). The between-farm dataset comprised a composite sample of soil and associated potatoes collected from each of the additional 17 farms, in each high and low soil-Se setting (34 samples in total) (Table 13). The analytical data were provided to the FSAS as part of the project.

For the purposes of data processing; the mean value for the Se concentration in the soil and food (potato) sample, for the within-farm datasets, was calculated and incorporated into the between-farm

soil dataset as a single data point per sample. This increased the number of data points in the between-farm dataset to 18, which was the number of sampling points calculated to be required statistically, at the sample plan construction stage. It was valid to do this because the between-farm and within-farm samples were collected and analysed in a similar way. The between-farm dataset consisted of physical composite soil samples, each comprised of eight sub-samples collected in the same field and homogenised before analysis. The composition of the between-farm dataset, used for statistical processing, is summarised in Table 14.

Statistical processing and presentation of the data generated by the project was carried out using Excel®, Statview® and R statistical software. Prior to statistical analysis, preliminary data exploration was carried out to determine whether any data transformation was required to make the data conform to the assumptions of the statistical techniques being used. The results demonstrated that, although log transformation of some of the food commodity datasets may have made them conform more closely to a normal data distribution, it did not improve the linear relationship between the variables, and would, therefore, not benefit the follow-up statistical analysis. In addition, transformed data were considered less intuitive for simple data presentation. As a result, statistical analyses were carried out on the original data without transformation.

Residual diagnostic plots were used to judge the performance of the pairwise t-tests, which looked reasonable. Statistical power calculations also demonstrated that the relatively small number of data points (8) in some of the food datasets were sufficient for statistical analysis in the majority of cases (Figure 3) (See Section 3.11 of this report).

Therefore, two-sided pairwise t-tests, with multiple comparison correction, were used in this study. False discovery rate (FDR) correction was used. FDR control is a statistical method used in multiple hypotheses testing to correct for multiple comparison. In a list of rejected hypotheses, FDR controls the expected proportion of incorrectly rejected null hypotheses. It is a less conservative procedure for comparison, with greater power than family-wise error rate control (Storey, 2002).

One-way analysis of variance (ANOVA) and a stepwise model selection based on Akaike Information Criterion (AIC) were used to quantify the relationships between the Se concentrations of grass, calabrese, potato and wheat samples and the various soil parameters.

Pearson correlation tests with FDR correction were used to assess relationships between the soil parameters. Pearson's correlation coefficient (range from –1 to 1) provides a measure of the strength of the association between the soil parameters. A positive value for the correlation implies a positive association, and vice versa. The closer the absolute correlation is to unity, the closer to a perfect linear relationship. Only test results below 5% significance levels (<0.05) were considered statistically significant.

In order to quantify the effects of soil parameters, bivariate linear regression analysis was used. The regression residual structure was evaluated graphically using the appropriate diagnostic plots (Crawley, 2003). Adjusted R^2 was documented to measure the goodness of fit of the linear regression model.

3. Results

3.1. Farmer Recruitment

The farmers who were approached to take part in this study were generally very receptive and interested in the project, with less than 5% declining to participate. The results of the chemical analysis in food and soil samples were reported back to each farmer, as an agreed part of their participation in the project. The experience of this preliminary survey is that farmers would be receptive to participation in any future, larger study.

3.2. Information on Commodity Destinations

One aspect of the project was to collate information on the destinations of the food commodities sampled, to give an indication of entry into the Scottish food market. The commodity destination results have been provided to FSAS as part of the field database generated from the project. The results are summarised in Table 15.

3.2.1. Calabrese and Potatoes

The calabrese and potatoes produced on the farms included in the study were all destined for the Scottish food market.

3.2.2. Wheat

Despite the fact that a biscuit-making variety of wheat was chosen for the study; wheat from both the selected farms went to distilling rather than food production.

3.2.3. Beef

The beef fillet steaks sampled for the project were destined for the Scottish food market. Indeed, a preliminary survey of fillet steaks available in supermarkets (carried out for the project) revealed these to be labelled as being exclusively British, and predominately Scottish, beef.

3.2.4. Milk

The milk sampled in this study was destined for local outlets.

3.3. Information on Fertiliser Use

Another aspect of the current project was to collate information on fertiliser usage practices. This has been reported to the FSAS, separately, as part of the field database generated for the project. Only the high-Se beef farmer (H02) applied Se fertiliser to his soil, whereas the use of phosphate- and

sulphate-based fertilisers was widespread. Other trace elements were applied as foliar sprays on the farms (Table 16).

3.4. Selenium Supplementation in Agriculture

The two beef and two milk farms selected for the present study provided Se mineral supplements to their cattle, in the form of standard multi-element feeds and mineral licks (Table 17). However, none of the farmers surveyed knew whether their feeds/licks contained Se; this information was ascertained from the bag labels. This was despite the fact that the low-Se beef farm L02 located in Aberdeenshire reported a history of Se deficiency on the farm, with cattle deaths due to WMD in 1975. Overt deficiency was treated at the time but no subsequent testing for Se deficiency has been carried out.

One of the high-Se potato farms (H22), located near Kelso also reported Se deficiency in sheep in the past, even though this farm was in a predicted high-Se area. This farm was located on the boundary between predicted high and low-Se areas, and thereby demonstrates the uncertainty associated with trying to predict soil-Se concentration on the basis of geological rather than known geochemical information. It also highlighted the fact that even in areas predicted to have higher Se concentrations than others; the concentrations of Se in the Scottish environment were generally low.

3.5. Overview of Project Soil and Food Results

This study did not aim to provide a representative survey of the Se concentration of Scottish soils and food products, because the samples were limited in number and spatial extent. However, it is useful to compare the results of the present project to those reported elsewhere. For the purposes of an initial overview, the results of the within-farm and between-farm datasets are taken together and summarised in Table 18. Soil, grass and food Se concentrations from other studies are listed in Tables 1, 3, 4 and 6 of this report, for comparison.

Sample Type	N	Range	Mean	Median
Total Soil-Se (mg kg^{-1})	114 from 44 farms	0.115 – 0.877	0.444	0.433
Water-soluble Soil-Se (µg kg^{-1})	114 from 44 farms	6.69 – 26.78	11.59	10.51
Soil pH	114 from 44 farms	4.11 – 6.59	5.22	5.23
Soil LOI (%)	114 from 44 farms	1.71 – 14.30	6.47	5.66
Grass Total Se (µg kg^{-1})	32 from 4 farms	3.42 – 22.24	8.64	7.04
Calabrese Total Se (µg kg^{-1})	16 from 2 farms	1.51 – 7.45	3.29	2.65
Potatoes Total Se (µg kg^{-1})	50 from 36 farms	0.00 – 9.71	2.28	1.87
Wheat Total Se (µg kg^{-1})	16 from 2 farms	3.57 – 62.70	23.10	19.72
Beef Total Se (µg kg^{-1})	16 from 2 farms	81.08 – 151.08	114.91	116.43
Summer Milk Total Se (µg kg^{-1})	16 from 2 farms	12.92 – 22.02	17.50	16.88
Winter Milk Total Se (µg kg^{-1})	16 from 2 farms	17.50 – 25.61	21.69	20.79

Grass and food samples are reported as fresh weight
Results are for the whole dataset = within-farm individual samples + between-farm composite soil and potato samples

Table 18. Overview of the project results.

3.5.1. Overview of Soil Results

Although the combined within-farm and between-farm datasets contained 114 soil samples, it should be noted that these were collected from only 44 farms (34 between-farm sites + 10 within-farm sites).

Quality Assurance Data

Table 19 presents the total, water-soluble Se, pH and LOI quality control data obtained during the analysis of the soil samples. For the analysis of total Se, the average recovery of analyte from the two quality assurance materials was 94% of the certified value. Tables 20 and 21 present data relating to the analytical precision, and reflect the high quality of sample pre-treatment stages (homogenisation, *etc*), as well as the analytical procedure itself.

Total Soil-Se

Total soil-Se concentrations in the dataset as a whole (n=114 samples) ranged from 0.115 to 0.877 mg kg^{-1} (Table 18). These values were within a similar range to those reported previously for Scottish soils from very limited datasets (Ure *et al*., 1979 and MacLeod *et al*., 1996) (Table 1). However, the results were narrower in range, and lower in average concentration, than those reported by Fordyce *et al*. (2009) for rural soils on the periphery of Glasgow (Table 1). The higher Se values in the Glasgow area reflected the influence of urban contamination on the rural environment around Glasgow, as well as the presence of coals and peaty soils in the Glasgow area.

Average Se concentrations from the present study (0.444 mg kg^{-1}) were comparable to world soil averages (0.400 mg kg^{-1}, Table 1) and to those found in over 19,500 soil samples from an extensive area of Eastern England (Humber-Trent; East Midlands and East Anglia) (0.400 mg kg^{-1}, Table 1), as part of the BGS Geochemical Baseline Survey of the Environment (G-BASE) project.

Although the present project aimed to target low and high-Se environments in Scotland; the range in total Se concentrations reported in Scottish soils was very narrow, and was more limited than those reported in the G-BASE Eastern England dataset; and other studies of soils from England, Wales, Northern Ireland, China, New Zealand and the USA (Table 1). This reflected the much wider spatial coverage of these datasets, over a greater range of geological settings and soil types, compared to the soils collected in the present study.

The total Se concentration of the majority of samples was below the suggested deficiency threshold in soils for the rearing of animals (0.6 mg kg^{-1}, Table 1). It was concluded that the total Se concentrations of the soils analysed by the present study are low, which was to be expected, as rock types that would give rise to high-Se soils (such as black shales) are rare and limited in their spatial extent in Scotland. The importance of Se to animal production is well known in Scottish veterinary and agricultural practice. The low productivity of animals not receiving enough Se is well known. Although

the farmers in this study were not aware that they were particularly supplementing Se, it is a standard component of the minerals licks and mineral supplements that are routinely given to animals.

Water-soluble Soil-Se

Water-soluble soil-Se concentrations ranged from 6.69 to 26.78 µg kg^{-1} (Table 18). These concentrations were much higher than those reported from Se-deficient areas of China (Fordyce, 2005) and were higher, on the basis of mean concentrations, than values noted in Chinese soils (Table 1). Results were comparable to ranges in water-soluble soil-Se concentrations from Se-deficient regions of India, but markedly lower than water-soluble soil-Se values in England and Wales (Table 1). Workers in China have suggested a link to human health deficiency threshold of 6 µg kg^{-1} for water-soluble soil-Se and a toxicity threshold of 20 µg kg^{-1} (Tan, 1989). The results of the present study were above the Chinese deficiency threshold; indeed the higher values exceeded the Chinese toxicity threshold. However, the range in concentration between these two thresholds is extremely narrow and Se deficiency has been reported in India at soil water-soluble concentrations above the suggested Chinese toxicity threshold. These results highlight the difficulty in applying thresholds to water-soluble soil-Se data, as very few studies have been carried out into water-soluble soil-Se concentrations.

Soil pH

Soil pH values ranged from 4.11 to 6.59, and were fairly typical of the acid and acid-neutral soils present over much of Scotland (Table 18). Selenium is more bioavailable in alkaline than acid conditions; therefore the acid-neutral nature of soils in the present study may inhibit the uptake of the element into plants and animals.

Soil Loss-on-Ignition

Soil loss-on-ignition was measured as an indicator of organic matter content, and values ranged from 1.71 to 14.3%. These values are indicative of moderate to high organic matter contents, typical of Scottish soils (SEERAD, 2006). Selenium is commonly associated with organic matter in soils. However, organic matter can also trap the Se in the soil, making it unavailable for plant uptake, in certain situations (Fordyce, 2005).

3.5.2. Overview of Grass Total Se

The Se concentration of grass from the milk and beef farms in the present study ranged from 3.42 to 22.24 µg kg^{-1}. This was lower than mean concentrations reported from countries such as Finland (Table 1); but samples were collected from four fields only, so were not representative of Scottish grass in general. However, the concentrations were below thresholds for forage intake of 40 µg kg^{-1} (Levander, 1986).

3.5.3. Overview of Food Commodities Total Se

Quality Assurance Data

Table 22 presents the quality assurance data obtained during the analysis of the food and vegetation samples. A number of different CRMs were used, so as to cover the range of analyte concentrations and matrix types experienced in this study. The average recovery of analyte from the nine CRMs was 106% of the certified values. The mean value for the recovery of analyte spiked into blank reagent (spike recovery) was 91%.

As 43 samples were subjected to duplicate analysis, the mean value and the relative standard deviation (RSD) % value is quoted (Table 23) rather than tabulating the individual data points (as was possible with the smaller number of soil replicates). The percentage mean proportion for the overall replicate analyses was 4.6%, and reflected the high quality of sample pre-treatment stages (homogenisation, *etc*), as well as the analytical procedure itself. The matrix which had significantly higher levels of imprecision was grass (11.9%), which could be explained by the fact that the material had not been washed (so the measurement included everything that the cattle were ingesting), plus it was not any easy matrix from which to create a homogenous sub-sample without drying and extensive grinding. These factors should be taken into account when designing any future studies.

Food Commodity Se Concentrations

Please note that, throughout this report, all data relating to the concentration of Se in food samples, is presented as fresh weight.

Calabrese

The Se concentration of the calabrese samples ranged from 1.51 to 7.45 µg kg^{-1} (Table 18), which was 10 times lower than results reported for calabrese in the USA (Table 4) but comparable to concentrations reported in green vegetables from the UK 2006 TDS (Table 6).

Potatoes

Potatoes from the present study contained, on average, 2.28 µg Se kg^{-1} (Table 18). Similarly low concentrations were reported in the UK 2006 TDS (Table 6). However, concentrations were significantly lower than those reported by Barclay *et al*. (1995) for potatoes imported to the UK (Table 4).

Wheat

The mean value of 23.10 µg Se kg^{-1} in wheat was comparable to that for Scottish wheat reported by Adams *et al*. (2002), but was lower than the reported Se concentration of wheat in East Anglia, or from the USA, Canada and Australia (Table 4).

Beef Fillet Steak

The beef fillet steak samples from the study were found to contain 81.08 – 151.08 µg Se kg^{-1} (Table 18). The mean concentration of 114.91 µg Se kg^{-1} was within the expected range for muscle meat (Table 3) but was lower than the UK 2006 TDS concentration of 140 µg kg^{-1} (Table 6). Values were higher than those previously reported, for Ayrshire beef, by Barclay and Macpherson (1992) (Table 4).

Summer and Winter Milk

Concentrations of Se in summer milk ranged from 12.92 to 22.02 µg kg^{-1}, and in winter milk from 17.50 to 25.61 µg kg^{-1} (Table 18). Values in winter milk were higher than in summer milk, as expected, due to the generally greater concentrations of Se found in dried hay, grain and silage fed to the cattle during winter months, as opposed to pasture fodder; plus increased Se-supplementation of the cattle during the winter. However, the difference in range between the two datasets was not statistically significant (see Section 3.8 of this report). The results from the present study were comparable to those reported previously for Scottish milk (Table 4) and the UK 2006 TDS (14 µg kg^{-1}) (Table 6).

3.6. Can Geology Be Used To Predict The Se Concentration Of Soil?

One of the questions that the project was designed to test was whether geological information alone could be used to predict the Se concentration of the soil in this, and in any future studies, in the absence of a national soil-Se geochemical dataset for Scotland.

3.6.1. Total Soil-Se Concentration Related to Geology-based Predicted High and Low-Se Areas

The use of geology to predict soil-Se concentration was first examined by comparing the results of the low-Se and high-Se within-farm dataset (Figure 4 and Table 24). These results, and two-sided pairwise t-tests with multiple comparison correction, demonstrated that for potato ($p<0.01$) and wheat ($p<0.001$) farm soils, the total Se concentrations in the predicted high-Se areas were significantly higher than the low-Se areas, as expected.

There was no significant difference in total soil-Se concentration between the high and low-Se areas for beef farm soils ($p=0.21$), despite the fact that soil Se-fertiliser was applied to the high-Se beef farm (H02).

Although there was no statistically significant difference in total soil-Se concentration between the high and low-Se areas for the milk farms ($p=0.18$); as Figure 4 demonstrates, concentrations were generally lower in the low-Se area.

Total concentrations of Se in soil from the predicted high-Se calabrese farm were significantly lower ($p<0.001$) than those from the low-Se calabrese farm*, i.e.,* the opposite of what was expected.

The relationships between soil-Se concentration and predicted high and low-Se areas were examined further using the between-farm dataset (Figure 5). The results also showed, and a two-sided t-test confirmed, that the total Se concentration of the soils in the predicted high-Se area were significantly higher ($p<0.05$) than the total Se concentrations of the soils in the predicted low-Se area.

However, once again, the range in concentration between the two was narrow, and there was much overlap between the two datasets. Not all the soils in the high-Se area contained more Se than the soils in the low-Se area. Only 12.6% (adjusted R^2) of the variance between the datasets was accounted for by the predicted Se area category, on the basis of bivariate linear regression. Although the between-farm soil dataset showed a greater range in total Se concentration (Figure 5) than any of the within-farm datasets (Figure 4), the difference in concentration ranges was narrow, given the much greater geographic spread of the between-farm dataset.

This shows that the selection of high and low-Se areas, on the basis of geology alone can, to a certain degree, be used to predict the total Se concentration of the soil; but that no striking contrast in total soil-Se concentration was observed between the two areas. In addition to this, even in the high-Se area, the majority of soils could be classed as being Se-deficient, as they are below the 0.6 mg kg^{-1} recommended threshold for grazing livestock (Fordyce, 2005) (Figure 5).

3.6.2. Water-soluble Soil-Se Concentration Related to Geology-based Predicted High and Low-Se Areas

In the science of geochemistry, it is not possible to predict the water-soluble concentration of any trace element (including Se) in soil from the total concentration. As outlined in Chapter 1 and Section 2.3.1 of this report, many factors control the amount of bioavailable or water-soluble soil-Se present. Therefore, it was not anticipated that the geology-based classification of high and low-Se areas would apply to the water-soluble soil-Se concentration. However, the success or otherwise of the high and low-Se area classification, in relation to water-soluble soil-Se concentration, was assessed.

Although water-soluble soil-Se concentrations were marginally higher in the low-Se area in the between-farm dataset (Figure 5); there was no statistically significant difference between the two areas ($p=0.71$) on the basis of a two-sided pairwise t-test with multiple comparison correction.

In the within-farm dataset (Figure 4 and Table 24), two-sided pairwise t-tests with multiple comparison correction demonstrated that water-soluble soil-Se concentrations were significantly lower in the low-Se areas in milk ($p<0.05$) and wheat ($p<0.001$) farm soils as expected. The water-soluble soil-Se results followed the trends in the total soil-Se concentration (Figure 4).

As the results in Figure 4 show; the soils from the two milk farms contained notably higher water-soluble soil-Se concentrations than the other farms. Soils from these two farms also showed the broadest range in water-soluble soil-Se concentrations. Possible reasons for these results are discussed in Chapter 4 of this report.

Water-soluble soil-Se concentrations were significantly higher in the low-Se area calabrese farm soils ($p<0.001$). However, this result followed the trend in total soil-Se concentrations. As explained in the preceding paragraphs of this report; the high-Se calabrese farm (H05) did not conform to the high and low-Se area classification due to the variable nature of the geology at this location.

Water-soluble soil-Se concentrations were also significantly higher in the low-Se area beef ($p<0.05$), and potato ($p<0.05$) farm soils, whereas total soil-Se concentrations measured in the same samples, showed the opposite trend (Figure 4).

Therefore, as expected, the water-soluble soil-Se concentrations showed no clear relationship with predicted high and low-Se areas and it was not possible to predict water-soluble soil-Se concentration on the basis of geology alone.

3.6.3. Factors That Control Total and Water-soluble Soil-Se Concentration

In addition to the total and water-soluble soil-Se concentrations; soil pH and LOI (as a measure of organic matter content) were determined during the study, to elucidate the geological controls on soil-Se concentrations further. As outlined in Chapter 1 of this report, Se is typically associated with organic matter in soils, and can be more bioavailable in alkaline, rather than acid, soils.

Soil pH and LOI results for the between-farm dataset are summarised in Figure 5 and the relationships between total and water-soluble soil-Se concentrations and these two parameters are shown in Figure 6. Pearson correlation tests with multiple comparison correction were applied to assess the relationships between the soil parameters in both the whole between-farm dataset and the high/low-Se area between-farm datasets (Table 25). Although total and water-soluble soil-Se concentrations showed a significant correlation in the dataset, as a whole ($p<0.01$), and were highly correlated in the low-Se area ($p<0.001$); they showed no significant correlation in the high-Se area ($p=0.08$). Therefore, total Se concentrations cannot be used to predict water-soluble soil-Se concentrations in all cases.

Neither total nor water-soluble soil-Se concentrations showed significant correlations with soil pH, which may have been due to the very narrow range of pH values observed.

As expected, total soil-Se and water-soluble soil-Se concentrations were highly correlated with soil LOI indicating that organic matter exerted a significant control on soil-Se concentrations (Table 25).

This explained the apparent joint correlation between total soil-Se and water-soluble soil-Se concentrations observed in the whole dataset described above. Indeed, based on bivariate linear regression, 56.1% (adjusted R^2) of the total Se and 48.0% (adjusted R^2) of the water-soluble soil-Se variance in concentration was accounted for by the soil organic matter content alone.

Therefore, the results of this study demonstrated that soil organic matter content had a greater control than geologically predicted high or low-Se areas on the Se concentration of the soil. However it should be noted that, in nature, the organic matter content of soil is also controlled to a significant degree by geological factors.

3.7. Grass and Foodstuff Se Concentrations, in Relation to Soil Se Status

The total Se concentrations of grass, calabrese, potatoes, wheat, beef and summer and winter milk in the within-farm datasets are summarised (as fresh weight) in Figure 7 and Table 26.

The results in Figure 7 show that Se concentrations in these commodities generally decreased in the order; beef fillet steak > wheat > winter milk > summer milk > grass > calabrese > potatoes. However two-sided pairwise t-tests with multiple comparison correction demonstrated that whilst beef fillet steak samples contained significantly more Se than the other food types ($p<0.001$); concentrations in wheat and winter milk ($p=0.73$) or summer milk ($p=0.19$) were not significantly different from each other. However, wheat and summer and winter milk values were significantly higher than concentrations in calabrese or potatoes ($p<0.001$). Total Se concentrations in calabrese and potatoes were not significantly different from each other ($p=0.88$). Although concentrations in winter milk were, as expected, marginally higher than ranges in summer milk due to the greater Se concentrations of cattle winter feed products; these differences were not statistically significant ($p=0.29$). Two-sided pairwise t-tests with multiple comparison correction showed no significant difference in grass total Se concentration between the beef and milk farms ($p=0.42$).

Overall, the significant differences in Se concentration between the various foodstuffs were summarised in decreasing order as follows: beef fillet steak > (wheat; winter milk; summer milk) > (calabrese; potatoes).

Indeed, on the basis of bivariate linear regression, 92% (adjusted R^2) of the variance in commodity Se concentration was accounted for by commodity type; indicating wide ranges in the Se concentration of the different foodstuffs, as expected. These results comply with previous food-Se studies in that that beef fillet steak as a red meat product was a good source of Se in the diet; and the Se concentrations in wheat were higher than in vegetable crops.

