An initial study to assess the use of geological parent materials to predict the Se concentration in overlying soils and in five staple foodstuffs produced on them in Scotland.

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Abstract

Evidence suggests that dietary-intakes of the essential element selenium have fallen in Scotland in recent years, due to changing sources of bread-making wheat. The Scottish environment is thought to be Se-poor due to the geology and climate. This initial study assessed whether geological parent-materials could be used to predict relatively high and low soil-Se areas in Scotland and whether differences in soil-Se were reflected in foodstuff-Se produced on them. Samples (n = 8 per farm) of wheat, calabrese (broccoli), potato, beef-steak, milk, cattle pasture (grass) and soil were collected from pairs of farms (one in each high/low predicted Se area (PSA)). Potatoes and soils were collected from a further 34 farms in high/low PSAs to assess a greater geographical zone. Total soil-Se ranged from 0.115 to 0.877 mg kg^{-1} but most samples (90%) could be classed as Se-deficient (< 0.6 mg kg^{-1}), irrespective of PSA. Total soil-Se was significantly higher ($p<0.05$) in the high than in the low PSAs as expected; however, the difference between the two was small (mean 0.48 and 0.37 mg kg^{-1}, respectively). Water-soluble-soil-Se (6.69 to 26.78 µg kg^{-1}) concentrations were not significantly different between the two PSAs ($p=0.71$). Soil loss-on-ignition (indicating organic matter content) correlated significantly with total and water-soluble soil-Se ($p<0.001$) and exerted a greater control than parent-material on soil-Se. Significant differences between the PSAs for beef-Se ($p<0.001$), wheat-Se ($p<0.001$), calabrese-Se ($p<0.01$) and beef-farm grass-Se ($p<0.05$) indicated partial success of the parent-material soil-Se prediction. However, only wheat-Se ($p<0.001$) and potato-Se ($p<0.001$) correlated significantly with total soil-Se. The results suggest that soil-Se concentrations in the main agricultural areas of Scotland are generally low. Given the low Se concentrations also reported in the food commodities; further investigations may be warranted to fully characterise the Se-status of Scottish produce and dietary-Se intakes in Scotland.

Keywords: selenium, pH, organic-matter, soil, grass, foodstuffs, Scotland

1. Introduction

The naturally occurring element selenium (Se) is of interest because it is essential to human and animal health in trace amounts (Lower Reference Nutrient Intake = 40 µg day^{-1}; DOH, 1991) but can be harmful in excess (safe upper intake level = 450 µg day^{-1}; EVM, 2003). In humans and animals, approximately 25 essential selenoproteins have been identified and dietary-Se 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. Selenium deficiency has also been implicated in a number of conditions including heart disease, cancer and HIV-AIDS (WHO, 1996; Rayman, 2005; Reilly, 2006; Navarro-Alarcon and Cabrera-Vique, 2008; Rayman, 2008). In animals, clinical signs of Se-deficiency include reduced appetite/growth/fertility and muscle weakness; generally described as white muscle disease (WMD) (Levander, 1986). In most cases, food forms the main source of Se for humans because concentrations in water and air are generally low (WHO, 1987; Fordyce, 2005; Fordyce, 2007; Johnson et al., 2010).

The concentration of Se in foodstuffs depends primarily upon the Se concentration of the soil on which the raw food was grown or reared (Fleming, 1980; WHO, 1987; Fordyce, 2005). In many soils, geology has a fundamental control on the distribution of Se, as rocks form the parent materials from which soils are derived via the process of weathering. The Se concentration of most soils is very low 0.01 - 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, 2005). Selenium bioavailability to plants and animals 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, irrigation, etc. 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$^{(6+)}$ is more mobile, soluble and less-well adsorbed

in soils than the organic forms selenomethionine and selenocysteine or the more reduced inorganic forms selenite$^{(4+)}$; elemental-Se$^{(0)}$ or selenide$^{(2-)}$. Therefore, oxidising, alkaline conditions that favour the formation of selenate improve bioavailability, whereas selenite and selenide formed under reducing acid conditions are less bioavailable (Fleming, 1980; Jacobs, 1989; Neal, 1995; Fordyce, 2005). Total soil-Se concentrations < 0.6 mg kg^{-1} are generally considered indicative of deficiency for livestock production, 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).

In addition to the complex relationships governing Se uptake from soil, different species of plants and animals also assimilate Se to varying degrees. Plants can be divided into three groups: Se-accumulators, 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). Some common crops 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). In animals, Se tends to concentrate in the liver, kidneys and, to a lesser extent, in the muscle (Levander, 1986; WHO 1987). Hence, offal, red meat and poultry were identified as good sources of Se in the 2006 UK Total Diet Survey (TDS) along with fish, Brazil nuts, bread and cereals (FSA, 2009).

In Scotland, as in the rest of the UK, there is evidence that Se intake has been declining in recent years. Concentrations of Se in the Scottish diet fell by 50% between 1974 and 1994 from 60 to 32 µg day^{-1} (Macpherson et al, 1997). 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}

blood plasma Se in 1994. Data from the most recent National Diet and Nutrition Survey (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 but the number of participants in Scotland was small. The decline in Se intake is partly attributed to falling intakes of red meat/ offal but is primarily linked to changes in the sources of bread-making wheat from North America to the UK and Europe (Macpherson et al., 1997). Canadian and American wheat tends to contain more Se, as it is grown over black shale and volcanic tuff rock types on the prairies, which are rich in the element. In contrast, wheat from the UK is lower in Se as Se-rich rock types and soils are less common; and acid-neutral soils are typical as in much of Northern Europe (Fordyce, 2005; Broadley et al., 2006; Johnson et al., 2010).

Although there is no existing national soil-Se geochemical dataset for Scotland to indicate the range of Se concentrations across the country; it is recognised that the Se status of Scottish soils is likely to be low due to their acid nature, geological parent materials and the climate (Fordyce, et al., 2008). If the Se status of Scottish soils is low and this is reflected in the foodstuffs produced on them, then current moves towards the procurement of locally-produced foods (Scottish Government, 2009) may have a potentially deleterious impact on the Se status of the general population in Scotland.

Therefore, this initial study was designed to establish whether, in the absence of a soil-Se dataset for Scotland, it would be possible to use existing information on the underlying geological parent materials to predict the Se status of the overlying soil, and of the foodstuffs produced on it. It was also designed to provide a preliminary indication of the differences in Se concentration between soils and foodstuffs in predicted high and low-Se areas and whether it was possible to measure these accurately. In addition, the project collected data regarding factors that have a significant influence over the agricultural utilisation of soil-Se, e.g., soil pH and organic matter content and water-soluble-Se content. As such, this study

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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, from these data, it was hoped that the sponsors (Food Standards Agency Scotland) would gain an insight into the relationship between the Se content of Scottish soil and locally produced foods. It also provided information on the feasibility of using a similar experimental design to perform more widespread assessments of the Scottish Se status, prior to formulating future policies.

2. Selection of High and Low Selenium Areas for the Present Study

Information on the Se status of the Scottish environment is limited but the data available suggest that it is likely to be low in Se. 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}). Adams et al. (2002) also 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 more than 60 µg kg^{-1}).

Very few previous studies have been carried out into the Se concentrations of Scottish soils. Ure et al. (1979) reported total Se concentrations of 0.02 to 0.36 mg kg^{-1} in 10 arable top soils selected from different parent material types. MacLeod et al. (1996) reported total Se concentrations of 0.55 to 0.76 mg kg^{-1} in four arable top soils in Aberdeenshire. However, due to differences in analytical methodology, it is difficult to draw conclusions about the Se concentration of particular soil types from these very limited studies.

As part of the British Geological Survey's (BGS) Geochemical Baseline Survey of the Environment (G-BASE) project peri-urban/rural soil samples were collected at a sample density of 1 per 2 km^2 around the Glasgow conurbation. Total-Se concentrations in the 241

rural top soils ranged from 0.10 to 6.60 mg kg^{-1} (Figure 1). However, the uppermost concentrations reported in this dataset were associated with contamination on the periphery of the urban environment and concentrations in 'natural' soils around Glasgow were estimated to range between 0.10 and 2.00 mg kg^{-1} (Fordyce et al., 2010).