3.8. Grass and Foodstuff Relationships with Predicted High and Low-Se Areas

As Figure 7, for the within-farm dataset, and Figure 8, for the between-farm dataset show; Se concentrations in the grass and food commodities were generally higher in the high-Se areas than the low-Se areas. However, two-sided pairwise t-tests, with multiple comparison correction, demonstrated that differences in total Se concentrations for milk-farm grass ($p=0.18$), summer milk ($p=0.08$); winter milk ($p=0.10$); within-farm potatoes ($p=0.36$) and between-farm potatoes ($p=0.08$) were not statistically significant. In contrast, the differences in Se concentration between beef-farm grass ($p<0.05$); calabrese ($p<0.01$); wheat ($p<0.001$) and beef ($p<0.001$) were statistically significant. Indeed, bivariate linear regression demonstrated that 50.8%; 69.6% and 71.5% of the variance in Se concentration in calabrese, wheat and beef samples, respectively, was explained by the predicted Se area category.

In summary, calabrese, wheat and beef Se concentrations were significantly higher in the high-Se areas as expected. Although milk and between-farm potato samples showed similar trends; there were no statistically significant differences in milk or potato Se concentration between the high and low-Se areas.

3.9. Grass and Foodstuff Relationships with Soil Se Concentrations

Although the grass and food commodity Se concentrations, in general, reflected the predicted high and low-Se areas; relationships with the measured soil-Se concentrations were more complex. Statistical analysis of the relationships between soil parameters and commodity Se concentrations were possible for samples collected at the same sites as the soils only; *i.e.* calabrese, potato, wheat and grass samples. The relationships between the soil and commodity Se concentrations of these samples are shown in Figure 9.

ANOVA and a stepwise model selection were used to quantify the relationships between the Se concentrations of grass, calabrese, potato and wheat samples and the various soil parameters (Table 27). The results showed no significant correlations between any of the parameters in soil and the Se concentration of the grass (milk farm) or within-farm potato samples. This reflected the limited variation in Se concentration in the soils between the high and low-Se areas for these foodstuffs.

Wheat and between-farm potato Se results correlated significantly with total soil-Se ($p<0.001$). Indeed; 72.8% (R^2) of the variance in wheat concentration in the within-farm dataset was accounted for by the total soil-Se concentration. The data distributions also demonstrated that the wheat and between-farm potato Se concentrations followed the trend in soil-Se concentrations as expected (Figures 4, 7 and 8).

Calabrese Se concentrations showed a significant negative correlation ($p<0.01$) with total soil-Se concentrations (Table 27), as is demonstrated by Figures 4 and 7. The calabrese Se concentration was higher in the high-Se area, despite lower soil-Se concentrations in this area. Possible reasons for these results are discussed in Chapter 4 of this report.

Grass (beef farm) samples show a significant positive correlation with soil pH (<0.001) (Table 27). The data distributions shown in Figures 4 and 7 reveal that despite the similar soil-Se concentrations between the high and low-Se beef farms; the grass in the high-Se beef farm contained markedly more Se. Possible reasons for these results are discussed in Chapter 4 of this report. The beef fillet steak Se concentrations reflect the concentrations in the grass rather than the soil, and are higher in the high-Se area (Figure 7).

In summary, wheat and between-farm potato Se concentrations show a significant relationship with the total Se concentration of the soil. The high-Se beef-farm grass Se concentrations are higher than expected compared to soil-Se concentrations. The Se concentrations in calabrese from the high-Se farm are also higher than expected from the measured soil values.

3.10. Transfer of Se from the Soil to Grass and Food

The ratios (expressed as percent) of the total Se concentration in grass, calabrese, potato (within- and between- farm) and wheat, versus the total and water-soluble Se concentration of their associated soils, are shown in Figure 10. The results suggested that, of all the crop samples, the transfer factor for Se from the soil was greatest for wheat. Indeed, the wheat Se concentrations were 300 – 600 % of the water-soluble soil-Se concentration in the soil, indicating that other weakly sorbed portions of the total soil-Se may be available for plant uptake. There is no evidence that the transfer factor for calabrese, as a secondary Se-accumulator plant, is greater, relative to other crop types. The results also confirm that concentrations in the high-Se beef farm grass samples are markedly higher, relative to soil concentrations, than the other beef or milk farms as discussed in Chapter 4 of this report.

The total Se concentrations of beef fillet steak and milk samples cannot be compared directly to the soil samples, as they were not collected in the same geographic location in any given field. However, the average total Se concentration for these commodities in each of the farms can be calculated and compared to the average soil-Se concentrations for each farm. The results demonstrate that beef fillet steak samples contain the highest Se concentrations relative to soil concentrations of any of the commodity types containing over 1000% of the water-soluble soil-Se concentrations in soil. This is to be expected as Se accumulates in the muscle meat of the animal and the cattle are fed supplements which contain Se (Figure 11).

3.11. Evaluation of Sampling Plan Effectiveness

With regard to the construction of the sampling plan, presented in the proposal; the statistical significance (p value) was set at 5%, which is a standard level to detect significant differences. In addition, a power statistic of 80% was assumed (Figure 1).

By exploring the collected data set, it was possible to revisit the sampling plan, and to assess its appropriateness for future studies. Statistical power calculations depend on the following components:

1. Standardised effect size: (a) effect size and (b) variation / standard deviation
2. Sample size (N)
3. Significance level (alpha = 0.05)

For the two-sided pairwise t-tests, between high and low soil-Se status, the statistical power was computed, and the values calculated for those parameters where the tests resulted in a significant difference between the two predicted environments (Figure 3), and are summarised here;

Total Soil-Se level	=	79%
Se in Calabrese	=	99.1%
Se in Grass	=	83%
Se in Wheat	=	99.9%

The above data confirm that the sampling strategy was appropriate for the requirements of the study, *i.e.,* eight replicates, for the within-farm samples, were sufficient to achieve statistically significant results. Although some of the datasets reached an even a higher power *e.g.,* wheat = 99% (suggesting that smaller sample sizes could be taken for certain commodities), the results also show that a commodity such as milk would require a larger sample size to be taken in any future study, and that potatoes should not be considered for inclusion in any future study.

4. Discussion

4.1. Predicted High and Low Soil-Se Concentrations

This project was designed to explore the feasibility of relating predicted soil geological parent material Se concentrations against the measured concentrations in overlying soils, and the foods grown on them, in Scotland.

This study has shown that, in the absence of national soil-Se geochemical maps for Scotland; geological information, alone, can be used to predict differences in the Se concentration of Scottish soils, although only in relatively general terms. The average total Se concentration in high-Se areas (0.484 mg kg^{-1}) was greater than that in low-Se areas (0.372 mg kg^{-1}). However, the prediction did not work in every case, as the range of total soil-Se concentrations measured in this study was very narrow (≤ 0.877 mg kg^{-1}), making it difficult to distinguish relatively high and low-Se areas. Indeed, even in the high-Se areas, the majority of soils could be classed as Se-deficient as they are below the 0.6 mg kg^{-1} recommended threshold for grazing livestock (Fordyce, 2005).

Maps showing the distribution of total and water-soluble soil-Se concentrations from the 44 farms in the combined within and between-farm dataset are presented in Figures 12 and 13. These confirm the lack of obvious spatial association between the predicted high-Se areas and demonstrably higher total soil-Se concentrations. As Figures 12 and 13 highlight; because potato samples were selected for the between-farm study; the majority of samples were collected from the east coast of Scotland in the main arable growing area between the Arbroath-Montrose basin, north of Dundee and East Lothian to the east of Edinburgh. In these areas, the main rock types defined as potentially high-Se soil parent materials were the Carboniferous rocks of the Midland Valley of Scotland.

These rock types are highly variable and comprise cyclical sequences of coals, shales, mudstones and siltstones (all likely to be higher in Se) with interbedded sandstones and limestones (likely to be low in Se). At the start of the project, the relationship between the Carboniferous rocks and the concentrations of Se in the soils associated with them was poorly defined. Therefore, even though the actual Se concentration was unknown, they were classified as high-Se, because that was the expectation.

However, the results for the high-Se calabrese farm soil (H05) - which was located on Carboniferous rock types, near Kingsbarns in Fife - did not fit the predicted trend. Soil total Se concentrations were higher on the low-Se farm (L05) (mean 0.421 mg kg^{-1}) than on the high-Se farm (H05) (mean 0.308 mg kg^{-1}). The high-Se calabrese farm (H05) soils were noted as sandy in texture, and contained the lowest organic matter content (mean 3.66%) of any of the farm datasets. This suggested that these soils were collected on a sandstone-dominated part of the geological sequence and the sandy nature

of the soils and low organic matter content probably accounted for the lower than expected Se concentrations in these soils. Therefore, using the additional information gained during this study (soil-Se relationships to soil texture/ organic matter); it should be possible to further refine the definitions of high and low-Se areas within the Carboniferous sequence on the basis of the geological information held by BGS, thereby improving the accuracy of the high and low-Se area classifications. The Macaulay Research Institute (MI) also holds the National Soils Inventory dataset for Scotland. Whilst this dataset does not, at present, contain systematic information for Se; it is in the process of being re-surveyed. Selenium concentration data for approximately 400 soil samples across Scotland will be available in the next 2-3 years (MI, 2009). Inclusion of information from the MI National Soils Inventory would also improve the accuracy of the high and low-Se area classifications.

As indicated above, the Carboniferous rock types of the Midland Valley of Scotland were the main rock types predicted to be high-Se within the area of coverage of the present study. The results demonstrated higher soil-Se concentrations to some extent; but the increase in soil-Se concentration, over this parent material, was marginal.

Significant areas of high-Se soil parent materials were not identified in the main arable production regions of Scotland examined within this project. However, the predicted high-Se soils of Ayrshire and Aberdeenshire were not surveyed as part of this project, but should be included in any future study, to ensure completeness of the dataset. Having said that, it is unlikely that the Ayrshire predicted high-Se area will contain significantly higher soil-Se concentrations because it is underlain by the same Carboniferous rock types as the east coast of Scotland. The only difference is that, because of the wetter climatic conditions in the Ayrshire areas; more organic-rich soil types form, which may contain higher concentrations of Se. Similarly, it is unlikely that the metamorphic rock types underlying the predicted high-Se area of Aberdeenshire will result in soils with markedly greater Se concentrations than elsewhere in lowland Scotland.

With regard to the between-farm section of the study, it is expected that the variability observed in the soil-Se dataset was not affected by potatoes having been the crop chosen for investigation. This statement is based on the fact that both calabrese and wheat were also grown at similar locations, as was highlighted from further interrogation of the on-farm questionnaire (crop rotation section).

This study also showed that soil organic matter content had a greater control over the Se concentration of a soil than the geological prediction of high or low-Se area. However it should be noted that in nature, the organic matter content of soil is also controlled, to a significant degree, by geological factors.

High organic matter soils tend to occur in the upland areas of Scotland developed over poorly draining rock types. Higher Se concentrations in organic-rich upland soils have been found in the very similar

geological and climatic environment of Northern Ireland (GSNI, In Prep), so it is logical to assume the same will apply in Scotland. It is possible therefore that, future, wider-scale surveys of upland Scottish soils may reveal greater total soil-Se concentrations, which in turn may be utilised to produce meat-based commodities, containing higher concentrations of Se. However the relationships between soils and foodstuffs produced in these areas (mainly lamb) would have to be assessed, because organic matter can also bind Se in the soil, making it less mobile under certain conditions.

In summary, this study has demonstrated measurable variations in the Se concentrations of Scottish soils reflecting differences in soil geological parent materials. However, these differences were marginal and this study confirmed previous investigations indicating that the Se concentrations of Scottish soils were generally low. Indeed, the evidence from this study is that the Se concentrations of soils in the main agricultural production areas of Scotland are likely to be low, as there are no spatially extensive high-Se soil parent materials in these locations.

4.2. Relationships between Se in Soil and Foodstuffs

The second major aim of this project was to assess whether differences in soil-Se status were reflected in the foodstuffs produced from them.

The results showed that wheat and between-farm potato sample Se concentrations were significantly correlated with the total soil-Se concentrations and beef-farm grass, calabrese, wheat, and beef sample Se concentrations were significantly correlated with predicted Se setting.

The calabrese Se results reflected the predicted Se area despite the fact that the total and water-soluble soil-Se concentrations showed the opposite trend (See Section 3.9 of this report). The reasons for the disparity between soil-Se concentrations and calabrese Se concentrations were unclear but may have related to marginally different ages in the crops at the time of sampling, even though sampling was carried out within a week of predicted harvest date. Differences in trace element fertiliser application (the L05 soils received Cu and Mn) or the lower organic matter content of the high-Se calabrese farm soil (H05) may also have influenced the uptake of Se into the calabrese on the high-Se farm, despite lower soil-Se concentrations. Further investigations would be required to establish why the relative concentrations of Se in the calabrese samples from these two farms (L05 and H05) did not reflect those in the soil.

Despite similar soil-Se concentrations in the high and low-Se beef farms; the grass in the high-Se beef (H02) farm contained markedly more Se (mean 13.47 µg kg^{-1}) than the low-Se beef farm (mean 7.07 µg kg^{-1}). It was possible that the higher pH conditions of the high-Se beef farm soils made the Se more bioavailable. However, these results may also have reflected the application of sodium selenite to the fields on the high-Se beef farm (H02).

The results also showed that soils from the two milk farms contained notably higher water-soluble soil-Se concentrations (means 18.17 and 22.51 µg kg^{-1} for low and high farms respectively) than the other farms. The low-Se milk farm (L03) was located in Dumfriesshire, and the high-Se milk farm (H03), in Lanarkshire. Although it was difficult to draw conclusions from only two farms; the results suggested that a factor related to milk production was causing elevated concentrations of water-soluble soil-Se. Further studies would be required to investigate the cause of the higher water-soluble soil-Se concentrations on the milk farms.

All the food commodities (calabrese, potato, beef fillet steak and milk) with the exception of wheat collected for the present study were destined for the Scottish food market, indicating that food produced in Scotland is consumed locally. Therefore, is likely to contribute to the Scottish diet. However it was not scientifically valid to extrapolate dietary intakes from Scottish produce on the basis of the limited number of samples included in the present study.

In summary, the data showed that the concentrations of Se in a soil on which a plant was grown were, in general, reflected in the concentrations measured in the resulting plant material. However, as the uptake of Se is affected or controlled by many other environmental and physiological processes, care must be taken if attempting to predict how a particular plant may respond in a predicted high or low-Se location. For the animal-based samples, similar comments apply, but there are an even greater range of factors influencing the geology/soil/food relationship. A further limitation to providing a detailed discussion of the individual commodity datasets was that, with the exception of potatoes, the data only related to a comparison of samples from two single farms.

4.3. Potential Options for Increasing the Se Content of Scottish Foodstuffs

4.3.1. Application of Se Fertiliser

The results of the present study showed that only the high-Se beef farmer (H02) applied Se fertiliser to his soil. In contrast, the use of phosphate- and sulphate-based fertilisers was widespread, and other authors have suggested that these may interfere with the uptake of Se in agricultural crops (Broadley *et al*., 2006).

Therefore, on the basis of the very limited results from the present study; the use of Se-fertilisers does not appear to be widespread in Scottish agriculture, but this would have to be verified by a wider-scale study.

This study has demonstrated (as have several previous investigations) that the Se concentration of wheat and cereals are greater than in most vegetable crops. This finding, coupled with the fact that

cereals form a major dietary component, means that they are a significant source of the element in the diet (FSA, 2009).

As a result, many of the schemes designed to raise the dietary Se intake of deficient populations have focussed on enhancing the Se concentration of cereal crops through the application of Se-fertilisers to the soil, or to the plants themselves. This approach has been adopted successfully in countries like New Zealand (Oldfield, 1999) and Finland (Eurola *et al*., 2003).

Following concerns about very low concentrations of Se intake in the Finnish diet; a national programme was initiated in 1984 to increase the Se concentration of foodstuffs by adding sodium selenate fertilisers to crops and pasture. Mean daily intakes rose from 45 µg day^{-1} in 1980 to 110 – 120 µg day^{-1} between 1987 and 1990 and 90 µg day^{-1} when fertiliser levels were stabilised in 1992. This programme targeted both crops and grazing animals, and successfully increased the Se status of the human population by 45% (Eurola *et al*., 2003).

However, it should be noted that in some circumstances, Se added as fertiliser to the soil can become trapped in the soil and remains unavailable for plant uptake. Therefore, foliar application of Se-fertilisers may be a more efficacious approach (Fordyce, 2005).

As a result of concerns about low-Se concentrations in the UK diet; Se-fertilisers for soil application, to enhance the Se concentration of UK wheat, are currently being developed by the Biofortification through Agronomy and Genotypes to Elevate Levels of Selenium (BAGELS) project lead by Nottingham University (Adams, 2008) (Hawkesford and Zhao, 2007). In the likely absence of true high-Se environments in Scotland; this type of approach may prove necessary to increase the Se concentration of the Scottish diet in the future.

However, wheat from both the farms studied during the present project was destined for distilling and not for food production. Indeed, approximately 50% of Scottish wheat goes to distilling. The remainder is, in part, milled for biscuit flour, as well as for bread making, but is mixed with imported grain due to insufficient supply and quality. Alternatively, if the wheat is of poor quality, it is used for animal feed. Due to the climate, Scottish wheat has a lower protein content than English wheat. In wet harvest years, Scottish wheat often fails to meet bread making requirements. It is not clear from the preliminary investigations carried out during the present study whether Scottish wheat forms a major constituent of bread-making in Scotland.

Consequently, applying Se-fertiliser to Scottish cereals may not increase dietary intake if these cereals do not form a major dietary component. **Therefore, the significance of Scottish wheat and other cereal crops in the Scottish diet warrants further investigation.**

4.3.2. Selenium Supplements in Agricultural Animals

Although the importance of Se in animal production is well understood within the veterinary and agricultural sectors, the farmers in the present study did not consciously or specifically supplement their animals with Se. However, since Se is a standard constituent of the routinely used, multi-element mineral licks and supplements, all the animals in the present study had been (unknowingly) supplemented with Se. That Se supplementation is not more specific is somewhat surprising, because the Se concentrations measured in the grass samples from the present study were all below the 40 µg kg^{-1} deficiency threshold for grazing livestock (Levander, 1986). Although the limited sample size available to this study cannot be considered representative of Scotland, the results suggest that the Se status of grazing animals in Scotland may be of potential concern.

According to the Scottish Agricultural College (pers. commun.) it is left to individual farmers to decide whether or not to supplement their animals. The practice may vary depending on the animal; for example, it may be more prevalent in beef and milk farming than in sheep farming. However, the amount of Se supplementation carried out in animal husbandry in Scotland is not documented, and could be an issue worthy of further investigation.

The Se fertilisation of crops and pasture carried out in Finland described in Section 4.3.1 of this report was shown to increase the Se content of animal products such as meat, milk and eggs in the Finnish diet (Eurola *et al*., 2003). Similarly, the provision of Se-rich feed to hens has been used to produce Se-enriched eggs that can deliver ~50% of the recommended daily intake of Se to populations. These are being marketed in countries such as Ireland, Malaysia, Thailand, Australia and here in the UK (Fisinin *et al*., 2008).

On the basis of the limited information collected in this present study; it is unlikely that farmers actively supplement Se to their animals specifically, but Se supplementation, as part of standard multi-element products, maybe quite widespread. However, these results and the scope to increase dietary intake via animal supplementation would have to be confirmed by wider-scale studies.

4.4. Logistical Experience from the Present Study

4.4.1. Farmer Participation

In general, farmers were very willing to take part in the study, and few problems are envisaged with farmer recruitment in any larger scale studies considered in the future.

4.4.2. Varietal Differences

Although this project aimed to sample the same varieties of crop throughout, to minimise variability; two varieties of potatoes had to be selected to provide enough farms to survey for the present project. Therefore, the only logistical difficulties foreseen in carrying out wider surveys are that several

varieties of each vegetable may have to be included in any study to generate a representative dataset. However, if this was done, between-variety differences in Se utilisation would need to be established.

4.4.3. Meat Sampling

Although processed meat products such as mince, pies, sausages *etc*. were likely to be more commonly consumed in the Scottish diet than beef steak; enquiries during the present project revealed it would not be possible to relate these products to individual farms due to the mixing of meat from various sources, at the abattoir stage. The majority of meat in Scotland is slaughtered and processed at the main licensed abattoirs, not on individual farms. Butchers rarely slaughter animals, as they generally buy carcasses directly from abattoirs. As butchers generally only have one or two carcasses from the same farm on their premises in any given month, it would not have been possible to have collected the eight separate meat samples from each farm required in the present project, through butchery outlets. **If larger surveys were to be carried out in the future; the experience of this study was that sampling unprocessed cuts direct from the abattoir was the only practical way to obtain meat samples that could be traced back to individual farms. The abattoirs were accustomed to dealing with research projects of this type.**

4.4.4. General Statistical Overview

The statistical evaluation of the datasets obtained within the current study, showed that the overall approach used here would be appropriate for use in the development of a sampling plan for any future project with similar aims.

Analysis of the variance measured in the within-farm replicate samples of wheat and calabrese, suggested that either of these commodities would be suitable for use as the target foodstuff in a project looking at a wider geographical coverage. In the study reported here, eight replicates were collected, but statistical analysis of the collected data suggested that the quality of the data would not have been affected if a minimum of three and five samples had been collected for wheat and calabrese, respectively. However, the caveat accompanying this statement is, that the above suggestion was based upon data from only two farms (per commodity), and may therefore not be representative of what would be found in a survey of a larger number of farms.

5. Conclusions

This study aimed to asses whether differences in soil-Se concentration could be determined on the basis of predicted low and high soil geological parent materials in the main agricultural growing areas of Scotland. The study also investigated whether differences in soil-Se concentration were reflected in the Se content of the foodstuffs produced in predicted low and high-Se environments.

On the basis of the results reported in this study, we have shown that, in the absence of national soil-Se geochemical maps for Scotland; geological information alone can, in general terms, be used to predict differences in the Se concentration of Scottish soils. Although the approach worked well enough to be applied to future studies; it should be noted that these predictions will never be accurate unless supported by data from a larger scale, systematic geochemical map of Scottish soil-Se. Low resolution data (400 samples for the whole country) will become available in the next 2-3 years as part of the Macaulay Research Institute National Soils Inventory.

This study has demonstrated measurable variations in the Se concentrations of Scottish soils reflecting differences in soil geological parent materials. However, these differences were marginal, and this study confirmed previous investigations, indicating that the Se concentrations of Scottish soils were generally low *i.e.,* below the 0.6 mg kg^{-1} recommended threshold for grazing livestock.

The results of this project demonstrated, to varying degrees, that the Se concentrations of foodstuffs related to those of the soils on which they were produced as follows:

- wheat and between-farm potato sample Se concentrations were significantly correlated with the total soil-Se concentration.
- calabrese, wheat, beef and beef-farm grass Se concentrations were significantly correlated with predicted Se area.
- of the crop commodities sampled, the transfer factor of Se from the soil was greatest for wheat.
- there was no evidence that the transfer factor of Se from the soil was greater for calabrese, as a secondary Se-accumulator plant, relative to other crop types.

However, Se concentrations in the foodstuffs did not always match the trends observed in the soil, highlighting the difficulty in trying to predict the Se content in foodstuffs on the basis of soil-Se concentrations alone.

This study has also shown that:

- the Se concentrations of the grass samples were all below the 40 µg kg^{-1} deficiency threshold for grazing livestock.

- concentrations of Se in the food samples decreased in the order: beef fillet steak > (wheat; winter milk; summer milk) > (calabrese; potato) (statistically significant differences between sample types are shown in brackets). Total Se concentrations were notably higher in the beef fillet steak samples (5-times as high) than any other commodity as expected.
- the concentration of Se in milk was similar to that reported in the 2006 UK Total Diet Survey.
- although the concentrations of Se in calabrese and potatoes were only marginally lower than data reported in the 2006 UK Total Diet Survey, the calabrese results were approximately 10 times lower than those reported from the USA and the potato results were five times lower those for potatoes imported into the UK.
- selenium concentrations in wheat samples were similar to those reported from Scotland by other studies, but lower than from other parts of the UK and 20 times lower than wheat from Canada.

Therefore, the Se concentrations of soil, grass, wheat, calabrese, potato and beef fillet steak samples determined in this study were comparable or slightly lower than concentrations reported from other parts of the UK or the world.

It was not scientifically valid to extrapolate dietary Se intakes from Scottish produce on the basis of the small dataset generated by the present study. Nonetheless, as Scottish soils are likely to be largely Se-deficient, this may have an implication for dietary intakes, if significant amounts of locally-produced foods are consumed.

Given the relatively low concentrations of Se reported in this study; further investigations are warranted in order to fully characterise the Se concentration of Scottish produce and the concentrations of Se in the Scottish diet. This may lead to consideration of soil-Se-supplementation, *via* fertiliser applications, as has been performed in a number of other countries.

6. Recommendations and Future Directions

On the basis of the low-Se concentrations in soils and foodstuffs identified in the present study, in combination with the evidence of low-Se status in the Scottish population from the 2004 NDNS, further investigations to more fully characterise the Se content of Scottish produce and the diet of people in Scotland are recommended.

The health and policy implications of the current trend towards the consumption of locally-produced foods should be considered.

Specific recommendations arising from the present study are that:

- any future studies using geological parent materials to predict high and low-Se areas should include a more detailed classification of the Carboniferous rock types across Scotland based on the anomalous evidence from the calabrese farm soils gained in the present study.

- the relationships between soil-Se status and food commodities in the predicted high-Se areas of Aberdeenshire and Ayrshire should be included in any future study.

- larger scale, systematic geochemical mapping of soil-Se concentrations, such as the BGS G-BASE programme or the Macaulay Research Institute National Soils Inventory would aid the identification of high and low-Se environments in Scotland.

- the organic-rich upland soils of Scotland may contain more Se than soils in the main arable areas. Therefore, the links between these soils and foodstuffs, such as lamb, produced on them may warrant further investigation, to determine whether or not they represent a significant source of Se in the Scottish diet.

- any project aiming to relate the Se concentration of Scottish meat to the soils on which it was produced should be carried out in partnership with the main abattoirs, as it is only at this stage of the food production process that meat samples, which can be related back to specific farms, can be procured.

- a wider-scale survey of dietary-important commodities, to determine the Se status of these products in Scotland, possibly focussing on wheat.

- the importance of Scottish-grown cereal crops to the Scottish diet be investigated further; as cereal crops form the focus of most Se-biofortification programmes.

- should Scottish cereals prove not to be an important dietary component in Scotland; other methods to increase the Se status of the population such as the application of Se-fertilisers to pasture or provision of mineral licks and Se-supplements to grazing animals to increase the Se content of milk and meat or the production of Se-enriched eggs may be worthy of investigation.

- any strategies to increase the Se status of the Scottish population should be mindful of emerging evidence from the USA of links between moderately high Se intakes (~200 µg day^{-1}) and an increased risk of diabetes.

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8. Glossary

AIC	Akaike Information Criterion
ANOVA	Analysis of Variance
BAGELS	Biofortification through Agronomy and Genotypes to Elevate Levels of Se
BGS	British Geological Survey
BNG	British National Grid
CRM	Certified Reference Material
CONTEST	Contaminated Land Proficiency Testing Scheme
Defra	Department for Environment, Food and Rural Affairs
DOH	Department of Health
EU	European Union
EVM	Expert Group on Vitamins and Minerals
FDR	False Discovery Rate
Fera	Food and Environment Research Agency
FSA	Food Standards Agency
FSAS	Food Standards Agency Scotland
G-BASE	Geochemical Baseline Survey of the Environment
GIS	Geographic Information System
GPS	Global Positioning System
GSH-Px	Glutathione Peroxidase
GSNI	Geological Survey of Northern Ireland
HCl	Hydrochloric Acid
HIV-Aids	Human Immunodeficiency Virus - Acquired Immune Deficiency Syndrome
HGCA	Home Grown Cereals Authority
HNO_3	Nitric Acid
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IGGE	Institute of Geophysical and Geochemical Exploration
LRNI	Lower Reference Nutrient Intake
LOD	Limit of Detection
LOI	Loss on Ignition
LOQ	Limit of Quantification
MAFF	Ministry of Agriculture Fisheries and Food
MI	Macaulay Research Institute
NDNS	National Diet and Nutrition Survey
NIST	National Institute of Standards and Technology
OS	Ordnance Survey
QA	Quality Assurance
QC	Quality Control
RNI	Reference Nutrient Intake
RSD	Relative Standard Deviation
SACN	Scientific Advisory Committee on Nutrition
SARI	Scottish and Agricultural Research Institutes
SASA	Science and Advice for Scottish Agriculture
SD	Standard Deviation
SEERAD	Scottish Executive Environment and Rural Affairs Department
TDS	Total Diet Survey
UK	United Kingdom
UKAS	United Kingdom Accreditation Service
USA	United States of America
WHO	World Health Organisation
WMD	White Muscle Disease
WRc	Water Research Plc.

Appendix 1 Tables and Figures

Table 1. Soil and grass Se concentrations reported from around the world

Total Soil-Se mg kg^{-1}	Count	Minimum	Maximum	Median	Mean	Reference
World Soils (non seleniferous)		0.010	2.000		0.400	Fordyce (2005)
World Deficient					< 0.600	Fordyce (2005)
England/Wales (General)		0.010	4.700			Thornton *et al*. (1983)
New Zealand (General)		0.100	4.000			Oldfield (1999)
USA (General)		0.100	4.300			Jacobs (1989)
Finland (General)		0.1	8.3	0.146		Reimann and Caritat (1998)
China (General)		0.020	3.810			Tan (1989)
Northern Ireland	6937	0.100	7.800	0.700	0.800	GSNI (In Prep)
Eastern England	19562	0.000	20.100	0.300	0.400	BGS (2009)
Scotland	10	0.020	0.360			Ure *et al*. (1979)
Scotland (Aberdeenshire)	4	0.546	0.761			MacLeod *et al*. (1996)
Scotland Glasgow rural soils	241	0.100	6.600	0.900	1.000	Fordyce *et al*. (2009)
Water-soluble Soil-Se µg kg^{-1}	**Count**	**Minimum**	**Maximum**	**Median**	**Mean**	**Reference**
England/Wales (General)		50	390			Fordyce (2005)
India Se Deficient		19	66			Fordyce (2005)
China Se Deficient	100	0.03	5			Fordyce (2005)
China (General)	354	0.6	109.4	6.4	4	Tan *et al*. (2002)
Grass Total Se µg kg^{-1}	**Count**	**Minimum**	**Maximum**	**Median**	**Mean**	**Reference**
USA grass		10	40			Jacobs (1989)
Chile grass (dry weight)		30	40			Contreras *et al*. (2005)
Germany grass (dry weight)					25	Gierus *et al*. (2003)
Finland grass/silage (dry weight)	56	3	83		28	Eurola *et al*. (2003)

Table 2. Thresholds for Se in soils

Category	Total Se in Top Soil mg kg^{-1}	Water-soluble Se in Top Soil µg kg^{-1}
Deficient	< 0.125	< 3
Marginal	0.125 – 0.175	3 - 6
Moderate	0.175 – 0.400	6 - 8
High	> 0.400	> 8
Excessive	> 3	> 20

From Tan (1989)

Table 3. Typical Se concentrations of selected food types

Foodstuff	Se Concentration µg kg^{-1}
Organ Meats	400 to 1500
Seafood	400 to 1500
Muscle Meats	100 to 400
Agricultural Crops	< 1000 (dry weight)
Cereals and Grains	< 100 to > 800
Dairy Products	< 100 to 300
Fruits and Vegetables	< 100

From WHO (1987)

Table 4. Some concentrations of Se in UK foodstuffs and comparative data from elsewhere

Foodstuff	Se Concentration µg kg^{-1}	Comments	Reference
Wheat	25 (dry weight) 33 (dry weight) 25 (dry weight)	n = 180 (1982) n = 187 (1992) n = 85 (1998) Mean concentration UK bread-making wheat *(Triticum aestivum L)*	Adams *et al*. (2002)
Wheat	< 40 (dry weight)	Scotland : results from survey above	Adams *et al*. (2002)
Wheat	40 - > 60 (dry weight)	East Anglia: results from survey above	Adams *et al*. (2002)
Wheat	457 370	n = 190 (1981) n = 290 (1983) Mean concentration USA bread-making wheat	Adams *et al*. (2002)
Wheat	760	Mean concentration of wheat from 12 locations over 3 years, Manitoba, Canada (1993)	Adams *et al*. (2002)
Wheat	155 (5 – 720)	n = 170 Mean and range, South Australia wheat 2000 - 2001	Lyons *et al.* (2005)
Wheat flour	60 – 69 (wet weight) 77 – 99 (wet weight)	n = 66 Irish white flours n = 40 Irish wholemeal flours	Murphy and Cashman (2001)
Wheat flour	59	n = 32 (1989) Mean concentration Scottish flour	Barclay and Macpherson (1992)
Wheat flour	511	n = 32 (1989) Mean concentration Canadian flour used in Scottish bread making	Barclay and Macpherson (1992)
White bread Wholemeal bread	66 (wet weight) 86 – 129 (wet weight)	n = 18 Irish white bread n = 52 Irish brown breads	Murphy and Cashman (2001)
White bread Wholemeal bread 	21 (fresh weight) 118 (fresh weight) 30 (fresh weight) 130 (fresh weight)	n = 3 EU blended flour n = 4 Canadian blended flour n = 4 EU blended flour n = 3 Canadian blended flour Mean concentration in foods available in Scotland	Barclay and Macpherson (1992)
Wholemeal bread Currant bread	90 (fresh weight) 35 (fresh weight)	Mean UK, survey of 700 foodstuffs	Barclay *et al*., 1995
Bread	37 53	1994 Mean UK survey 119 food types 1995 Mean UK survey 119 food types	MAFF (1997)
Milk (whole)	15 (fresh weight)	n = 6 From Scottish creameries Mean concentration in foods available in Scotland	Barclay and Macpherson (1992)
Milk (whole)	18 (wet weight)	n = 10 Irish Milk	Murphy and Cashman (2001)
Eggs (whole)	175 (fresh weight)	n = 6 From Ayrshire sources Mean concentration in foods available in Scotland	Barclay and Macpherson (1992)
Beef (4 different cuts)	38 (fresh weight)	n = 4 From Ayrshire sources Mean concentration in foods available in Scotland	Barclay and Macpherson (1992)
Beef (raw)	81 (wet weight)	n = 14 Irish beef	Murphy and Cashman (2001)
Beef	76 (fresh weight)	n = 4 Mean UK, survey	Barclay *et al*., 1995
Kidney	1460 (fresh weight)	n = 28 Mean UK, survey	Barclay *et al*., 1995
Crab meat	840 (fresh weight)	n = 20 Mean UK, survey	Barclay *et al*., 1995
Vegetables	< 20 (fresh weight)	n = 140 Mean UK, survey	Barclay *et al*., 1995
Dairy products	< 20 (fresh weight)	n = 316 Mean UK, survey	Barclay *et al*., 1995
Potatoes	16 (fresh weight)	n = 18 Mean imported, UK survey	Barclay *et al*., 1995
Brazil nuts	2540 (850 – 6860) (fresh weight)	n = 14 Mean and range, UK survey. Natural UK purchased	Barclay *et al*., 1995
Calabrese	49.3 – 84.7	Range 30 varieties USA	Farnham *et al*., 2007

Table 5. Estimated population dietary exposure to Se from UK Total Diet Surveys

Year	Se μg day^{-1}
1985	63
1991	60
1994	43
1995	39
1997	39
2000	32 - 34
2006	48 - 58

From FSA (2009)

Table 6. Selenium concentrations in the main food groups consumed in the UK from the 2006 Total Diet Survey

Food Group	Se Concentration μg kg^{-1}	Contribution to UK Dietary Intake %	Comments
Fresh Fruits	< 5	1	The foods making up the 20 groups were bought from retail outlets in 24 randomly selected towns throughout the UK in the TDS for 2006. The food samples were prepared and cooked according to normal consumer practice. Equal quantities of samples from each town were mixed for each food group to obtain the national composite samples.
Fruit Products	< 5	<1	
Beverages	< 5	11	
Green Vegetables	7	<1	
Potatoes	< 10	2	
Canned Vegetables	14	1	
Milk	14	6	
Other Vegetables	18	3	
Oils & Fats	< 30	1	
Sugar & Preserves	< 30	3	
Dairy Products	30	4	
Bread	60	11	
Misc. Cereal	70	16	
Carcase Meat	140	5	
Meat Product	140	15	
Poultry	170	6	
Eggs	190	4	
Nuts	300	2	
Fish	420	10	
Offal	770	1	

From FSA (2009) Limit of Detection 30 μg kg^{-1}

Table 7. Mean blood plasma Se in adults aged 19 – 64 years in the UK

Men	Blood Plasma Se μmol L^{-1} (μg L^{-1})	Women	Blood Plasma Se μmol L^{-1} (μg L^{-1})
Scotland men	1.09 (86)	Scotland women	1.07 (84)
UK Men (low incomes)	1.05 (83)	UK Women (low incomes)	1.01 (78)
UK Men (standard incomes)	1.12 (88)	UK Women (standard incomes)	1.12 (88)
All UK men	1.11 (88)	All UK women	1.10 (87)

From the National Diet and Nutrition Survey of 1347 adults aged 19 - 64 (Ruston *et al.*, 2004)

Table 8. Wheat purchased by Scottish flour mills and Se concentration of flour and associated bread.

Wheat Origin	Wheat (Tonnes) 1987	Wheat (Tonnes) 1995	Flour Se µg kg^{-1}	Bread Se µg kg^{-1}
UK	288 000	250 000		
EU	120 000	130 000	48 (low protein)	36
			163 (medium protein)	112
Canada	88 000	57 000	280 (medium protein)	550
			610 (wholemeal)	680

From Macpherson *et al*. (1997)

Table 9. Scottish Se daily dietary intake and blood plasma Se concentrations

Measure	1974	1985	1994
Se intake µg day^{-1}	60	43	32
Se plasma µmol L^{-1} (µg L^{-1})	nd	1.50 (118) (n = 354)	0.86 (68) (n = 478)

From Macpherson *et al*. (1997) nd = no data

Table 10. Sampling design adopted for the project.

Sampling Design		Rationale
Within-farm Variability Dataset:		This part of the project was to assess on an individual farm basis the relationships between the Se status of the five food commodities of interest to the project and the soils on which they were grown in the high versus low-Se areas to test whether differences in soil and food Se concentration as a result of geological conditions could be assessed. The data obtained would directly reflect the precision achievable using the sample collection, preparation and analysis protocols. If the data from the exercise showed that the variability from this stage alone was significant, it would inform the FSAS about the feasibility of performing a larger-scale project in the future.
High (H) Selenium Soils	**Low (L) Selenium Soils**	
Farm-1 (H): Wheat (x 8) + soil (x 8)	Farm-6 (L): Wheat (x 8) + soil (x 8)	
Farm-2 (H): Potato (x 8) + soil (x 8)	Farm-7 (L): Potato (x 8) + soil (x 8)	
Farm-3 (H): Calabrese (x 8) + soil (x 8)	Farm-8 (L): Calabrese (x 8) + soil (x 8)	
Farm-4 (H): Milk (summer x 8) + soil (x 8) + grass (x 8) + milk (winter x 8)	Farm-9 (L): Milk (summer x 8) + soil (x 8) + grass (x 8) + milk (winter x 8)	
Farm-5 (H): Beef (x 8) + soil (x 8) + grass (x 8)	Farm-10 (L): Beef (x 8) + soil (x 8) + grass (x 8)	
Between-farm Variability Dataset:		This part of the project was to assess the range of Se concentration in one example food commodity and associated soils across a greater geographic area of Scotland. The data were to assess the wider geological/pedological controls on the Se concentration of soils and food products. This was to inform the FSAS about the feasibility of carrying out larger-scale studies in the future to assess the range of Se concentrations in food produced in different regions of Scotland.
High (H) Selenium Soils	**Low (L) Selenium Soils**	
17 Farms (H): Potato (x 1 composite of 8 sub-samples per farm) + soil (x 1 composite of 8 sub-samples per farm)	17 Farms (L): Potato (x 1 composite of 8 sub-samples per farm) + soil (x 1 composite of 8 sub-samples per farm)	

Table 11. Predicted low and high soil parent material types for the present study

Low-Se Parent Material	High-Se Parent Material
Gabbro	Volcanic Tuff
Basalt	Andesitic Lava
Diorite	Pelite
Granite	Marl
Felsite	Slate
Quartzite	Shale and Black Shale
Psammite	Clay
Greywacke	Mudstone
Gneiss and High Grade Metamorphic Rocks	Siltstone
Grit	Phosphatic Rock
Clean Sandstone	Coals and Coal Measures
Devonian Sandstone	Carboniferous Limestone
Permo-Triassic Sandstone	Carboniferous Sandstone
Limestone	

Table 12. Summary of the within-farm soil, grass and food dataset

Low-Se Area	High-Se Area	Sample Totals	
8 x Calabrese Samples from one farm 8 x Calabrese Soil Samples from one farm	8 x Calabrese Samples from one farm 8 x Calabrese Soil Samples from one farm	Calabrese	16
8 x Potato Samples from one farm 8 x Potato Soil Samples from one farm	8 x Potato Samples from one farm 8 x Potato Soil Samples from one farm	Potato	16
8 x Wheat Samples from one farm 8 x Wheat Soil Samples from one farm	8 x Wheat Samples from one farm 8 x Wheat Soil Samples from one farm	Wheat	16
8 x Beef Samples from one farm 8 x Beef Grass Samples from one farm 8 x Beef Soil Samples from one farm	8 x Beef Samples from one farm 8 x Beef Grass Samples from one farm 8 x Beef Soil Samples from one farm	Beef	16
8 x Summer Milk Samples from one farm 8 x Winter Milk Samples from one farm 8 x Grass Samples from one farm 8 x Milk Soil Samples from one farm	8 x Summer Milk Samples from one farm 8 x Winter Milk Samples from one farm 8 x Grass Samples from one farm 8 x Milk Soil Samples from one farm	Summer Milk	16
		Winter Milk	16
		Beef Grass	16
		Milk Grass	16
		Soil	80

Table 13. Summary of the between-farm soil and potato dataset

Low-Se Area	High-Se Area	Sample Totals	
17 x Potato Samples from different farms	17 x Potato Samples from different farms	Potato	34
17 x Potato Soil Samples from different farms	17 x Potato Soil Samples from different farms	Soil	34

Table 14. Composition of the between-farm dataset used for data processing

Between-farm Dataset Low-Se Area	Between-farm Dataset High-Se Area
17 x physical composite potato farm soils	17 x physical composite potato farm soils
17 x physical composite potato results	17 x physical composite potato results
5 x statistical composite (average) Low-Se within-farm soils	5 x statistical composite (average) High-Se within-farm soils
1 x statistical composite (average) Low-Se within-farm potato	1 x statistical composite Low-Se (average) within-farm potato

Table 15. Market destination of food commodities from the present project

Farm Identifier	Sample Type	Variety/Breed	Commodity Destination
L01	Wheat	Consort	Distilling
H01	Wheat	Consort	Distilling
L02	Beef	Aberdeen Angus Beef fillet	Scotbeef/Fresh Meat Market
H02	Beef	Aberdeen Angus Beef fillet	Scotbeef/Fresh Meat Market
L03	Summer and winter milk	Friesian Holstein	Arla Foods who supply Asda (liquid milk market)
H03	Summer and winter milk	Friesian Holstein	Sorn Milk Limited, liquid milk market
L04	Potatoes	Maris Piper	Pre-pack
H04	Potatoes	Maris Piper	Fresh Market
L05	Calabrese	Parthenon	Fresh Market
H05	Calabrese	Parthenon	Fresh Market
L06	Potatoes	Maris Piper	Fresh market/Pre-pack
L07	Potatoes	Maris Piper	Pre-pack
L08	Potatoes	Maris Piper	Not recorded
L09	Potatoes	Maris Piper	Ware
L10	Potatoes	Maris Piper	General ware
L11	Potatoes	Maris Piper	Seed & ware
L12	Potatoes	Maris Piper	General ware
L13	Potatoes	Maris Piper	Fresh market
L14	Potatoes	Saxon	Pre-pack
L15	Potatoes	Saxon	Supermarkets
L16	Potatoes	Saxon	Pre-pack
L17	Potatoes	Saxon	Pre-pack
L18	Potatoes	Saxon	Pre-pack
L19	Potatoes	Saxon	Fresh market
L20	Potatoes	Saxon	Pre-pack
L21	Potatoes	Saxon	Pre-pack
L22	Potatoes	Saxon	Pre-pack Tescos
H06	Potatoes	Maris Piper	Pre-pack
H07	Potatoes	Maris Piper	Pre-pack
H08	Potatoes	Maris Piper	Fresh market
H09	Potatoes	Maris Piper	Pre-pack
H10	Potatoes	Maris Piper	Bagging and chipping
H11	Potatoes	Maris Piper	Processing
H12	Potatoes	Maris Piper	Farm shop
H13	Potatoes	Maris Piper	Fresh market
H14	Potatoes	Saxon	Pre-pack
H15	Potatoes	Saxon	Supermarkets
H16	Potatoes	Saxon	Pre-pack
H17	Potatoes	Saxon	Pre-pack
H18	Potatoes	Saxon	Pre-pack
H19	Potatoes	Saxon	Prepack, bulk & bags
H20	Potatoes	Saxon	Fresh market/Pre-pack
H21	Potatoes	Saxon	Pre-pack and seed
H22	Potatoes	Saxon	Pre-pack

Table 16. Summary of fertiliser use on farms in the present study

Farm Identifier	Sample Type	Soil Fertiliser	Foliar Fertiliser	Soil-Se Fertiliser
L01	Wheat	N, P, K		
L02	Beef	N		
L03	Summer and winter milk	N, P, K, S		
L04	Potatoes	N, P, K	Zn	
L05	Calabrese	N, P, K, S	Cu, Mn	
L06	Potatoes	N, P, K	Mg	
L07	Potatoes	N, P, K		
L08	Potatoes	N, P, K		
L09	Potatoes	N, P, K	B, Ca	
L10	Potatoes	N, P, S		
L11	Potatoes	N, P, K	Mn, Zn	
L12	Potatoes	N, P, K		
L13	Potatoes	N, P, K		
L14	Potatoes	N, S	Zn	
L15	Potatoes	N, P, K		
L16	Potatoes	N, P, K		
L17	Potatoes	N, P, K, S		
L18	Potatoes	N, P K, S		
L19	Potatoes	N, P, K		
L20	Potatoes	N, P, K, S		
L21	Potatoes	N, P, K, S	Zn	
L22	Potatoes	N, P, K		
H01	Wheat	N, P, K, S		
H02	Beef	N, P, K		2.82 g ha^{-1} (3 times per year)
H03	Summer and winter milk	N, P, K, S		
H04	Potatoes	N, P, K		
H05	Calabrese	N, P, K		
H06	Potatoes	N, P, K		
H07	Potatoes	N, P, K		
H08	Potatoes	N, P, K, S	B, Ca, Mn	
H09	Potatoes	N, P, K, S	Zn	
H10	Potatoes	N, P, K, S	Mn	
H11	Potatoes	N, P, K, S	Ca, Mn	
H12	Potatoes	N, P, K, S		
H13	Potatoes	N, P, K		
H14	Potatoes	N, P, K	Mn	
H15	Potatoes	N, P, K		
H16	Potatoes	N, P, K, S	Ca, Mn	
H17	Potatoes	N, P, K		
H18	Potatoes	N, P, K, S	Mn	
H19	Potatoes	N, P, K		
H20	Potatoes	N, P, K		
H21	Potatoes	N, P, K, S	Mn	
H22	Potatoes	N, P, K, S		

N = Nitrogen P = Phosphorus K = Potassium
S = Sulphur B = Boron Ca = Calcium
Zn = Zinc Mg = Magnesium Mn = Manganese

Table 17. Summary of Se supplementation of cattle surveyed in the present project

Farm Identifier	Commodity	Se Supplemented Feed	Se Mineral Licks
L02	Beef	Aug 2007 until April 2008: 0.45 mg sodium selenite/animal/day May to July 2008: No supplemented feed Aug 2008 to slaughter: 0.6 mg sodium selenite/animal/day	1.25 mg sodium selenite/animal/day Summer only (May to July)
H02	Beef	Late Sep 2007 to late April 2008: 2 mg sodium selenite/animal/day May to Sep 2008: No supplemented feed Late Sep 2008 to slaughter: 3 mg sodium selenite/animal/day	1.2 - 1.75 mg sodium selenite/animal/day all year round
L03	Summer Milk	3.24 to 4.05 mg sodium selenite/animal/day	None
L03	Winter Milk	8.63 to 9.46 mg sodium selenite/animal/day	0.24 mg sodium selenite/animal/day
H03	Summer Milk	8 mg of Se/animal/day	None
H03	Winter Milk	8 mg of Se/animal/day	None

Table 18. Overview of the project results.