A recent study (Shand and Hillier; 2009) of total Se (aqua regia digest) concentrations in 44 Scottish agricultural soils over various geological parent materials reported values in the range 0.19 to 1.46 mg kg^{-1} (mean 0.63 mg kg^{-1}). However, these data were publically available only after the end of the current project; hence were not included in the project design stage.

Given the small size of the Ure et al. (1979) and MacLeod et al. (1996) studies and the specific geographic focus of the G-BASE Glasgow project; it was not possible to define high and low-Se soils across Scotland for the present study on the basis of known Se concentrations. Therefore, a scheme was devised that utilised existing geological and geochemical knowledge to rank various rock types as being either high or low-Se soil parent materials. No rock Se data were available for Scotland, but from studies around the world it was known that sedimentary rocks tend to 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. 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, with exceptionally high concentrations being reported in Se-rich coals in China (6000 mg kg^{-1}) (Plant et al., 2004; Fordyce, 2005).

Within the main agricultural production regions of Scotland, 2000 different rock types were identified using the BGS DiGMapGB-50® solid geology database for Scotland. These were

classified as predicted high or low-Se soil parent materials (Table 1) based on typical Se concentrations in various rock types from around the world and the limited soil-Se data available for Scotland. Note: the terms 'high' and 'low' refer to concentrations relative to each other, rather than predicting the absolute concentration of Se in a soil (Fordyce et al., 2008). This information was incorporated into a geographic information system (GIS) where it was used to select farms for the study. Since many factors, not just the underlying geology, affect the concentration of Se in an overlying soil, the prediction of high/low-Se areas was subject to significant levels of uncertainty.

3. Methods

3.1 Project design

The following food commodities were chosen for this study because they were both widely-grown and locally-consumed in Scotland: wheat, potatoes, calabrese (broccoli), cow's milk and beef steak (including grass from the fields in which the milk and beef cattle were grazing). However, due to the lack of existing data on soil and foodstuff Se contents in Scotland, the likely differences between high and low-Se concentrations, and ability to measure these accurately in the foodstuffs, was unknown prior to the project. Therefore, for the purposes of this initial study, a controlled approach was adopted to compare the Se concentrations in each of the food types and associated soils from a pair of farms; one in a predicted low-Se area and one in a predicted high-Se area. Since this project design would give very limited information on the geographical variability of Se concentrations across Scotland; a larger soil and foodstuff dataset was collected also for one of the food commodities: potatoes. Therefore, the project was organised into two sections; one testing in detail the ability to measure relationships between soil-Se and foodstuff-Se grown on individual farms in areas of predicted high and low-Se. This was the main aspect of the study, and will be referred to as the Main Study. The second section of work designed to evaluate the variability in soil-Se and foodstuff-Se in predicted high and low-Se environments over a wider geographical area, based on potato sampling, is referred to as the Between-

Farm Study. Potatoes were chosen for this aspect of the project as they were easy to collect, and widely grown across Scotland.

3.1.1   Calculation of replicate sampling numbers:

The number of replicate samples required to asses differences between 'high' and 'low' soil-Se and foodstuff samples in both the Main and Between-Farm Studies was determined using a statistical power calculation assuming a 'power' of 80% and a statistical significance level of 5% (Thomas, 1997). Based on experience from the extensive food survey work undertaken at the Food and Environment Research Agency (Fera) over the last 20 years, the calculation assumed that the Se concentration data for the soils and foodstuffs would have aspects of inherent variability, ranging from 5% (representing the analytical/sample variation) to 60% (incorporating possible farm/site and commodity/variety variation). Therefore, to minimise variability resulting from the latter source, replicate samples of a single variety of each food commodity were taken from individual farms, in the predicted high and low-Se areas. Figure 2 is a graphical representation of the calculation output, showing that, to detect a two-fold difference in Se concentrations in food from predicted high and low soil-Se areas, with a moderate level of variation (35%) in the overall precision, eight samples would be required for each food commodity, per Se area for the Main Study. The statistical power calculation also indicated a dataset size of 18 was required to accurately assess Between-Farm variability. Therefore, additional sets of potatoes, and soils, were collected from a further 17 farms (in both the predicted high and the low soil-Se areas, i.e., 17 x 2 = 34 samples) for the Between-Farm Study. The project design is summarised in Table 2.