Sample Type	N	Range	Mean	Median	Comment
Total Soil-Se mg kg^{-1}	114 from 44 farms	0.115 – 0.877	0.444	0.433	**Similar to other results for Scottish soils. Values are low, with only 10 % samples exceed the 0.6 mg kg^{-1} soil deficiency threshold (Fordyce, 2005)**
Water-soluble Soil-Se µg kg^{-1}	114 from 44 farms	6.69 – 26.78	11.59	10.51	**Higher than Se-deficient areas of China, lower than soils in England and Wales**
Soil pH	114 from 44 farms	4.11 – 6.59	5.22	5.23	**Typical for Scottish soils**
Soil LOI %	114 from 44 farms	1.71 – 14.30	6.47	5.66	**Typical for Scottish soils**
Grass Total Se µg kg^{-1}	32 from 4 farms	3.42 – 22.24	8.64	7.04	**Below 40 µg kg^{-1} deficiency threshold in forage (Levander, 1986)**
Calabrese Total Se µg kg^{-1}	16 from 2 farms	1.51 – 7.45	3.29	2.65	**Comparable to green vegetables in UK Total Diet Survey 2006; 10 times lower than USA**
Potatoes Total Se µg kg^{-1}	50 from 36 farms	0.00 – 9.71	2.28	1.87	**Comparable to UK Total Diet Survey 2006 but five times lower than potatoes imported to the UK (Barclay *et al.*,1995)**
Wheat Total Se µg kg^{-1}	16 from 2 farms	3.57 – 62.70	23.10	19.72	**Comparable to other results for Scottish wheat; lower than other parts of the UK**
Beef Total Se µg kg^{-1}	16 from 2 farms	81.08 – 151.08	114.91	116.43	**Lower than UK Total Diet Survey 2006**
Summer Milk Total Se µg kg^{-1}	16 from 2 farms	12.92 – 22.02	17.50	16.88	**Comparable to UK Total Diet Survey 2006**
Winter Milk Total Se µg kg^{-1}	16 from 2 farms	17.50 – 25.61	21.69	20.79	**Comparable to UK Total Diet Survey 2006**
The total Se concentrations in foods decreased in the order beef fillet steak > (wheat, winter milk, summer milk) > (calabrese, potato)					

Grass and food samples are reported as fresh weight
Results are for the whole dataset = within-farm individual samples + between-farm composite soil and potato samples

Table 19. Results for soil analysis quality control procedures

Analytical CRM Solution	Total Soil-Se µg L^{-1}	Analytical CRM Solution	Water-soluble Soil-Se µg L^{-1}
SRM1643e-1	11.77	SRM1643e-1	10.89
SRM1643e-2	11.22	SRM1643e-2	11.45
SRM1643e-3	11.25	SRM1643e-3	11.30
SRM1643e-4	10.90	SRM1643e-4	11.29
Mean	*11.29 ± 0.36*	*Mean*	*11.23 ± 0.24*
Reference Value	*11.97*	*Reference Value*	*11.97*
% Recovery	*94 %*	*% Recovery*	*94 %*
Solid Soil CRM	**Total Soil-Se mg kg^{-1}**	**Solid Soil CRM**	**Water-soluble Soil-Se µg kg^{-1}**
GSS-4	0.629	GSS-4	12.27
GSS-4	0.596	GSS-4	13.13
GSS-4	0.594	GSS-4	12.35
GSS-4	0.581	GSS-4	12.68
GSS-4	0.575	GSS-4	12.43
GSS-4	0.568	GSS-4	12.36
GSS-4	0.608	*Mean*	*12.54 ± 0.32*
GSS-4	0.647	*Reference Value*	*nd*
Mean	*0.600 ± 0.027*		
Reference Value	*0.640*		
% Recovery	*94 %*		
Quality Control Standard	**Soil pH**	**Quality Control Standard**	**LOI %**
QC1-1	6.98	LLC-1	3.02
QC1-2	6.99	LLC-2	2.31
QC1-3	6.98	LLC-3	3.13
QC1-4	7.01	*Mean*	*2.82*
QC1-5	6.97	*Target Value*	*2.85 ± 0.90 (± 2 std dev)*
QC1-6	6.97	QC1-1	8.32
QC1-7	6.99	QC1-2	8.17
Mean	*6.98*	QC1-3	8.15
Target Value	*7.03 ± 0.11 (± 2 std dev)*	*Mean*	*8.21*
		Target Value	*8.39 ± 0.33 (± 2 std dev)*

SRM1643e = National Institute of Standards and Technology (NIST) CRM 'Trace Elements in Water'
GSS-4 = Institute of Geophysical and Geochemical Exploration (IGGE), China Soil CRM
QC1 and LLC = BGS internal Quality Control Standards for soil pH and LOI determinations
nd = no data

Table 20. Results of replicate soil sample analyses

Se Area	Farm Type	Sample Number A	Total Soil-Se mg kg^{-1} Replicate A	Sample Number B	Total Soil-Se mg kg^{-1} Replicate B	Mean	Mean Proportion %
Low	Wheat	L01-7A	0.210	L01-9A	0.230	220	9.0
Low	Wheat	L01-8A	0.206	L01-10A	0.202	204	2.0
High	Calabrese	H05-7A	0.307	H05-9A	0.299	303	3.3
High	Calabrese	H05-8A	0.302	H05-10A	0.311	306	2.9
Se Area	**Farm Type**	**Sample Number A**	**Water-soluble Soil-Se µg kg^{-1} Replicate A**	**Sample Number B**	**Water-soluble Soil-Se µg kg^{-1} Replicate B**	**Mean**	**Mean Proportion %**
Low	Wheat	L01-7A	6.71	L01-9A	7.11	6.91	5.8
Low	Wheat	L01-8A	7.07	L01-10A	7.32	7.19	3.5
High	Calabrese	H05-7A	7.14	H05-9A	6.87	7.01	4.0
High	Calabrese	H05-8A	8.05	H05-10A	8.18	8.12	1.7
Se Area	**Farm Type**	**Sample Number A**	**Soil pH Replicate A**	**Sample Number B**	**Soil pH Replicate B**	**Mean**	**Mean Proportion %**
Low	Wheat	L01-7A	5.26	L01-9A	5.27	5.27	0.2
Low	Wheat	L01-8A	5.32	L01-10A	5.35	5.34	0.6
High	Calabrese	H05-7A	5.35	H05-9A	5.37	5.36	0.4
High	Calabrese	H05-8A	5.49	H05-10A	5.49	5.49	0.0
Se Area	**Farm Type**	**Sample Number A**	**Soil LOI % Replicate A**	**Sample Number B**	**Soil LOI % Replicate B**	**Mean**	**Mean Proportion %**
Low	Wheat	L01-7A	4.97	L01-9A	4.79	4.88	3.5
Low	Wheat	L01-8A	4.73	L01-10A	4.86	4.80	2.8
High	Calabrese	H05-7A	3.16	H05-9A	3.26	3.21	3.0
High	Calabrese	H05-8A	3.42	H05-10A	3.43	3.42	0.4

Mean Proportion = Difference/Mean %

Table 21. Results of soil ICP-MS duplicate analyses

Sample Number	Total Soil-Se mg kg^{-1}	Total Soil-Se Duplicate mg kg^{-1}	Mean	Mean Proportion %
L01-4A	0.215	0.207	0.211	3.8
L04-2A	0.415	0.422	0.418	2.4
L05-4A	0.413	0.420	0.416	2.4
L21-1A	0.397	0.385	0.391	2.9
H04-1A	0.590	0.557	0.573	5.7
H10-1A	0.548	0.558	0.553	1.8
H20-1A	0.199	0.189	0.194	5.0
H02-8A	0.527	0.532	0.530	0.9
Sample Number	**Water-soluble Soil-Se µg kg^{-1}**	**Water-soluble Soil-Se Duplicate µg kg^{-1}**	**Mean**	**Mean Proportion %**
L01-6A	7.66	8.08	7.87	5.5
L04-8A	12.86	12.42	12.64	3.5
H03-1A	21.30	21.13	21.21	0.8
H04-7A	10.48	10.35	10.41	1.3
H05-10A	8.18	7.69	7.94	6.2
L02-4A	13.53	13.34	13.44	1.4

Mean Proportion = Difference/mean %

Table 22. CRM values (measured and certified) for the analysis of Se in foods

Typical Diet CRM	Selenium µg kg^{-1}
NIST 1548a	269
NIST 1548a	249
NIST 1548a	254
NIST 1548a	237
Mean	*252*
Certified value	*245 ± 28*
% recovery	*103%*
Non-fat Milk Powder CRM	
NIST 1549	130
NIST 1549	124
Mean	*127*
Certified value	*110 ± 10*
% recovery	*115%*
Bovine Liver CRM	
NIST 1577a	702
NIST 1577a	781
Mean	*741*
Certified value	*710 ± 70*
% recovery	*104%*
Whole Milk Powder CRM	
NIST 8435	141
NIST 8435	119
Mean	*130*
Certified value	*131 ± 14*
% recovery	*99%*
Durum Wheat Flour CRM	
NIST 8436	1314
Certified value	*1230 ± 90*
% recovery	*107%*
Bovine Muscle CRM	
NIST 8414	78
Certified value	*76 ± 10*
% recovery	*102%*
Bovine Muscle CRM	
BCR 184	184
Certified value	*183 ± 12*
% recovery	*100%*
Apple Leaves CRM	
NIST 1515	56
Certified value	*50 ± 9*
% recovery	*112%*
Peach Leaves CRM	
NIST 1547	137
NIST 1547	*141*
NIST 1547	*140*
Mean	*139*
Certified value	*120 ± 9*
% recovery	*116%*

Table 23. Results of food commodity duplicate analyses

Commodity	Number of Duplicates	Mean Concentration µg kg^{-1}	Mean Proportion (%)
Potato	11	3.1	3.4%
Wheat	4	22.3	3.5%
Calabrese	5	3.5	2.9%
Beef	6	120.6	3.4%
Milk	8	20.4	3.5%
Grass	9	8.9	11.9%

Table 24. Summary soil analytical results for the within-farm sample locations

Farm ID	Stats	Total Soil-Se mg kg^{-1}	Water-soluble Soil-Se µg kg^{-1}	Soil pH	Soil LOI %
L01	Count	8	8	8	8
Wheat	Minimum	0.204	6.91	5.20	4.52
	Maximum	0.281	8.30	5.55	5.57
	Median	0.222	7.69	5.35	4.94
	Mean	0.232	7.68	5.36	5.05
L02	Count	8	8	8	8
Beef	Minimum	0.483	10.55	4.81	7.91
	Maximum	0.573	14.14	5.04	10.30
	Median	0.505	13.53	4.95	9.07
	Mean	0.510	12.97	4.93	9.15
L03	Count	8	8	8	8
Milk	Minimum	0.290	12.71	4.43	6.14
	Maximum	0.679	22.24	5.00	9.81
	Median	0.517	18.22	4.79	8.46
	Mean	0.508	18.17	4.76	8.16
L04	Count	8	8	8	8
Potato	Minimum	0.368	9.14	5.26	4.16
	Maximum	0.463	13.77	5.53	5.67
	Median	0.412	12.33	5.38	4.58
	Mean	0.407	11.92	5.39	4.64
L05	Count	8	8	8	8
Calabrese	Minimum	0.399	11.18	5.11	5.56
	Maximum	0.440	13.94	5.53	6.19
	Median	0.420	12.35	5.24	5.87
	Mean	0.421	12.43	5.26	5.84
H01	Count	8	8	8	8
Wheat	Minimum	0.370	8.57	5.20	6.41
	Maximum	0.589	10.53	5.58	14.30
	Median	0.498	9.84	5.30	9.56
	Mean	0.492	9.66	5.35	9.71
H02	Count	8	8	8	8
Beef	Minimum	0.424	10.18	4.65	4.75
	Maximum	0.543	13.37	5.89	6.04
	Median	0.480	11.78	5.29	5.40
	Mean	0.485	11.71	5.35	5.36
H03	Count	8	8	8	8
Milk	Minimum	0.485	19.10	4.68	10.00
	Maximum	0.673	26.78	5.07	13.50
	Median	0.580	22.65	5.03	11.25
	Mean	0.585	22.51	4.96	11.76
H04	Count	8	8	8	8
Potato	Minimum	0.481	9.08	4.73	4.84
	Maximum	0.792	11.77	5.82	6.53
	Median	0.559	10.46	5.22	5.75
	Mean	0.577	10.33	5.25	5.72
H05	Count	8	8	8	8
Calabrese	Minimum	0.283	7.01	5.05	3.21
	Maximum	0.336	9.27	5.92	4.28
	Median	0.308	8.48	5.40	3.56
	Mean	0.308	8.35	5.37	3.66

Table 25. Pearson Correlation coefficients for relationships between soil parameters in the between-farm dataset.

	Whole Between-farm Data Set		Low-Se Area		High-Se Area	
	Cor	p value	Cor	p value	Cor	p value
Total Soil-Se vs Water-soluble Soil-Se	**0.46**	**<0.01**	**0.71**	**<0.001**	0.43	0.08
Total Soil-Se vs Soil pH	0.13	0.38	-0.12	0.61	0.25	0.27
Total Soil-Se vs Soil LOI	**0.76**	**<0.001**	**0.87**	**<0.001**	**0.69**	**<0.001**
Water-soluble Soil-Se vs Soil pH	-0.06	0.70	-0.07	0.72	-0.04	0.87
Water-soluble Soil-Se vs Soil LOI	**0.70**	**<0.001**	**0.74**	**<0.001**	**0.72**	**<0.001**
Soil pH vs Soil LOI	-0.08	0.61	-0.09	0.68	-0.10	0.66

Significant correlations shown in bold

Table 26. Summary of total Se concentrations in grass and foodstuffs in low and high-Se settings.

Total Se µg kg^{-1}	Low-Se Area					High-Se Area				
	Count	Minimum	Maximum	Mean	Median	Count	Minimum	Maximum	Mean	Median
Grass Whole Dataset	16	3.74	11.18	6.53	6.58	16	3.42	22.24	10.74	8.61
Grass Beef Farms	8	3.74	11.18	7.07	6.53	8	6.64	22.24	13.47	10.99
Grass Milk Farms	8	4.04	7.72	6.00	6.58	8	3.42	14.78	8.00	6.67
Calabrese	8	1.51	2.65	2.25	2.39	8	2.65	7.45	4.32	4.12
Potato	8	1.36	4.85	2.96	2.92	8	1.56	4.34	2.46	2.30
Wheat	8	3.57	14.43	9.57	9.68	8	25.01	62.7	36.63	32.95
Beef	8	81.09	117.68	95.95	94.96	8	115.18	151.08	133.86	135.69
Summer Milk	8	14.69	19.26	16.33	16.07	8	12.92	22.02	18.67	19.56
Winter Milk	8	17.5	25.61	20.57	19.92	8	20.19	25.41	22.8	22.73
Between-farm Potatoes	18	0.00	2.96	1.62	1.49	18	1.25	9.71	2.61	1.90

Grass and food results reported fresh weight

Table 27. Significant correlations (based on ANOVA) between soil parameters and commodity Se concentrations collected at the same location.

Commodity Type	Soil Parameter	Correlation	% Variance R^2	Significance p-value
Beef-farm grass, n = 16	pH	0.81	47.7	<0.001
	Water-soluble Se	-0.55	30.1	<0.01
Milk-farm grass, n = 16	NS	NS	NS	NS
Calabrese, n = 16	Total Se	-0.69	47.5	<0.01
Wheat , n = 16	Total Se	0.85	72.8	<0.001
	LOI	0.64	15.8	<0.001
Within-farm potatoes, n = 16	NS	NS	NS	NS
Whole between-farm dataset for potatoes, n = 36	Total Se	0.58	33.5	<0.001
Low-Se between-farm potatoes, n = 18	LOI	-0.24	23.8	<0.05
High-Se between-farm potatoes, n = 18	Total Se	0.64	41.0	<0.01

NS = no significant correlations with any soil parameters were evident

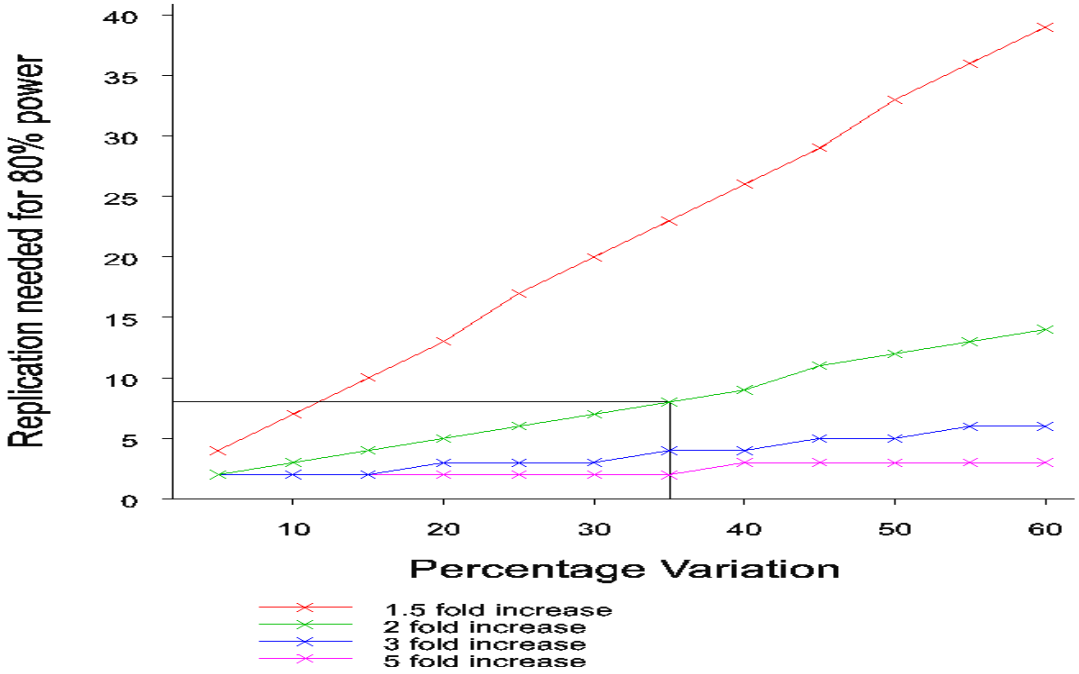


Figure 1. Number of samples required to achieve 80% statistical power per commodity for the two soil types.

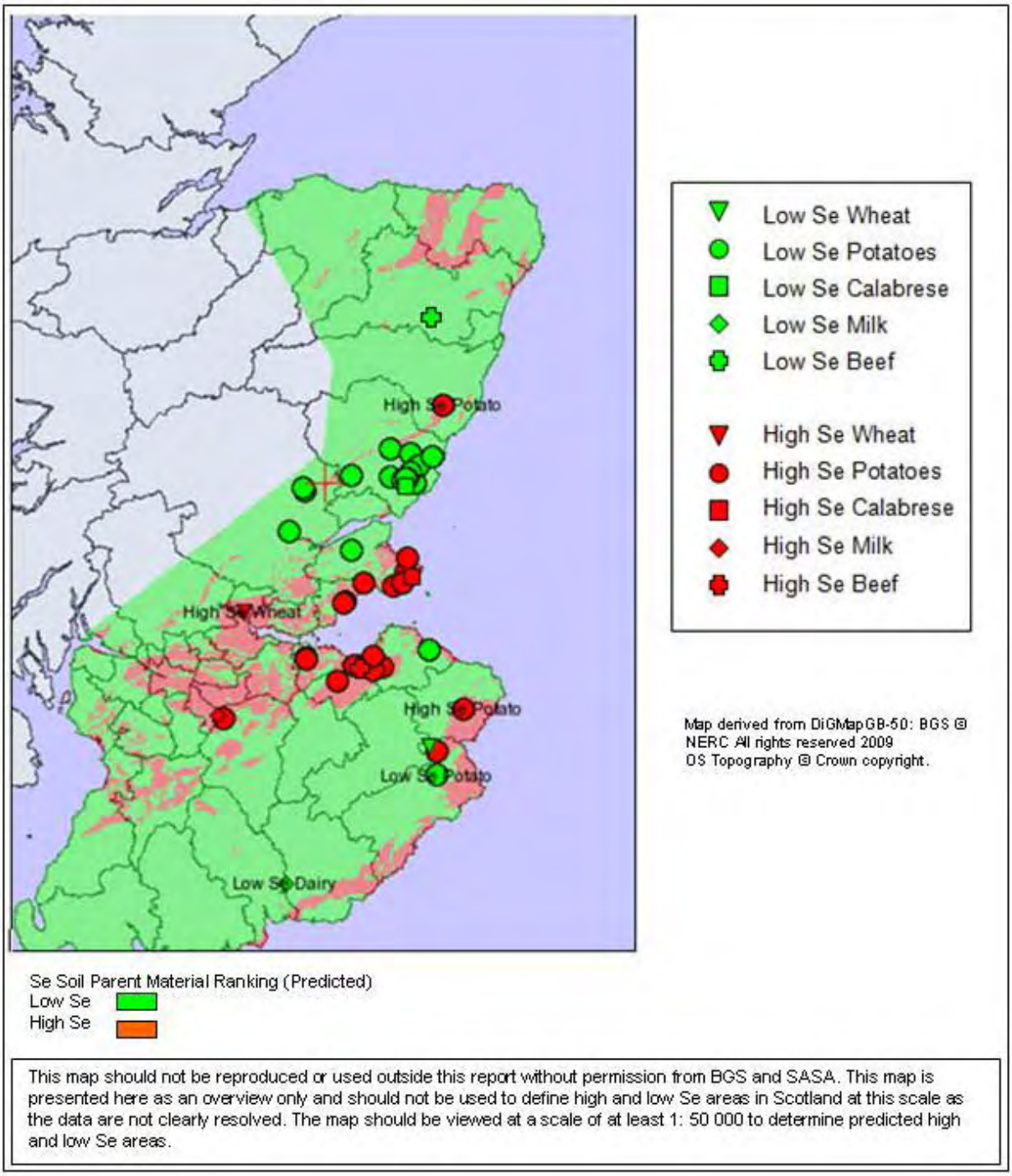
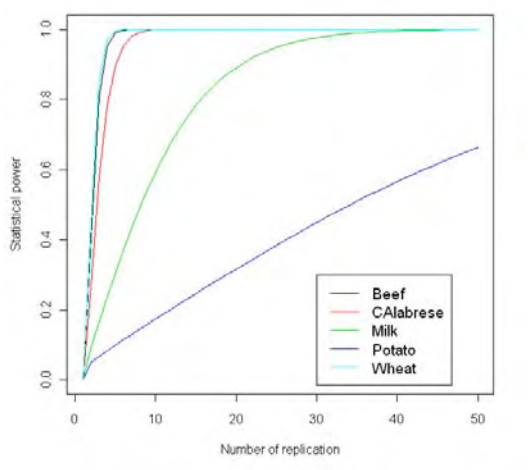
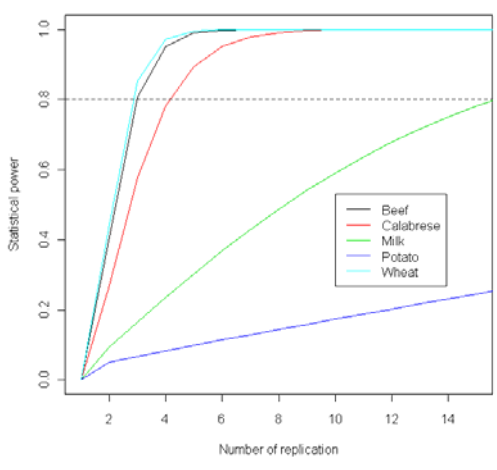


Figure 2. Map of predicted high and low-Se soils in the main agricultural growing areas of Scotland based on bedrock geology and sample sites for the present study.



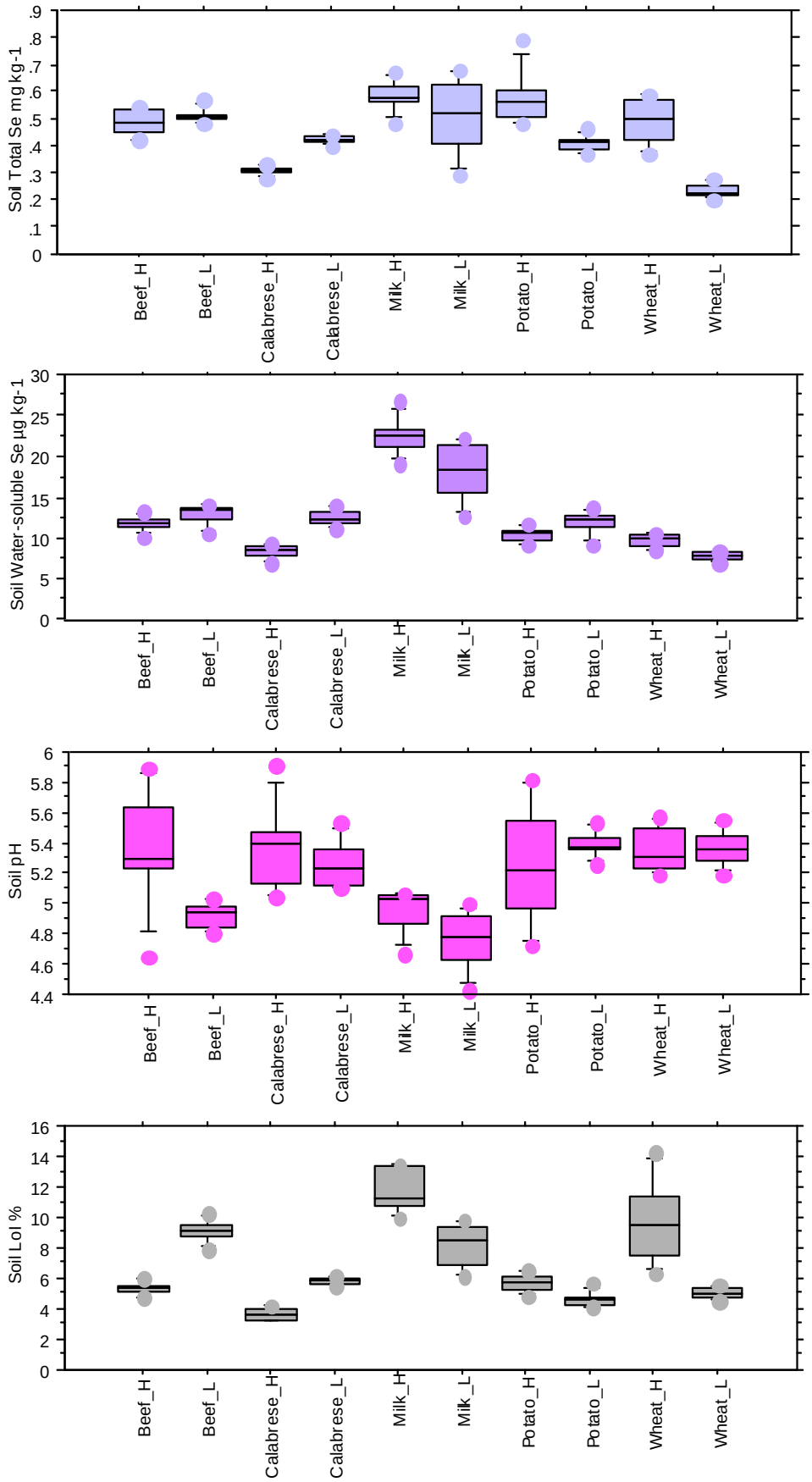
a) Full graph



b) Close-up sub-figure of a)

	Sample Size	Effect Size	Pooled SD	Statistical Power
Beef	8	37.9	12.2	**99.9%**
Calabrese	8	2.07	0.89	**99.1%**
Milk (summer)	8	2.35	2.27	48.5%
Potato	8	0.50	1.04	14.2%
Wheat	8	27.07	8.16	**99.9%**

Figure 3. Statistical power achieved per commodity for the two soil types on the basis of the eight samples in each dataset in the present study.

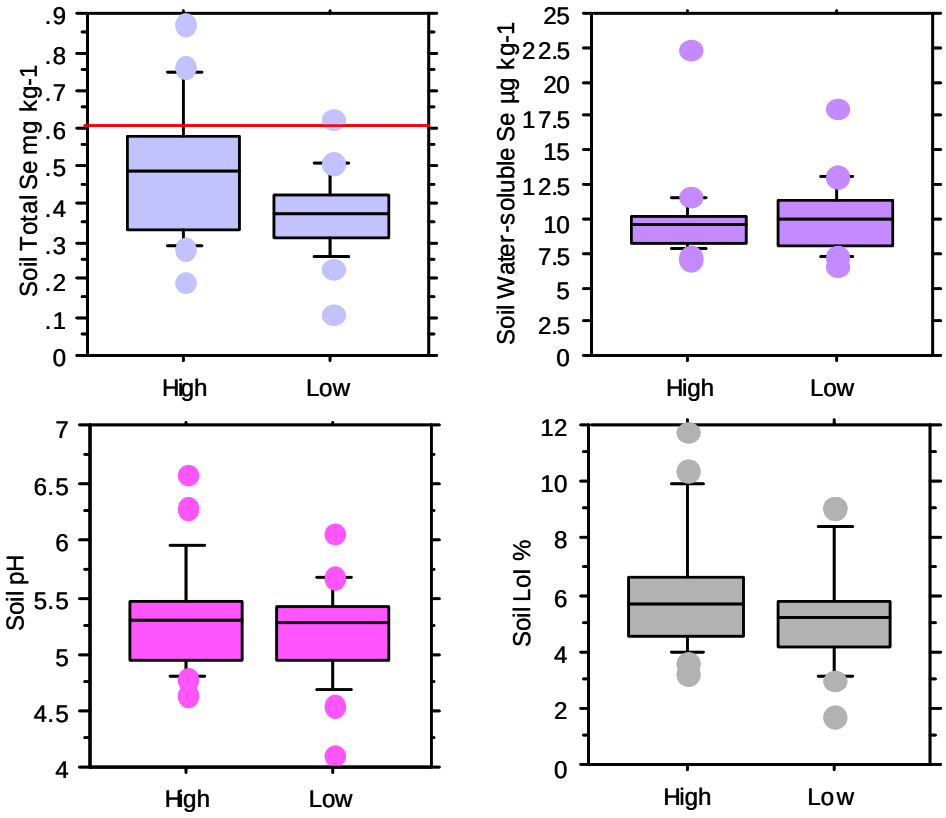


L = predicted low-Se area H = predicted high-Se area

Outliers are represented as points. N = 8 in each dataset

Box and whisker plots showing the 10th, 25th, 50th, 75th and 90th percentiles of the data distributions.

Figure 4. Box and whisker plots of the within-farm soil analytical results.



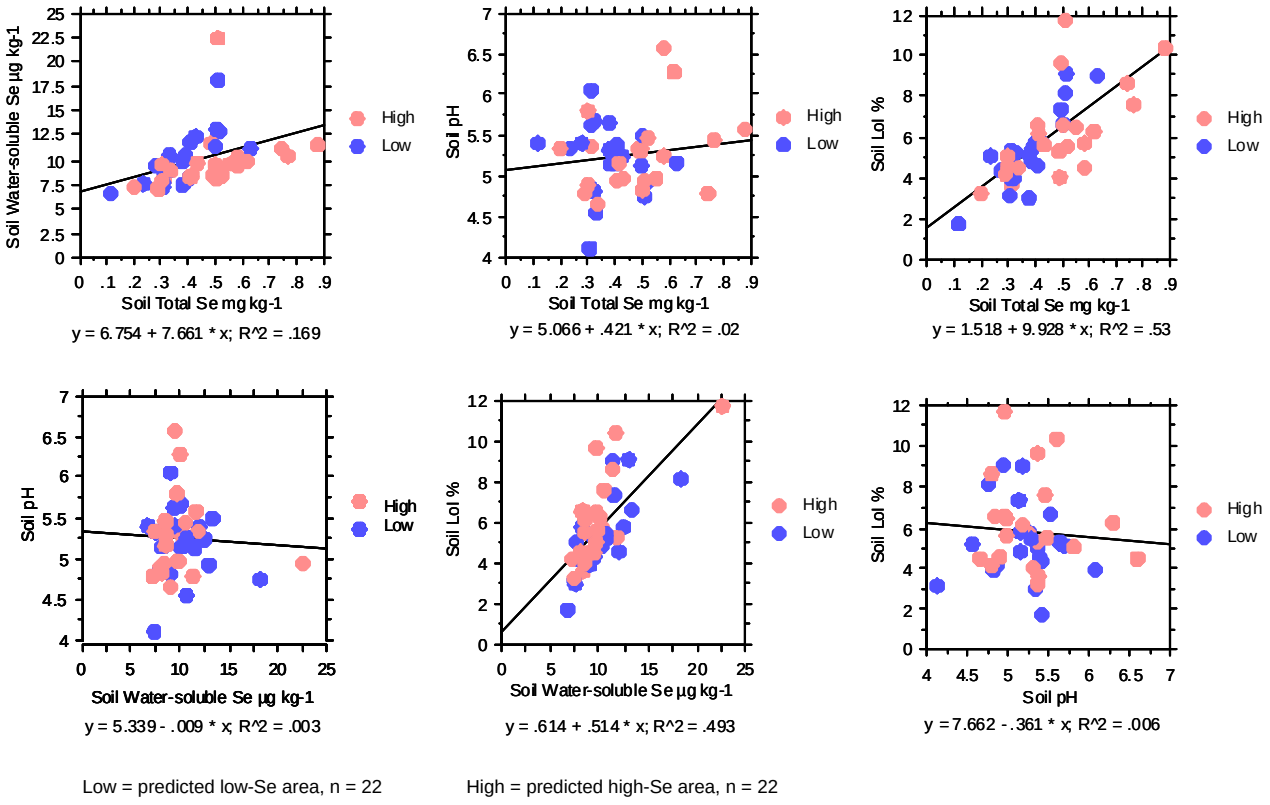
Low = predicted low-Se area
Outliers are represented as points.

High = predicted high-Se area
Soil-Se Deficiency threshold

Box and whisker plots showing the 10th, 25th, 50th, 75th and 90th percentiles of the data distributions.

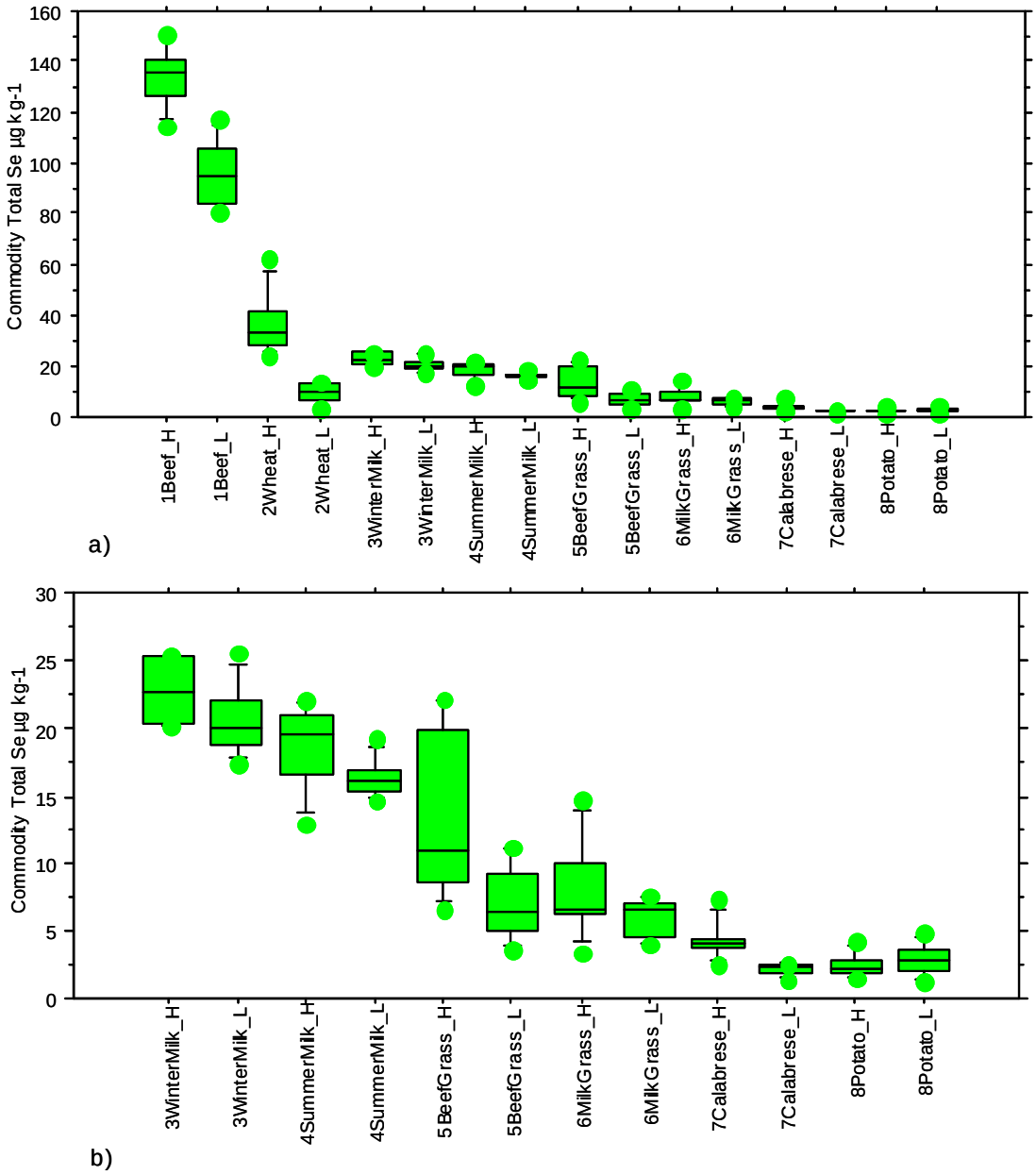
Analysis	Low-Se Area					High-Se Area				
	Count	Minimum	Maximum	Mean	Median	Count	Minimum	Maximum	Mean	Median
Total Soil-Se mg kg^{-1}	22	0.115	0.626	0.372	0.373	22	0.199	0.877	0.484	0.491
Water-soluble Soil-Se µg kg^{-1}	22	6.69	18.17	10.20	10.01	22	7.17	22.51	9.88	9.53
Soil pH	22	4.11	6.06	5.21	5.27	22	4.66	6.59	5.28	5.29
Soil LOI %	22	1.71	9.15	5.33	5.15	22	3.26	11.76	6.21	5.67

Figure 5. Box and whisker plots and summary statistics of the between-farm soil analytical results.



Low = predicted low-Se area, n = 22 High = predicted high-Se area, n = 22

Figure 6. Linear regression plots of soil parameters in the between-farm dataset



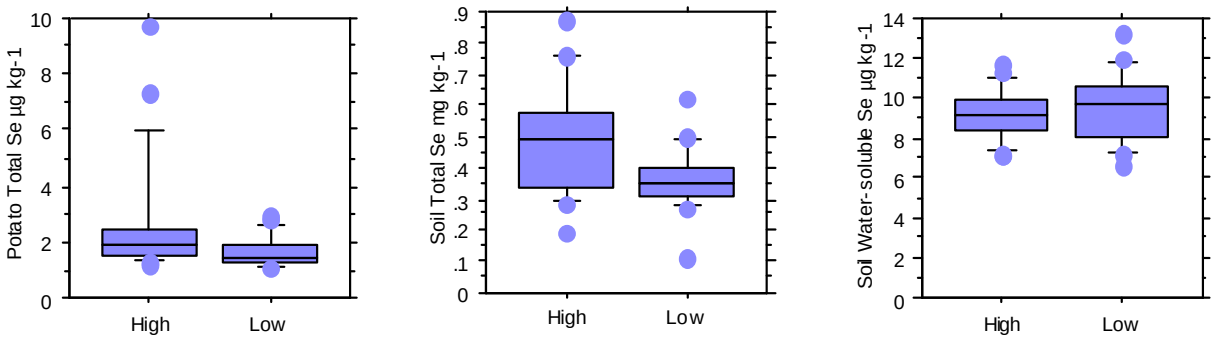
a) All commodities b) Low-Se concentration commodities

L = predicted low-Se area H = predicted high-Se area n = 8 in each dataset

Commodity results reported fresh weight Outliers are represented as points.

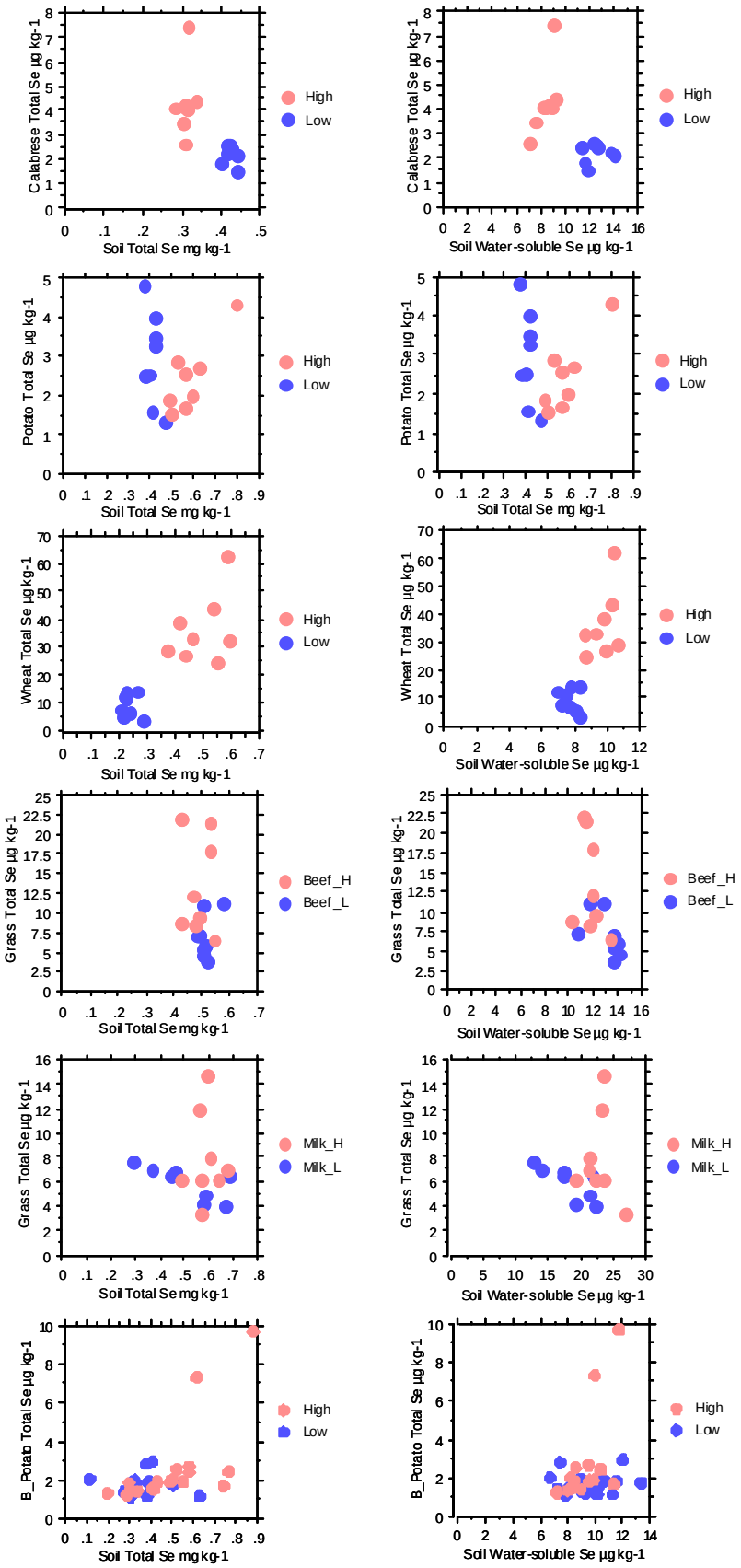
Box and whisker plots showing the 10th, 25th, 50th, 75th and 90th percentiles of the data distributions.