3.2   Identification of suitable farms

Using information from the Scottish Agricultural Census Database (Scottish Government, 2008), a total of 10 farms were selected for the Main Study – five in predicted high-Se areas and five in predicted low-Se areas. For the Between-Farm Study, 17 farms were selected for

each Se environment. Figure 3 shows a map of the sampling sites and predicted high and low-Se areas.

3.3 Commodity selection and sample collection

3.3.1 Main Study

To minimise sources of variability, other than predicted Se environment; crop variety; crop maturity; breed of animal; age of animal; and cut of beef collected from the cattle, were standardised. The calabrese variety, Parthenon, was selected as it is widely grown in Scotland. Similarly, Maris Piper was selected as it is the most common potato variety grown for human consumption in Scotland, (SASA, unpublished data). A widely grown variety of biscuit-making winter wheat (Consort) was selected for the study (Struthers, 2007). Lean fillet Aberdeen Angus beef steak was selected to minimise differences in Se concentration between fat and muscle in the meat cut. The cattle were 20 to 24 months old at slaughter. Both of the dairy farms included in the study had herds of Friesian/Holstein-cross cows. Samples of milk were collected in August from both farms.

To minimise sources of variability further, the eight replicate samples of soils and each food commodity were collected from the same field on each farm. However, within each field, samples were collected from as wide a distribution as possible across the field. On the dairy farms, the soil and grass samples were collected from the field grazed by the cattle in the previous 24 hours prior to milk sampling to capture the rapid turnaround of Se from grass intake into cows' milk. On the beef farms, the soil and grass samples were collected from the field grazed prior to cattle slaughter, within 0-3 months of slaughter and not beyond 6 months from slaughter.

Samples of top soil (5-20 cm depth) were collected using a Dutch hand-held auger. Each soil sample comprised 5 auger-heads of soil. In the case of the wheat, calabrese and potato farms, soils were collected directly from the root-base of the plant sampled for the study. In

the case of dairy and beef farms each soil sample comprised five auger-heads taken from the centre and corners of a 2 x 2 m square. Namely, from eight 2 x 2 m squares distributed widely across each pasture field.

Grass samples were collected from the same 2 x 2 m squares 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 milk collection. Grass from beef pasture sites was collected in early autumn, prior to cattle slaughter. Soil-splash was not removed from the grass samples, as it was deemed important to obtain an indication of the total amount of Se being consumed by the cattle. However, root material was not included.

The ears of wheat from one plant; individual heads of calabrese and individual potato tubers comprised a single sample of each of these food types. Care was taken to ensure that the heads, in the case of calabrese, and tubers, in the case of potatoes, were of similar size in the predicted low and high-Se farms and that all crops were sampled at maturity, just prior to harvest. Only potato tubers of 45-65 mm diameter were collected, in order to reduce potential Se concentration differences arising from different skin-to-flesh ratios. Milk (60-100 mL) was collected directly from freshly-washed teats of eight different cows on each farm. Single cuts of fillet steak (150-200 g) were collected from carcasses at the local abattoir from eight different cows from each farm. All samples were subjected to rinsing off soil, air-drying and chilling prior to being distributed to the laboratory.

3.3.2 Between-Farm Study

Crop variety and maturity were also standardised for the Between-Farm potato study. However, it was not possible to identify enough farms growing the Maris Piper variety to give a large enough dataset. Therefore, both Maris Piper and Saxon varieties were included in the project. Saxon was selected as the second variety as it produces tubers of comparable size

to Maris Piper and is widely grown in Scotland (SASA, unpublished data). Equivalent numbers of the two varieties were collected from each Se area. At each of the 34 Between-Farm sites (17 farms in each Se area), one composite soil and one composite potato sample was collected.

At each farm, top soil (5-20 cm depth) was collected using a Dutch hand-held auger. The composite comprised eight auger-heads of soil collected from the base of each of the eight potato plants selected for study. Soil from the eight auger-heads was thoroughly homogenised on a plastic sheet, before collection in a Kraft® paper bag.

Eight potato tubers were collected from different plants from the same field on each farm. Any adhering soil was washed off, and the tubers were dried prior to storage. These eight tubers were combined (including skins) to create a single sample from each farm.