Figure 7. Box and whisker plots of commodity total Se concentrations in the within-farm dataset.



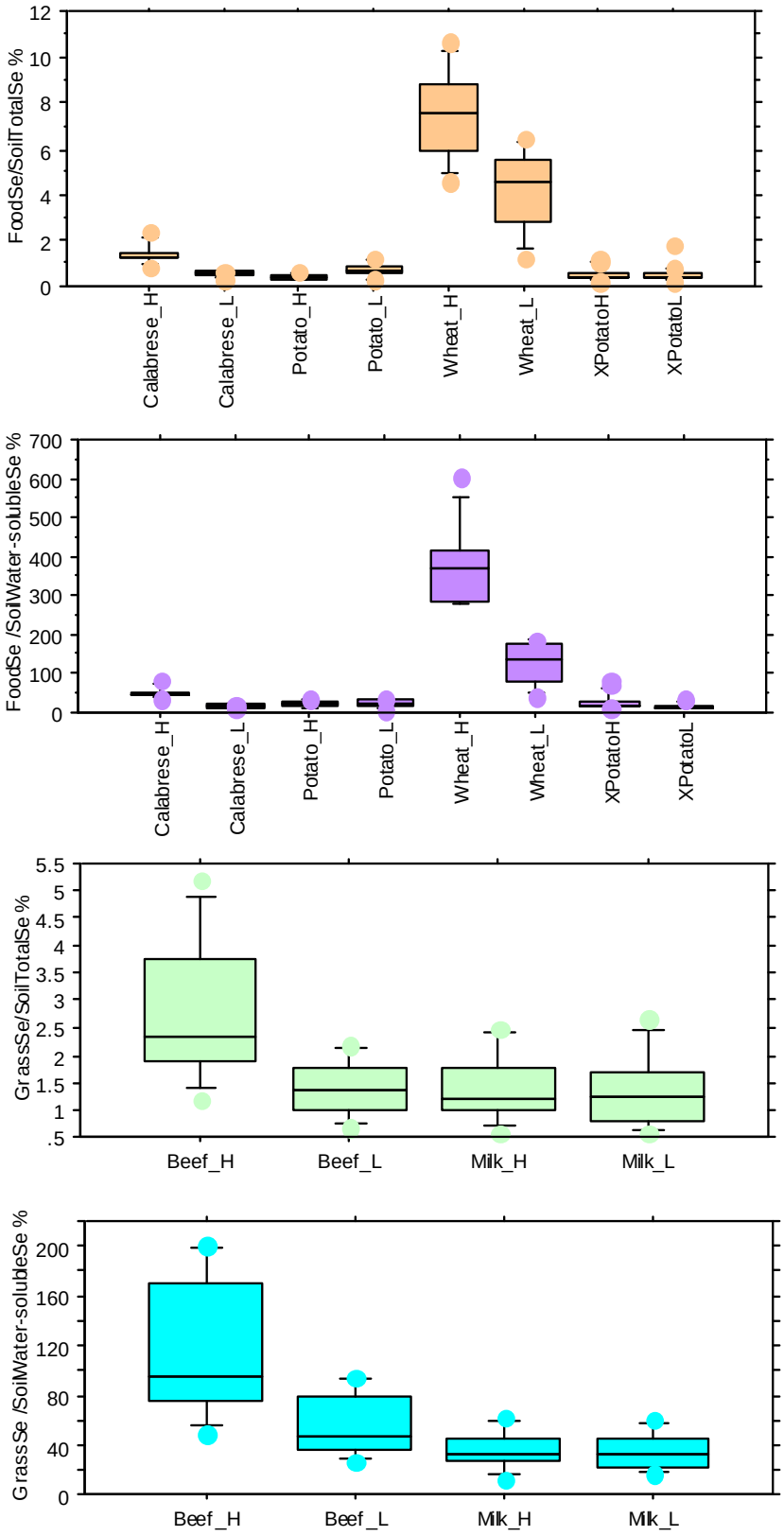
Low = Low-Se area, n = 18 High = High-Se area, n = 18 Potato results reported fresh weight
Box and whisker plots show the 10th, 25th, 50th, 75th and 90th percentiles of the data distributions.
Outliers are represented as points.

Figure 8. Box and whisker plots of potato and associated soil-Se concentrations in the between-farm dataset.



Low = predicted low-Se area, n =8 High = predicted high-Se area, n = 8
Beef_L = Low-Se beef farm Beef_H = High-Se beef farm Milk_L = Low-Se milk farm Milk_H = High-Se beef farm
B_Potato = between-farm potato dataset: n Low = 18; n high = 18 Grass and food results reported fresh weight

Figure 9. Plots of food and grass Se concentration versus total and water-soluble soil-Se concentration collected at the same locations.



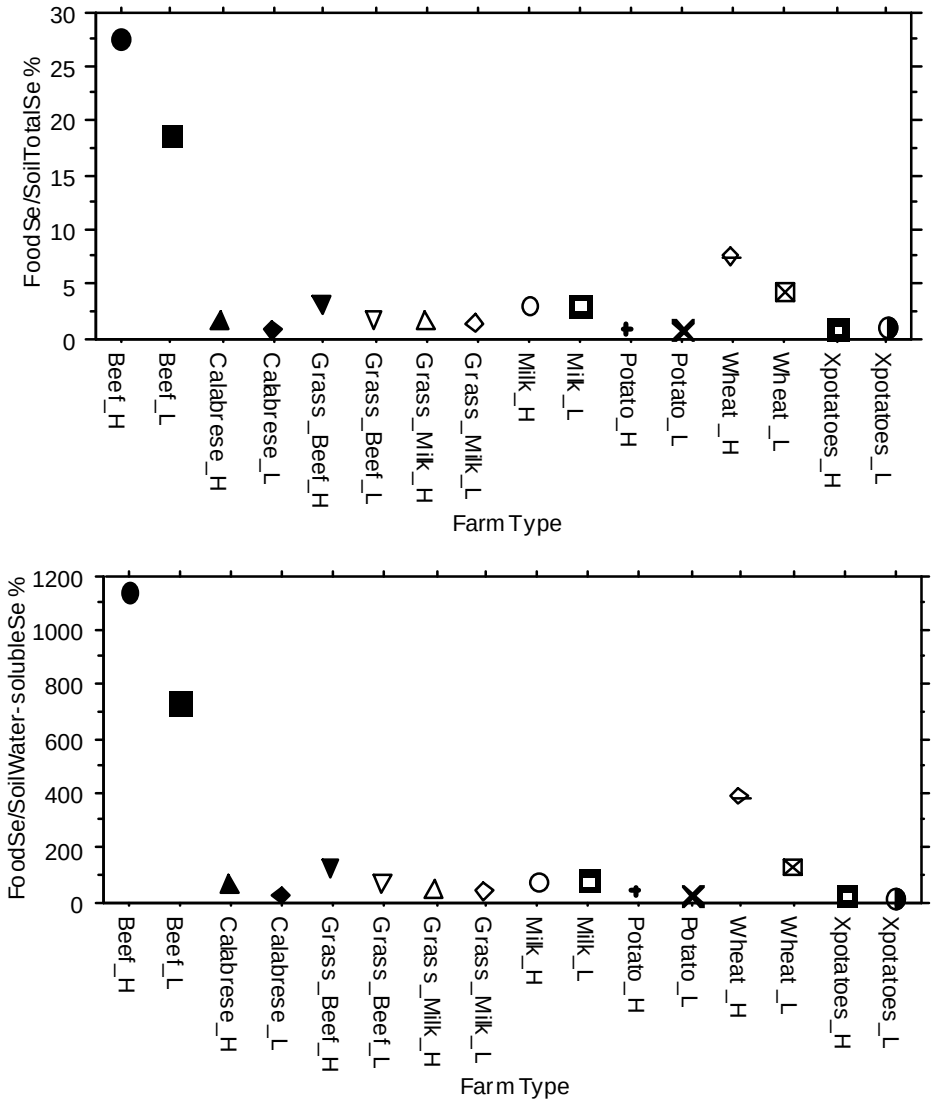
L = Low-Se area, n = 8 H = High-Se area, n = 8

XPotatoL = Low-Se between-farm samples, n = 18 XPotatoH = High-Se between-farm samples, n = 18

Box and whisker plots show the 10th, 25th, 50th, 75th and 90th percentiles of the data distributions.

Outliers are represented as points.

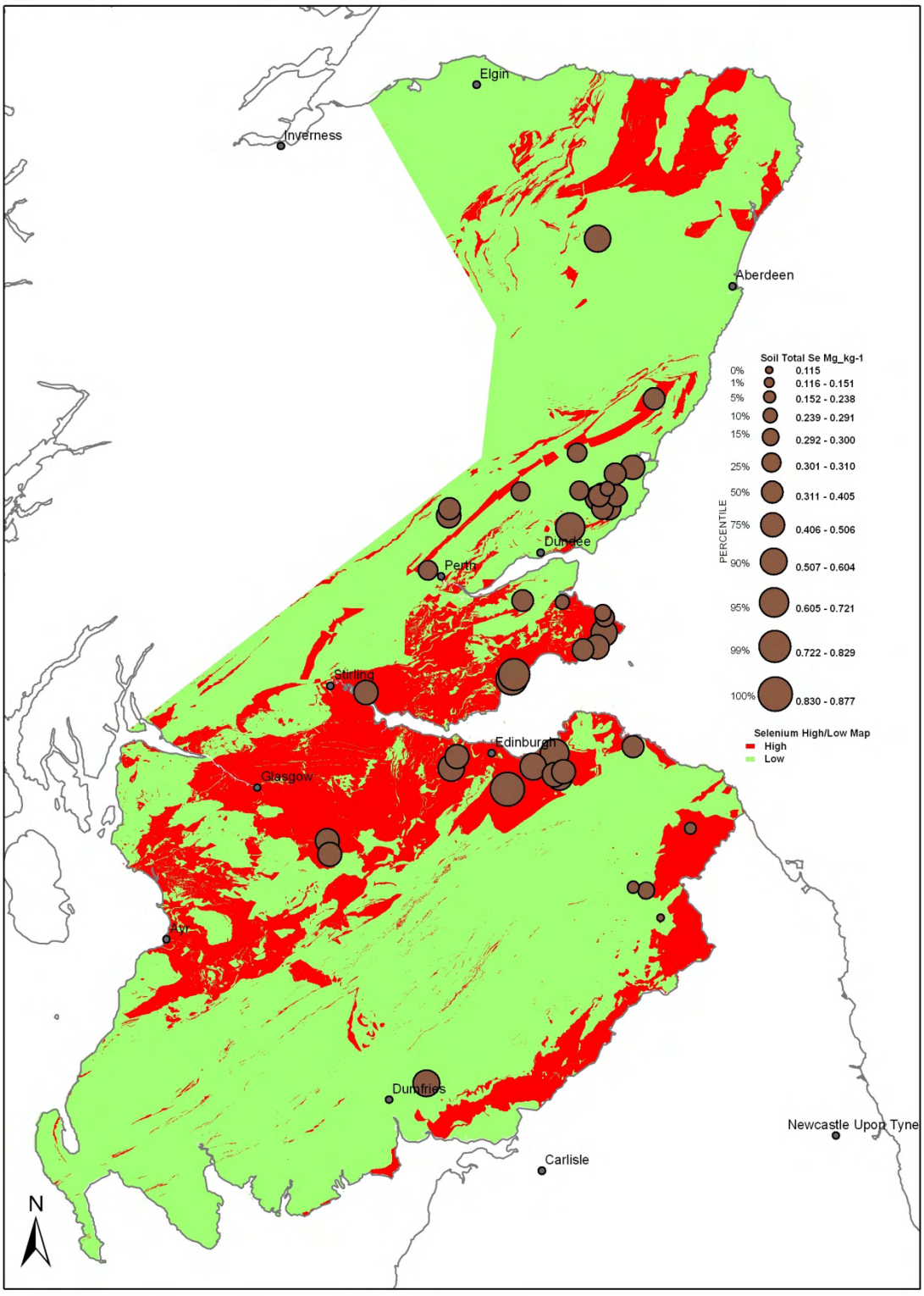
Figure 10. Box and whisker plots of the ratios of grass, calabrese, potato and wheat total Se concentrations to total and water-soluble soil-Se concentrations in their associated soil samples collected at the same locations.



L = Low-Se area H = High-Se area XPotatoes = between-farm potato samples

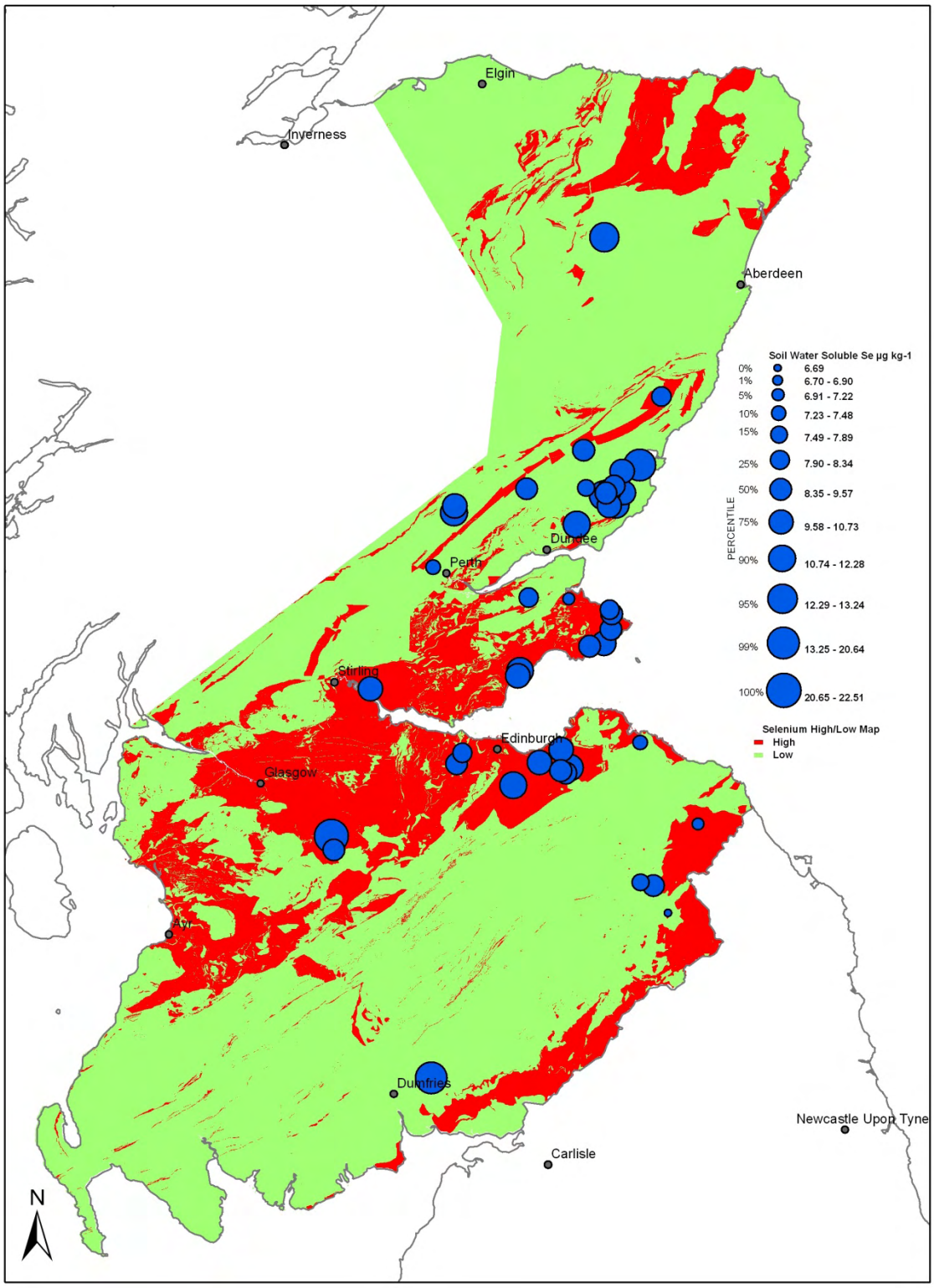
Each point = ratio of average of 8 in each dataset, except XPotatoes, n = 17 in each dataset

Figure 11. Plots of the ratios of average grass and food commodity total Se concentrations to average total and water-soluble soil-Se concentrations for each of the within-farms datasets.



N = 44. Results for the eight samples on each of the within-farm locations have been averaged and assigned a grid reference in the centre field where they were collected. Results are plotted on predicted high and low-Se areas.

Figure 12. Graduated symbol map of total soil-Se concentrations determined for the present study.



N = 44. Results for the eight samples on each of the within-farm locations have been averaged and assigned a grid reference in the centre field where they were collected. Results are plotted on predicted high and low-Se areas.

Figure 13. Graduated symbol map of water-soluble soil-Se concentrations determined for the present study.

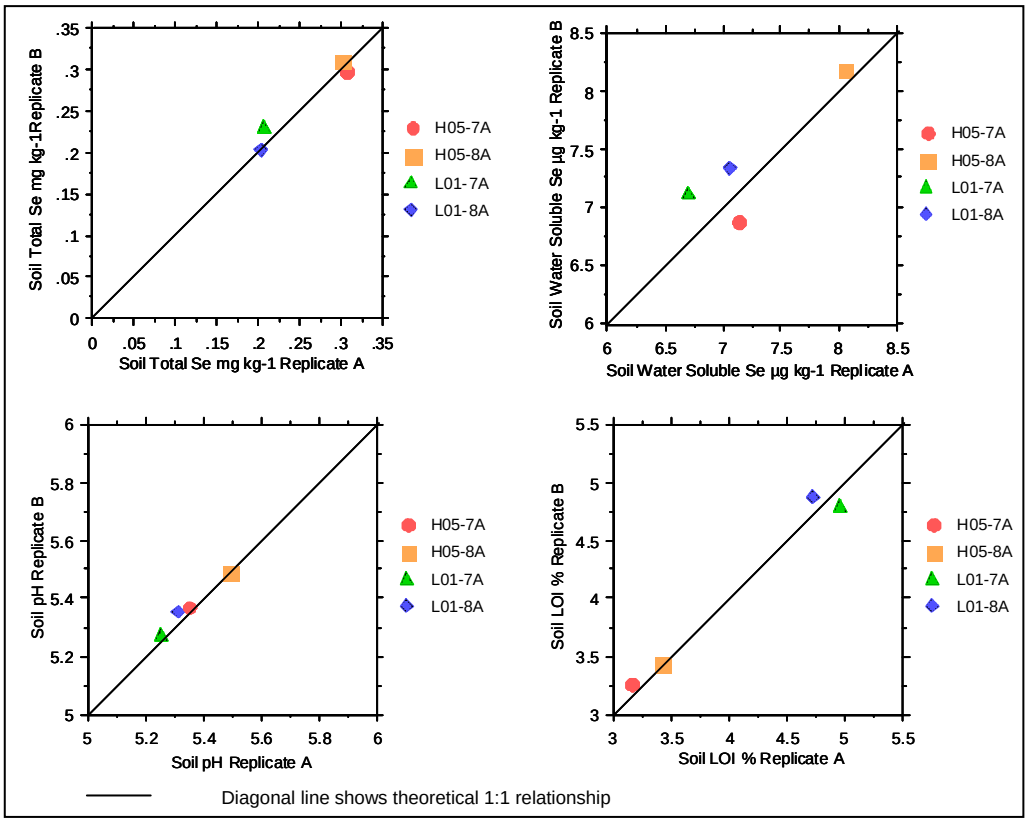


Figure 14. Plot of replicate soil sample analyses

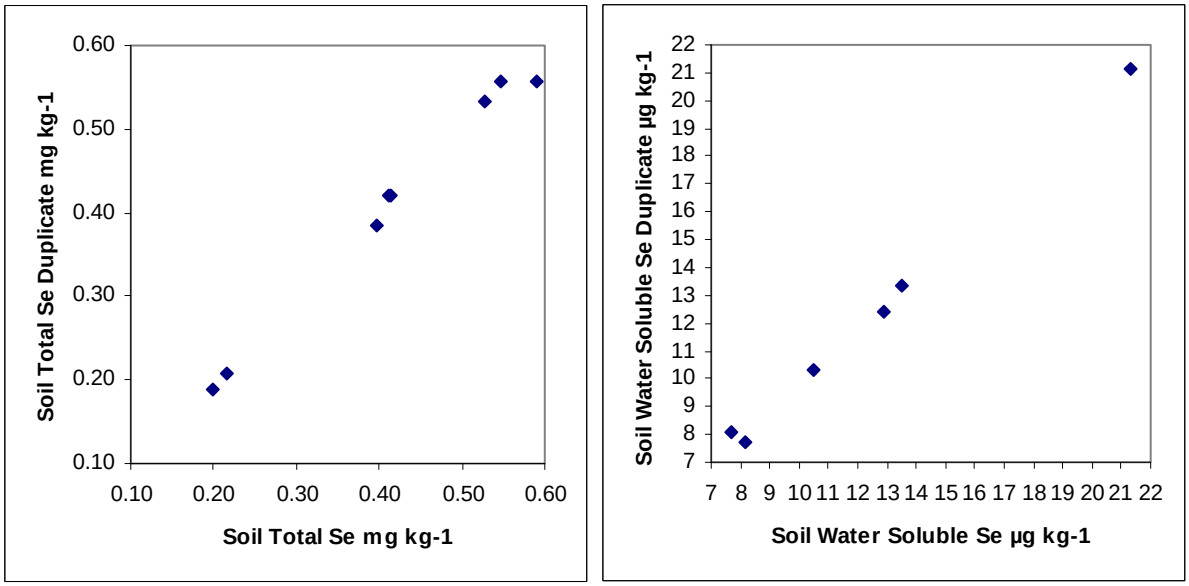


Figure 15. Plots of soil ICP-MS duplicate analyses.

Appendix 2 **Soil Sampling Protocol for the Se in Scottish Soil and Food Products Project - S14042**

1. Sampling Design

The aim of the project is to collect the following suite of samples:

Within-farm Variability Sampling

High (H) Soil-Selenium Setting	**Low (L) Soil-Selenium Setting**
Farm-1 (H): Wheat (x 8) + soil (x 8)	Farm-6 (L): Wheat (x 8) + soil (x 8)
Farm-2 (H): Potato (x 8) + soil (x 8)	Farm-7 (L): Potato (x 8) + soil (x 8)
Farm-3 (H): Calabrese (x 8) + soil (x 8)	Farm-8 (L): Calabrese (x 8) + soil (x 8)
Farm-4 (H): Milk (summer x 8) + soil (x 8) + grass (x 8) + milk (winter x 8)	Farm-9 (L): Milk (summer x 8) + soil (x 8) + grass (x 8) + milk (winter x 8)
Farm-5 (H): Beef (x 8) + soil (x 8) + grass (x 8)	Farm-10 (L): Beef (x 8) + soil (x 8) + grass (x 8)

Between-farm Variability Sampling

High (H) Soil-Selenium Setting	**Low (L) Soil-Selenium Setting**
17 Farms (H): Potato (x 1 composite per farm) + soil (x 1 composite per farm)	17 Farms (L): Potato (x 1 composite per farm) + soil (x 1 composite per farm)

The aim of the project is to collect:

1. Soil and grass samples from the fields grazed by the cattle producing the beef and milk samples for the project

2. Soil and vegetation samples together from within the same field on each farm

2. Equipment

2.1 Equipment Provided By BGS

- Handheld Dutch soil sample augers with a 1 m shaft and 0.15 m auger head
- Kraft™ paper soil sample bags
- Sample storage crates
- Soil Sampling Protocol
- Sample Numbering Scheme
- Plastic self seal bags (1 per farm) to lay the soil samples on for assessment and for mixing the between-farm composite samples from potato fields

2.2 Equipment Provided by SASA

- Global Positioning Systems (GPS)
- Mobile phones
- Selenium Soil and Food Products Questionnaire and Sampling Record Sheet
- Pens including permanent marker pens for labelling up soil bags
- Maps
- Health and Safety field equipment including weather-proof clothing normally adopted for farm surveys
- Non-powdered nitrile gloves
- Large bag or rucksack to transport samples from field to vehicle
- Lab towel and bottle of water to make soil texture assessments and clean hands between samples
- Grass clippers

2.3 Equipment Provided by Fera

- Food Sample Numbering Labels
- Food Sampling Protocol
- Food and Grass Sampling Kit

3. Farm Information Database

- The information documented on the Sampling Record Sheet and the Selenium Soil and Food Products Questionnaires should be entered into an Excel Spreadsheet database to record the details for each sample site at the end of sampling.

- This information will be merged with the chemical data generated for each sample to form the overall project database.

4. Soil Sampling Strategy for Within-farm Variability

4.1 Site Selection for Soil and Food Samples from Vegetation Production

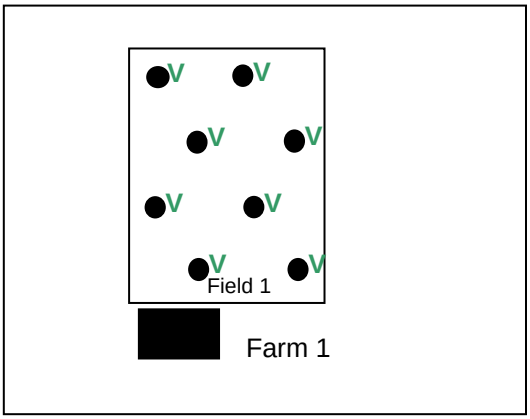
- Vegetation Soil and Food Samples - a field growing the correct variety must be (where possible) selected for sampling.

- The same variety of vegetable must be collected in both the low and high-Se settings.

- All eight soil and food samples must be (where possible) collected from one field.

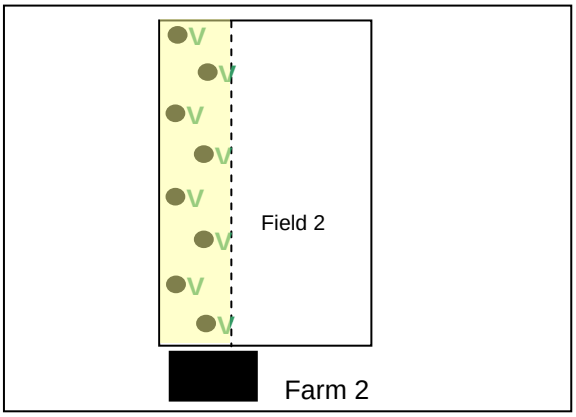
4.1.1. Calabrese and Potato Soil and Food Samples

- These crops are likely to be grown in one field or in strips in a field. If the crop is grown in one field, the eight soil and food samples should be collected from as wide a spread as possible in the field (Figure A (i)). If the crop is grown in strips within a field, the eight soil and food samples should be collected from a wide spread in each strip (Figures A (ii) and A (iii)).



● = Soil sample site V = Vegetation sample site

Figure A (i) Ideal soil and vegetation sampling design with 8 samples taken from 1 field



Samples are spaced as wide apart as possible within each field

Figure A (ii) Ideal soil and vegetation sampling design where 8 samples have to be taken in 1 strip in 1 field

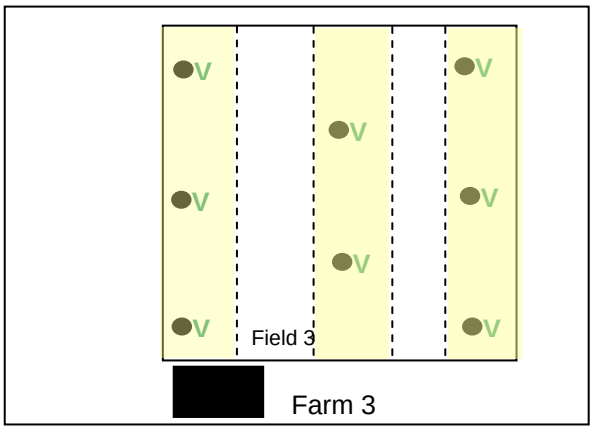


Figure A (iii) Ideal soil and vegetation sampling design with 8 samples collected from several strips in 1 field.

4.1.2. Wheat Soil and Food Samples

- Wheat is likely to be grown in one field therefore the eight soil and wheat samples must be (where possible) collected from as wide a spread as possible in the field.

- Where the crop has been harvested this can be done on a systematic basis across the field. (Figure A (i)).

However, if the crop is standing in the field at the time of sampling, this design should be modified so that soil and wheat samples are collected from along the tram lines in the field to avoid damaging the crop (Figure A (iv)).

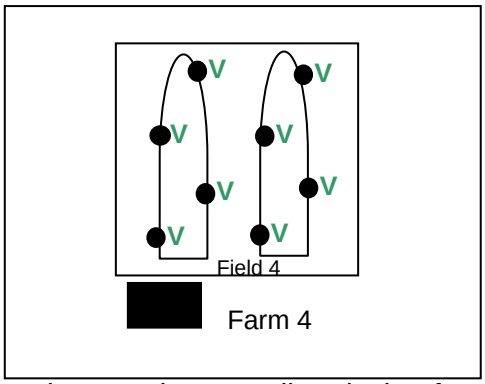


Figure A (iv) Ideal soil and vegetation sampling design for standing crops. 8 samples are taken from the tram lines in 1 field.

4.2. Site Selection for Soil Samples from Milk Production

- Milk Soil Samples – the field grazed by the cattle within the previous 24 hours must be (where possible) sampled.
- The eight soil and associated grass samples should be collected from one field from as wide a spread as possible in the field (Figure A (i)).

4.3. Site Selection for Soil Samples from Beef Production

- Beef Soil Samples – the fields grazed by the cattle within no more than the previous six months (ideally 3 months) must be (where possible) sampled.
- Ideally, the fields grazed as close as possible to the time of indoor finishing or slaughter should be sampled.
- The eight soil and associated grass samples must be (where possible) collected from the area grazed by the cattle even if this comprises more than one field.
- If fields grazed by the cattle are adjacent, field boundaries may be effectively ignored to get as wide a spread as possible in the fields (Figure A (v)).

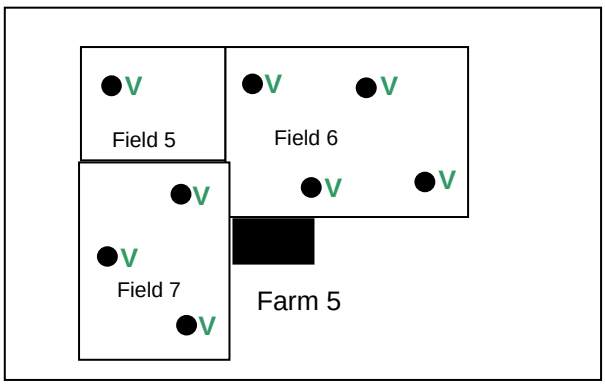


Figure A (v) Ideal soil sampling and associated grass sampling design for several fields were beef cattle have grazed.

5. Soil Sampling Strategy for Between-farm Variability

- Eight soil sub-samples (combined into one composite sample) and eight potato samples (collected to form one composite sample in the laboratory) should be collected from the same field on each farm.

- A field growing the correct variety must be (where possible) selected for sampling.

- The same variety of potato must be collected in both the low and high-Se settings.

- Potatoes are likely to be grown in one field or within one or several strips in a field. The samples must be (where possible) collected from the largest area of production on the farm whether this is a whole field; from the largest strip of several strips within a field or from one strip within a field.

- Each of the eight potato samples from a field should be collected to be combined into one sample later in the laboratory. In contrast, the soils collected from the base of each of the eight potato plants sampled in a field should be mixed at site on the plastic self seal bag provided to form one composite soil sample from the field.

- The aim is to collect one composite soil sample that is representative of the soil present at the base of each of the eight individual potato plants sampled in a field. However, not all the material from the eight sub-samples will fit into the soil collection bag.

- **Therefore it is very important that the eight soil sub-samples are mixed as thoroughly as possible prior to collection to provide a representative composite sample from which material can be used to fill the soil collection bag.**

All soil samples should be collected according to the procedures outlined in Section 6, which are modified from Johnson (2005).

6. Soil Sampling Procedures

- At each site, a top soil sample (0.05 – 0.20 m depth) (which is the plant root zone) of approximately 0.5 kg of material (a bag of sugar) must be (where possible) collected.
- Samples are collected using a handheld Dutch auger into Kraft™ paper soil sample bags as follows.

6.1. Avoiding Contamination

- **To avoid contamination hands must be clean and free from jewellery, plasters, sun screen or any hand creams or lotions.**
- **Hands should be cleaned with lab towel and water between the collection of each soil sample**
- **Samples must be (where possible) collected away from obvious sources of contamination including wire fences and roads and should avoid**

waterlogged areas. If these areas must be sampled this information should be recorded on the Sampling Record Sheet.

6.2 Sample Labelling

- The number of each sample should written on the Sampling Record Sheet and then on the soil bag with a permanent ink pen. Soil samples are coded A to indicate a top soil or 'A horizon' soil sample is being collected.

6.3 Soil Collection

6.3.1. General

- For the within-farm sampling the eight samples of soil collected should always be treated as independent samples and should never be made into composites.

- For the between-farm sampling of potato farms, the eight sub-samples of soil collected at the base of each potato plant should be mixed very thoroughly on the self seal plastic bag provided to make one composite sample for each field.

- **On arrival at a site within a field an initial auger sample should be collected and discarded so as to "clean" the auger head with soil from that site.**

- The auger should be vertical when used.

- Generally in pasture and some other fields a rootlet layer may extend down to 0.05 m at the very top of the soil, this to be avoided during collection as it is organic material not soil. In ploughed fields this organic rootlet layer may be absent but there may be vegetation litter at the surface. Therefore, at each auger hole, the auger is rotated down approximately 0 – 0.05 m to remove the surface vegetation and surface litter and roots, which are then discarded.

- The soil is collected below this layer to a depth of ~ 0.20 m from the same hole by rotating the auger head down into the hole until the top of the auger head is in line with the soil surface. With a 0.15 m auger head the sampling depth can be generalised to 0.05 to 0.20 m.

- The bottom depth of ~ 0.20 m is recorded on the Sampling Record Sheet.

- In the unlikely event that the soil is < 0.20 m deep, record the depth to which the soil sample can be taken.

- In sandy or dry soils, to avoid loss of the soil material from the auger, rotate the auger several times in the hole at the correct depth to pack the soil into the auger head.

- In all instances, every auger sample should be inspected when drawn out of the hole and extraneous material (weeds *etc*.) on the peripheries of the auger head should be removed and discarded.

- For the within-farm sampling, each of the eight soils within a field should be inspected by placing on a self seal plastic bag, to record colour, texture, organic matter, contamination and moisture for each one according to the schemes laid out in Section 6.4 and Sections 6.3.2 and 6.3.3 before collection in the paper sample bag. These observations should be noted on the Sampling Record Sheet.

- For the between-farm potato sampling, each of the eight soil sub-samples collected from the base of each potato plant should be collected onto a self seal plastic bag and mixed together very thoroughly to form one composite sample. Observations of colour, texture, organic matter, contamination and moisture should be recorded on the composite sample according to the schemes laid out in Section 6.4 and Sections 6.3.2 and 6.3.3 before collection in the paper sample bag. These observations should be noted on the Sampling Record Sheet.

    - Colour and soil moisture content are subjective assessments (Section 6.4). The organic matter content is also a subjective assessment (Section 6.4). Organic matter will appear in soils as black flecks through the soil; black carbonaceous lumps of material or as peaty compost-like material.

    - **Texture must be assessed with bare hands** using a standard soil survey scheme by feeling the soil between the fingers (Section 6.4). If the soil is not already reasonably damp, wet it before making the assessments using the bottle of water carried in the field.

    - It is important to note if no contamination is present

- Enough soil must be collected at each soil sampling site to fill the paper soil sampling bag according to the methods in Sections 6.3.2 and 6.3.3.

- The type of field from which the samples are collected (*e.g.* ploughed, harvested *etc*.) must be recorded on the Sampling Record Sheet. The field use may have changed since the commodities of interest were grown on it. Please note on the Sampling Record Sheet if fields are ploughed, harvested, replanted with a different crop *etc*. The mixture of crops in a field should also be noted on the Sampling Record Sheet.

- At the completion of each sample, the auger should be cleaned with the hand or scraped with the fork to remove any soil sticking to it before moving to the next sampling site.

- The soil sample bags should be sealed by folding over the top of the bag 3 times towards the back of the bag and closing the tabs towards the front. On completion of collection, the samples should stored upright in the crates provided until return to BGS. Samples should be allowed to air dry in the crates at temperatures of < 30^{o}C to avoid the volatilisation of Se. Normal climatic conditions mean that this temperature is unlikely to be exceeded. However, if the weather is particularly warm during sampling, the windows of vehicles used to store the soil samples during collection should be left open to avoid excess temperatures.

- Sometimes in very wet conditions, the paper soil bags can deteriorate and start to fall apart, in these cases re-bag the sample into a newly labelled sample bag and store as normal.

6.3.2. Collection of Soil Samples from Calabrese, Potato and Wheat Production

- Soils should be collected from the base of the plants from which vegetation samples have been taken.

- For the within-farm sampling, it may take four or five auger holes of 0.05 – 0.20 m material from the root zone underneath and around the base of the plant to fill the sample bag. The sample bag must be filled to give enough sample material. In

sandy soils it may take more auger-fulls to fill the bag, in clay soils it may take less.

- For the within-farm sampling, in order to carry out the observations on the soil, place each of the auger-fulls of soil on a self seal plastic bag and mix them. Then test soil texture, colour, organic matter content, moisture content and note any contamination present in the soil. Transfer the soil to the paper collection bag.

- For the between-farm sampling one auger-full of soil should be collected from each of the eight potato plants sampled in a field. This should give enough material to fill the soil collection bag regardless of the soil type. These eight sub-samples should be placed on a self seal plastic bag and mixed very thoroughly to form one composite sample. **It is important to mix this material thoroughly into a composite sample before collection as not all the material from the eight sub-samples will fit into the soil collection bag.** Then test soil texture, colour, organic matter content, moisture content and note any contamination present in the soil. Transfer the soil to the paper collection bag.

- To avoid contamination, a new self seal plastic bag should be used on each farm. For the within-farm sampling of eight soils from one field, it is acceptable to wipe down the self seal plastic bag with lab towel and water before moving to the next soil sampling site within the field.

- In the case of potato samples, the potatoes are likely to be dug up and collected before the soil sample. Therefore, the soil should be taken from material as close as possible to the 0 – 0.20 m root zone despite being disturbed.

- For the within-farm sampling, the national grid reference cited on the Sampling Record Sheet should be taken at each of the eight sampling sites using the GPS.

- For the between-farm sampling, the national grid reference cited on the Sampling Record Sheet should be at the potato plant at the centre of the area from which the potato plants have been selected for analysis. This may be the centre of a field, or the centre of a strip within a field.

- If the crops have already been harvested, food samples may have to be collected from storage facilities on the farm. In this case it will not be possible to relate the plants directly to the soil samples. Instead, soil samples should be collected using the 2 m square five auger-hole sampling plan described in Section 6.3.3 for Beef and Milk samples (See Figure B).

- In the case of calabrese and potato samples, soils should be collected from the strip or strips of land or whole field where the crop was grown (See Figures A(i), A1(ii) and A(iii)). Where potatoes and wheat were grown in more than one field, soil samples should be collected from the main or largest field where the crops taken for analysis were grown.

6.3.3. Collection of Soil and Associated Grass Samples from Beef and Milk Production

- **Avoid collecting soil and grass samples next to cow pats as these will contaminate the samples.**

- In this instance each of the eight soil samples collected in a pasture field or group of fields is made up of a composite of material from five auger holes located at the corners and centre of a 2 x 2 m square at each site (See Figure B). Grass

samples should be collected from within the same 2 x 2 m square from which the soil samples have been taken.

- In order to carry out the observations on the soil, place each of the five auger-fulls of soil on a self seal plastic bag and mix them. Then test soil texture, colour, organic matter content, moisture content and note any contamination present in the soil. Transfer the soil to the paper collection bag.

- If good full auger heads are collected from each of the five auger holes (*e.g.* in clay soils) more sample may be collected than can be fitted into the sample bag. In such instances, mix the soil thoroughly on the self seal bag and fill the paper collection bag with homogenised material.

- To avoid contamination, a new self seal plastic bag should be used on each farm. For the within-farm sampling of eight soils from one field, it is acceptable to wipe down the self seal plastic bag with lab towel and water before moving to the next soil sampling site within the field.

The national grid reference cited on the Sampling Record Sheet for each of the eight sampling sites should be that of the central hole in the five-hole sampling plan at each site.

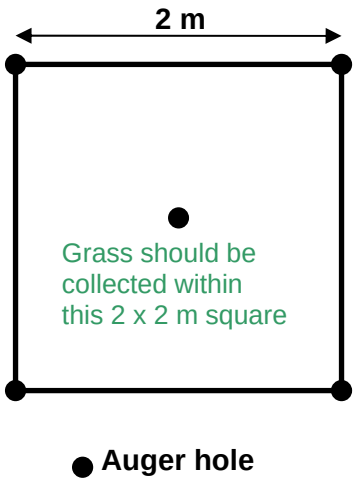


Figure B. Plan of composite auger holes for collecting a soil sample and associated grass sample from beef and dairy pasture fields.

6.4. Data Recording

The following information about the soil should be recorded on the Sampling Record Sheet:

Sample Number
From the Sample Number List

National Grid Reference
12 figure Easting and Northing grid co-ordinates taken using the GPS from either:
(i) The individual crop sampling site
(ii) The central auger hole in the five-hole sampling plan for pasture fields.

Soil Depth
Depth in m to the base of the top soil sample

Soil Texture
Sand – feels coarse and sandy, is loose
Silt – is finer than sand but still gritty when smeared and does not stick together to form a ball
Clay – is very fine and sticks together and is smooth and smears like plasticine
Sandy-Clay – is coarse but sticks together and cracks in a ball
Silty-Clay – is gritty but sticks together and cracks in a ball
Sandy-Silt – is finer than sand but coarser than silt, is gritty and loose and does not stick together

Field Type
Potato
Wheat
Calabrese
Dairy Pasture
Beef Pasture
Ploughed (please explain previous use)
Mixed (please explain crop mixture)
Harvested (please explain previous use)
Other – if the field of interest has been replanted with another unrelated crop please explain

Vegetation Sample Collected with the Soil
Potato
Wheat
Calabrese
Grass

Soil Colour
Black
Dark Brown
Light Brown
Red
Orange
Yellow
Green
Grey

Soil Contamination
Wire
Metal
Pottery
Glass
Brick
Coal/Clinker
Plastic
None – it's important to record no contamination

Soil Moisture Content
Dry
Damp
Waterlogged

Soil Organic Content
Low
Moderate
High

FSAS Se in Scottish Soil and Food Products Project S14042 Sample Number Scheme

Farm Identifier	Sample Identifier	Wheat Identifier	Beef Identifier	Milk Identifier	Potato Identifier	Calabrese Identifier	Grass Identifier	Soil Identifier	Notes
Within-farm Sampling:									
Low-Se Setting:									
L01	1	W						A	W = Wheat
L01	2	W						A	A = 'A Horizon' Top Soil
L01	3	W						A	
L01	4	W						A	
L01	5	W						A	
L01	6	W						A	
L01	7	W						A	
L01	8	W						A	
L02	1		B				G	A	B = Beef
L02	2		B				G	A	G = Grass
L02	3		B				G	A	
L02	4		B				G	A	
L02	5		B				G	A	
L02	6		B				G	A	
L02	7		B				G	A	
L02	8		B				G	A	
L03	1			M + M-W			G	A	M = Summer Milk
L03	2			M + M-W			G	A	M-W = Winter Milk
L03	3			M + M-W			G	A	
L03	4			M + M-W			G	A	
L03	5			M + M-W			G	A	
L03	6			M + M-W			G	A	
L03	7			M + M-W			G	A	
L03	8			M + M-W			G	A	
L04	1				P			A	P = Potato
L04	2				P			A	
L04	3				P			A	
L04	4				P			A	
L04	5				P			A	
L04	6				P			A	
L04	7				P			A	
L04	8				P			A	
L05	1					V		A	V = Calabrese (Vegetable)
L05	2					V		A	
L05	3					V		A	
L05	4					V		A	
L05	5					V		A	
L05	6					V		A	
L05	7					V		A	
L05	8					V		A	

Farm Identifier	Sample Identifier	Wheat Identifier	Beef Identifier	Milk Identifier	Potato Identifier	Calabrese Identifier	Grass Identifier	Soil Identifier	Notes
High-Se Setting:									
H01	1	W						A	
H01	2	W						A	
H01	3	W						A	
H01	4	W						A	
H01	5	W						A	
H01	6	W						A	
H01	7	W						A	
H01	8	W						A	
H02	1		B				G	A	
H02	2		B				G	A	
H02	3		B				G	A	
H02	4		B				G	A	
H02	5		B				G	A	
H02	6		B				G	A	
H02	7		B				G	A	
H02	8		B				G	A	
H03	1			M + M-W			G	A	
H03	2			M + M-W			G	A	
H03	3			M + M-W			G	A	
H03	4			M + M-W			G	A	
H03	5			M + M-W			G	A	
H03	6			M + M-W			G	A	
H03	7			M + M-W			G	A	
H03	8			M + M-W			G	A	
H04	1				P			A	
H04	2				P			A	
H04	3				P			A	
H04	4				P			A	
H04	5				P			A	
H04	6				P			A	
H04	7				P			A	
H04	8				P			A	
H05	1					V		A	
H05	2					V		A	
H05	3					V		A	
H05	4					V		A	
H05	5					V		A	
H05	6					V		A	
H05	7					V		A	
H05	8					V		A	

Farm Identifier	Sample Identifier	Wheat Identifier	Beef Identifier	Milk Identifier	Potato Identifier	Calabrese Identifier	Grass Identifier	Soil Identifier	Notes
Between-Farm Sampling:									
Low-Se Setting:									
L06	1				P			A	
L07	1				P			A	
L08	1				P			A	
L09	1				P			A	
L10	1				P			A	
L11	1				P			A	
L12	1				P			A	
L13	1				P			A	
L14	1				P			A	
L15	1				P			A	
L16	1				P			A	
L17	1				P			A	
L18	1				P			A	
L19	1				P			A	
L20	1				P			A	
L21	1				P			A	
L22	1				P			A	
High-Se Setting:									
H06	1				P			A	
H07	1				P			A	
H08	1				P			A	
H09	1				P			A	
H10	1				P			A	
H11	1				P			A	
H12	1				P			A	
H13	1				P			A	
H14	1				P			A	
H15	1				P			A	
H16	1				P			A	
H17	1				P			A	
H18	1				P			A	
H19	1				P			A	
H20	1				P			A	
H21	1				P			A	
H22	1				P			A	

For example the first within-farm potato sample from a high-Se soil has the identifier H041P and the associated soil sample H041A.

Appendix 3 **Food and Grass Sampling Protocol for the Se in Scottish Soil and Food Products Project S14042**

1. Sampling Design

The aim of the project is to collect the following sets of samples:

Within-farm Variability Sampling

High (H) Soil-Selenium Setting	**Low (L) Soil-Selenium Setting**
Farm-1 (H): Wheat (x 8) + soil (x 8)	Farm-6 (L): Wheat (x 8) + soil (x 8)
Farm-2 (H): Potato (x 8) + soil (x 8)	Farm-7 (L): Potato (x 8) + soil (x 8)
Farm-3 (H): Calabrese (x 8) + soil (x 8)	Farm-8 (L): Calabrese (x 8) + soil (x 8)
Farm-4 (H): Milk (summer x 8) + soil (x 8) + grass (x 8) + milk (winter x 8)	Farm-9 (L): Milk (summer x 8) + soil (x 8) + grass (x 8) + milk (winter x 8)
Farm-5 (H): Beef (x 8) + soil (x 8) + grass (x 8)	Farm-10 (L): Beef (x 8) + soil (x 8) + grass (x 8)

Between-farm Variability Sampling

High (H) Soil-Selenium Setting	**Low (L) Soil-Selenium Setting**
17 Farms (H): Potato (x 1 composite per farm) + soil (x 1 composite per farm)	17 Farms (L): Potato (x 1 composite per farm) + soil (x 1 composite per farm)

2. Equipment

2.1 Supplied by SASA

Linen sampling bags.
Paper sampling bags.
Cutting tools and spades

2.2 Supplied by Fera

Sample numbering labels (4 sets)
Plastic acid-clean bottles (125 ml capacity).
Plastic sealable bags.

3. Food Sampling Procedure

3.1 Wheat Samples

- Ears of wheat from a single plant will be collected and this will constitute a 'sample'. A minimum of 3 g of grain is required for analysis. 16 samples will be taken in total from 16 different plants (8 from each soil-Se setting).

- The wheat grains will be separated from the chaff and the chaff discarded.

- If there is moisture present on the grains due to rain for example, the grain should be left to dry on a clean, dust-free surface.

- Grain samples should then be transferred to labelled linen bags and stored in a cool, dry environment.

- **Duplicate samples:** a duplicate of each sample will be collected and retained by SASA until notification from Fera that the original samples have been received. The duplicates will be taken from the same plants as the original samples. If this is not possible, they should be taken from the adjacent plants so that soil conditions are comparable.

3.2 Brassica Samples

- A single head of calabrese for example constitutes a 'sample'. A minimum of 25 g is required for analysis. 16 samples will be taken in total from 16 different plants (8 from each soil-Se setting).

- Inedible parts of each sample will be removed (*e.g.* damaged or dead leaves, old outer leaves, woody stems). If necessary, any soil on the remaining plant should be rinsed off with tap water, shaken to remove excess water and carefully blotted dry using clean paper towel.

- The samples should then be transferred to labelled linen bags and stored at $^{+}4$°C.

- **Duplicate samples:** a duplicate of each sample will be collected and retained by SASA until notification from Fera that the original samples have been received. The duplicates will be taken from the same plants as the original samples. If this is not possible, they should be taken from the adjacent plants so that soil conditions are comparable.

3.3 Potatoes (within-farm and between-farm variation)

- For the within-farm variability study, a single tuber from a plant constitutes a 'sample'. 16 samples will be taken from 16 different plants (8 from each soil-Se setting). A minimum sample size of 25 g is required for analysis.

- The potatoes should be brushed free of soil, washed in tap water and blotted dry using clean paper towel.

- Samples should then be transferred to labelled paper bags and stored in a cool, dry environment.

- For the between-farm variation study, 8 samples will be collected from each soil-Se setting, as described above. In this case however, each set of 8 will be

combined to constitute a single sample per farm. There will be a total of 34 composite samples collected, each consisting of 8 tubers.

- **Duplicate samples:** a duplicate of each sample will be collected and retained by SASA until notification from Fera that the original samples have been received. The duplicates will be taken from the same plants as the original samples. If this is not possible, they should be taken from the adjacent plants so that soil conditions are comparable.

3.4 Milk (summer and winter)

- 16 samples (60-100 ml each) will be taken from 16 different cows during August (8 from each soil-Se setting). These samples constitute 'Summer Milk'. The same farms will be used to collect samples of milk during December and these samples will constitute 'Winter Milk' (16 samples in total).

- Where possible, the sample should be taken after the teat has been rinsed with tap water to remove any dirt or teat wash that could contaminate the milk.

- Samples should be collected in labelled acid clean plastic bottles (125 ml capacity), filling the bottles to just below the shoulder to allow room for expansion during freezing.

- The milk samples should be frozen as soon as possible after collection.

3.5 Beef

- A single cut of beef fillet (weight approx: 150-200 g) from a cow constitutes a single sample. 16 samples will be taken in total from 16 different cows (8 from each soil-Se setting).

- Samples should be transferred to labelled plastic bags and frozen as soon as possible after collection.

3.6 Grass

- Samples of grass will be collected from the summer milk (16 samples) and beef pasture fields (16 samples) in the high and low-Se settings (8 samples from each soil-Se setting). The grass will be sampled in the same 2 x 2 m square from which the soils will be taken. A minimum of 16 g is required per sample for analysis.

- Grass will be cut using clean scissors, stored in labelled sealable plastic bags and stored at $^{+}4$°C.

- Powder-free disposable gloves should be worn when handling the grass. The scissors should be rinsed with tap water and fresh gloves used between samples. Any soil should be removed from the grass before transferring to the labelled bags.

- **Duplicate samples:** a duplicate of each sample will be collected and retained by SASA until notification from Fera that the original samples have been received. The duplicates will be taken from the same area as the original samples. If this is not possible, they should be taken from the adjacent area so that soil conditions are comparable.

4. Transporting Food Samples to Fera

- Fresh samples (wheat, potatoes, brassicas and grass) must be delivered to Fera as soon as possible after collection (preferably within 5 days). Frozen samples (beef and milk) can be stored for longer if necessary.

- Brassica, potato and grass samples should be transported in cool boxes containing ice blocks. Some samples will be in linen or paper bags, so it is important to cover the ice blocks to prevent transfer of moisture from the blocks to the samples.

- Wheat should be transported in cool boxes without ice blocks, so ensuring the samples remain dry.

- Beef and milk samples should be transported frozen in cool boxes containing ice blocks.

Appendix 4 **Example of Se Content of Scottish Soil and Food Products Sampling Record Sheet**

Farm Identifier L01 **Date Visited**

Sample Number	Sample Type	Sample Taken (✓/x)	National Grid Reference[1]	Soil Parameters					
				Soil Depth [2]	Soil Texture [3]	Soil Colour [4]	Soil Organic Content [5]	Soil Moisture Content [6]	Soil Contamination[7]
L011A	Soil		E: N:						
L011W	Wheat								
L012A	Soil		E: N:						
L012W	Wheat								
L013A	Soil		E: N:						
L013W	Wheat								
L014A	Soil		E: N:						
L014W	Wheat								
L015A	Soil		E: N:						
L015W	Wheat								
L016A	Soil		E: N:						
L016W	Wheat								
L017A	Soil		E: N:						
L017W	Wheat								
L018A	Soil		E: N:						
L018W	Wheat								

1 12 figure Easting and Northing grid co-ordinates taken using a GPS from the soil and crop sample site, or from the central auger hole for pasture collections
2 Depth in m to the base of the top soil sample
3 Sand / Silt / Clay / Sandy-Clay / Silty-Clay / Sandy- Silt (refer to soil sampling protocol)
4 Black / Dark Brown / Light Brown / Red / Orange / Yellow / Green / Grey
5 Low / Moderate / High
6 Dry / Damp / Waterlogged
7 Wire / Metal / Pottery / Glass / Brick / Coal or Clinker / Plastic / None

Field Type/Description*

*Record crop type (types if a mix of crops), record if the field has been harvested/ploughed since the crop was grown or cattle were grazed.

Weather conditions at sampling

All samples collected as stated in sampling protocols Yes ☐

No ☐

If No, record deviations, sample numbers affected and reasons for deviations below:

Samples taken by Signature Date

Appendix 5 **Selenium Content of Scottish Soil & Food Products Questionnaire**

Farm Identifier: _____

Farm Details

Name:		Comments:
Address:		
Postcode		
Phone No.		Date of visit:
Map Ref:		Copy of Data to Farmer? Yes/No

Commodity Wheat / Calabrese / Potato / Beef / Milk

Variety/Breed _____

Field Name/Identification No. _____

Field size _____ (Ha/Ac)

Destination of Commodity _____

Milk/Beef Survey Only

What age are the animals in the sample? _____

How long have the animals been grazing on the field(s) sampled? _____

Were the animals fattened indoors before slaughter? Yes ☐ No ☐

Have the animals been exposed to mineral licks containing Se?
Yes ☐ No ☐ Unknown ☐

If yes, how often? _____

Have the animals been exposed to other Se-containing feeds/supplements/boluses?
Yes ☐ No ☐ Unknown ☐

If yes, how often? _____

Tillage

What form of tillage do you use? _____

(Ploughing/Direct drill/Min till)

Crop Rotation History

Year	Crop
2007	
2006	
2005	
2004	
2003	

Fertiliser Use

Have you applied fertiliser to the sample field in the previous 2 years?

Yes ☐ No ☐

If yes, please note fertiliser use below, including foliar feeds and trace element sprays:

Yr	Date	Fertiliser*	Rate	
			Amnt	Units

* Always record product name if available, if only N:P:K ratios are known ask if known if Se was contained as a secondary/trace element

Did any of the fertiliser contain Se? Yes ☐ No ☐ Unknown ☐

If yes, was the fertiliser a foliar application or applied directly to the soil (FO/SO) ☐

Has animal slurry been applied to the sample field in the last 2 years?

Yes ☐ No ☐

If yes, how often? _____

Source of Slurry (own cattle/bought in)? _____

Has sewage sludge been applied to the sample field in the last 2 years?

Yes ☐ No ☐

If yes, how often / rates? _____

When was the last lime application to the sample field? _____

Notes

Appendix 6 **Soil Analytical Methods**

1. Sample Preparation

1. Soils were air dried at < 30°C to avoid volatilisation of Se.
2. Soils were disaggregated and dry sieved through < 2 mm nylon mesh.
3. The < 2 mm material was homogenised and cone and quartered.
4. A 10 g split was taken from each sample for soil pH analysis.
5. A 30 g split was taken for agate planetary ball milling for 25 minutes until 95% of material was < 150 µm.
6. Of the 30 g milled material, a 2 g split was taken from each sample for soil LOI analysis.
7. Of the 30 g milled material, a 1 g split was taken from each sample for Total Se by Aqua Regia and ICP-MS analysis.
8. Of the 30 g milled material, a 3 g split was taken from each sample for Water-soluble soil-Se by Water and ICP-MS analysis.

2. Determination of Se in Soil Samples by ICP-MS

Total Soil-Se by Aqua Regia Digest

Sample Digest

1.00±0.01 g of milled soil were weighed into a labelled 50 ml calibrated test tube and 5.0 ml of deionised water added followed by 5.0 ml of aqua regia (3 HCl + 1 HNO_3). Air condensers were paced in the tops of the tubes. The tubes were transferred to a heating block and left at room temperature overnight before refluxing gently (usually at approximately 160 °C) for a minimum of two hours. The samples were allowed to cool, then the air condensers were rinsed and diluted to 50 ml with deionised water. The tubes were capped and shaken well and allowed to settle prior to decanting the supernatant liquid. Acid blanks and Certified Reference Materials (CRMs) were prepared with each batch.

Instrumentation

Measurements are made using an Agilent 7500cx ICP-MS instrument fitted with an octopole reaction system. The methodology for this instrument has been validated *via* the Cheeseman *et al*. (WRc Report NS30, 1989) protocol, by analysing standards and spiked samples in duplicate in 11 independent runs. The detection limit derived from this

validation was 0.015 µg L^{-1} in solution, which equates to 0.05 mg kg^{-1} total Se in the solid soil sample.

Analytical Method

A wash solution containing 2% v/v HNO_3 and 0.5% v/v HCl was prepared. At least three standards containing between 1 and 100 µg L^{-1} Se, plus a blank, from Claritas PPT multi-element ICP-MS Standard 2A (Spex CertiPrep, Inc) in an acid matrix to match that of the samples were prepared. The ^{78}Se concentrations in hydrogen mode using ^{72}Ge as an internal standard were determined. Data were captured by computer and concentrations calculated based on a calibration curve created by running known standards at the beginning of each run.

Water-soluble Soil-Se by Water Digest

Sample Digest

3.00±0.01 g of milled soil were weighed into a labelled 50 ml centrifuge tube and 30 ml of deionised water added, ensuring that all the powder was wetted thoroughly. The tube was capped tightly and shaken on an orbital shaker for 12 hours. The tube was centrifuged for 10 minutes at 3000 rpm before removing 10 ml of supernatant from half way down the tube to avoid any floating matter. The solution was acidified to 1% v/v HNO_3 and 0.5% HCl and Se determined by ICP-MS. Blanks, duplicates and Certified Reference Materials (CRMs) were prepared with each batch, although there are no CRMs certified for water leaches as far as we are aware. However, NIST SRM 1643e was included in every analytical run, to demonstrate the validity of the Se calibration.

Instrumentation

Measurements are made using an Agilent 7500cx ICP-MS instrument fitted with an octopole reaction system. The methodology for this instrument has been validated *via* the Cheeseman *et al*. (WRc Report NS30, 1989) protocol, by analysing standards and spiked samples in duplicate in 11 independent runs. The detection limit derived from this validation was 0.055 µg L^{-1} in solution, which equates to 0.055 µg kg^{-1} water-soluble soil-Se in the solid soil sample.

Analytical Method

A wash solution containing 2% v/v HNO_3 and 0.5% v/v HCl was prepared. At least three standards containing between 1 and 100 µg L^{-1} Se, plus a blank, from Claritas PPT multi-element ICP-MS Standard 2A (Spex CertiPrep, Inc) in an acid matrix to match that of the samples were prepared. The ^{78}Se concentrations in hydrogen mode using ^{72}Ge as an internal standard were determined. Data were captured by computer and concentrations

calculated based on a calibration curve created by running known standards at the beginning of each run.

3. Determination of Soil pH

Soil pH was determined by adding 10 g of < 2 mm sample to 25 ml of 0.01M $CaCl_2.2H_2O$ (calcium chloride). The mixture was shaken to form a slurry prior to analysis by pH electrode. This method of pH determination generally gives lower results (0.5 pH units) than water-based methods (Rowell, 1994).

4. Determination of Soil Loss-on-ignition (LOI)

2 g of milled soil material was heated in a furnace and kept at 450 °C for a minimum of 4 hours and the change in weight of the samples before and after heating was determined as the LOI.

5. Soil Analysis Quality Control

Replicate Samples

During the sample preparation process four of the soil samples were selected for replicate sample analysis (Replicate A, Table 20). These comprised two soils from the same field in the low-Se setting and two soils from the same field in the high-Se setting. A replicate split of milled soil material was taken from each of these samples and assigned a mock sample number (Replicate B, Table 20). The Replicate B samples were submitted 'blind' to the analysts to check the sample preparation and analytical procedures. Since both the Replicate A and Replicate B samples are derived from milled and homogenised material from the same soil sample, the analytical results for the two samples should be similar.

Results for the original sample and the replicate splits show good repeatability of the methods (Table 20 and Figure 14). Water-soluble soil-Se results show most variability as expected from this weak leaching method, but the variation is still within acceptable limits (< 10%).

The results for total Se and LOI demonstrate that the pairs of soils collected in the same field (L01-7A and L01-8A; H05-7A and H05-8A) are very similar in composition, whereas there is a clear distinction between soils collected in the low and high-Se areas. Conversely, the results for soil pH show a very narrow range between the high and low-Se areas, making distinction between the two pairs of samples less clear. Although the results for H05-8A demonstrate a higher water-soluble soil-Se concentration in the high-Se area than the low-Se soil samples L01-7A and L01-8A; the results for the other high-

Se sample H05-7A do not. This highlights the variable nature of water-soluble soil-Se concentrations, which do not necessarily relate to total soil-Se concentration (Figure 14). The results for the two splits of each of these samples were averaged and reported as the result for the original sample in the dataset used for statistical analysis for the project.

Analytical Duplicates

Analytical duplicate solutions were prepared and included with the ICP-MS soil analytical runs as a check on analytical repeatability or precision. Eight duplicates were analysed as part of the total soil-Se analysis and six duplicates as part of the water-soluble soil-Se analysis. The results demonstrate excellent repeatability of the analytical methods (Table 21 and Figure 15).

Certified Reference Materials and Quality Control Standards

To ensure data accuracy, certified reference materials (CRMs) and quality control standards were included in the soil Se, pH and LOI analytical runs.

National Institute of Standards and Technology (NIST) SRM1643e 'Trace Elements in Water' was run as a CRM during the determination of Se in water leaches and aqua regia digests. Four measurements were made of this standard as part of the total Se and as part of the water-soluble soil-Se analytical runs. The certified concentration of Se in this standard is 11.97 µg L^{-1}. Results demonstrate good accuracy of the methods with 94% recovery (Table 19).

GSS-4 Soil issued by the Institute of Geophysical and Geochemical Exploration (IGGE), China was run as a solid CRM. The reference concentration of Se for a total digest is 0.640 mg kg^{-1}. Eight measurements were made of this standard as part of the total Se analytical run and six measurements as part of the water-soluble soil-Se analytical run. Although this standard was run as part of the water-soluble soil-Se analysis, there are no international CRM values for water-soluble soil-Se determinations. However, the NIST water CRM results give an indication of accuracy for the water-soluble soil-Se leach solutions. Results demonstrate good accuracy of the total Se method with 94% recovery (Table 19). Results for the water-soluble soil-Se analysis of this CRM are also reported as these are of interest to the international literature (Table 19).

BGS internal quality control standards for soil pH and LOI were included in the soil analyses. Seven measurements of quality control standard QC-1 were made during the soil pH determinations. Three measurements of each of LLC-1 and QC-1 quality control standards were made during the LOI determinations. The results demonstrate good

accuracy of the methods (Table 19). The target values and two standard deviation (2SD) control limits were taken from BGS quality control charts. BGS regularly participates in the CONTEST contaminated land proficiency testing scheme for soil pH and LOI at 450°C, and had no failures for either determinand in 2008. The soil pH determinations are United Kingdom Accreditation Service (UKAS) accredited.

Appendix 7 **Food Analytical Methods**

Determination of Se in foods, by nitric acid digest followed by ICP-MS analysis:

1. Foodstuff Sample Preparation

1. Calabrese and potato samples were rinsed in Millipore-grade water.
2. Excess fat was removed from the beef samples
3. Using stainless steel knives, samples of calabrese, potatoes (skins on) and beef were cubed, and then completely homogenised using a Bucchi homogenisation system (ceramic blades, sealed bearings, *etc.*), a 50 g sub-sample taken, and stored until required for analysis.
4. Wheat samples were homogenised using a coffee grinder, with all the resulting material being stored until required for analysis.
5. Grass samples were finely chopped, using stainless steel knives on plastic boards, and a 50 g sub-sample taken, and stored until required for analysis.
6. Milk samples were carefully defrosted, and thoroughly shaken prior to analysis.

Sample Digestion

Aliquots of sample (0.5 ± 0.05 g to 3 ± 0.5 g, depending on water content) were digested in concentrated nitric acid (5 ml), using a high temperature, high pressure microwave digestion system. Each digestion batch was accompanied by a number of applicable CRMs (chosen to match the sample matrix), reagent blanks and spiked reagent blanks.

The digest liquor was quantitatively transferred to graduated test-tubes, made up to volume (10 ml) with deionised water, and then vortex mixed. An aliquot (0.3 ml) of this solution was then transferred to an autosampler tube, and an aliquot (2.7 ml) of diluent solution (propan-2-ol, 5% v/v, HNO_3, 2% v/v and In @25 ug ml^{-1}) added. The tube was then vortex mixed, ready for analysis by ICP-MS.

2. Instrumentation

Measurements were made using an Agilent 7500ce ICP-MS instrument fitted with an octopole reaction system. Analytical Method: A wash solution containing HNO_3 (2% v/v) and HCl (0.1% v/v) was prepared. Seven standards prepared using SCP SCIENCE PlasmaCal Se standard diluted using an acid/propan-2-ol matrix to match that of the

samples. The ^{78}Se concentrations in hydrogen mode using ^{115}In as an internal standard were determined. Data were captured by computer and concentrations calculated based on a calibration curve created by running known standards at the beginning of each run.

3. Analytical Method

A 10% audit (in duplicate) was performed within the study. Each analytical batch contained procedural blanks, a spiked procedural blank (for recovery estimate purposes) and certified reference materials. UKAS-accredited quality control QA/QC criteria, which have been established in this laboratory for multi-element surveys, are summarised below. These criteria include checks on instrument stability, spike recovery, replicate agreement, limit of detection (LODs) and CRM values.

Instrument Stability

Each batch included the re-measurement of a calibration standard at the end of the run. The re-measured standard had to be within ± 20% of the initial value to pass this check.

Spike Recovery

Data were accepted if the spike recovery was between 80 and 120%.

Replicate Agreement

Replicate values for a given sample must have a relative standard deviation of ≤ 20% or a SD of ≤ LOQ (limit of quantification); whichever is the greater (Table 23).

Reference Materials

The reference material results for each batch should be within the certified range or 25% of the quoted value, whichever is the greater (Table 22).

Limit of Detection

The limit of detection is defined as three times the standard deviation of the signal from procedural blanks, corrected for sample weight and dilution.