During the farm-survey process, a questionnaire was completed which contained information regarding; land usage (including fertiliser and pesticide application); previous field usage; plant/animal variety; final market for the food commodity and observations on soil conditions.

3.4   Soil sample analysis

Following air-drying (<30 °C to avoid volatilisation of Se) and sieving (<2 mm nylon mesh) soil samples were analysed for pH; loss-on-ignition (LOI) (as an indicator of organic matter content); total-Se (aqua regia digestion) and water-soluble Se. As outlined in the Introduction of this paper, the uptake of Se from the soil into plants and animals is influenced by many biogeochemical processes. Therefore, the water-soluble soil-Se concentration was determined, as a measure of the most mobile or bioavailable (to plants and animals) portion of Se in the soil. However, it should be noted that it may not reflect the entire bioavailable Se concentration of the soil. Soil pH and LOI also exert fundamental controls on the amount and bioavailability of Se in soil and so these parameters were also included in this study.

3.4.1 Total soil-Se analysis

Agate-ball-milled soil (< 150 µm; 1 g) was weighed into a calibrated test-tube (50 mL), and deionised water (5 mL) added, followed by aqua regia (3 HCl + 1 HNO_3) (5 mL). The mixture was left overnight and refluxed (160 °C, at least 2 hours) prior to the cooled solution being made up to volume with deionised water. Each analytical batch was accompanied by reagent blanks and certified reference materials (CRMs). Total Se quantification was by Inductively Coupled Plasma Mass Spectrometry (ICP-MS), using standard operating conditions.

3.4.2 Water-soluble soil-Se analysis

Agate-ball-milled soil (< 150 µm; 3 g) was weighed into a calibrated test tube (50 mL), and deionised water (30 mL) added. The mixture was shaken on an orbital shaker (12 hours) and then centrifuged (3000 rpm). An aliquot (10 mL) of supernatant was transferred to a test-tube and acidified with a nitric acid: hydrochloric acid solution (1: 0.5% v/v). Each analytical batch was accompanied by reagent blanks and CRMs (although the latter in solids are only certified for total Se, these were included in the water-soluble Se analysis as an in-house/within-project quality control material). Analysis of the Se concentration was by ICP-MS, using standard operating conditions.

3.4.3 Soil pH

Sieved soil (10 g, <2 mm) was added to a calcium chloride solution (25 mL, 0.01M $CaCl_2.2H_2O$). 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), but is widely used in environmental science. The soil pH analysis was performed using United Kingdom Accreditation Service (UKAS) accredited procedures.

3.4.4 Loss-on-ignition (LOI)

Agate-ball-milled soil (< 150 µm; 2 g) was heated in a furnace (450 °C, minimum of 4 hours). The change in weight of the samples before and after heating was deemed to be the LOI.

3.5   Food and grass analysis

Prior to homogenisation, the wheat grain was air dried; soil was rinsed from the calabrese and potatoes; and fat removed from the beef. Calabrese heads were trimmed (as for consumption) and the potatoes homogenised (including skins).

3.5.1   Total Se food and grass analysis

An aliquot (0.5 g dry, 3 g liquid) was dissolved in concentrated nitric acid (5 mL), using a microwave-assisted digestion system. Following cooling, the digest liquor was transferred to a graduated test-tube (10 mL), and made up to volume with deionised water. An aliquot (0.3 mL) of this solution was diluted (to 5 mL) with a mixture of propan-2-ol (5% v/v) and nitric acid (2% v/v) containing In (as an internal standard, for instrumental drift correction). Each analytical batch was accompanied by reagent blanks, spiked samples and CRMs. Total Se quantification was by ICP-MS, using standard operating conditions. All aspects of the analysis were performed using UKAS-accredited procedures.

3.6 Statistical analysis

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 demonstrate that no transformation was required to make the data satisfy the assumptions of the proposed statistical techniques. Two-sided t-tests, with pairwise testing and false discovery rate (FDR) multiple comparison correction were used to compare the data from predicted high and low-Se areas. FDR is a less conservative procedure for comparison, with greater power than familywise 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 measured in the various foodstuffs and grass samples, and the various soil parameters. Pearson correlation tests with FDR correction were used to assess relationships between the four soil parameters. In order to quantify the effects of soil parameters, bivariate linear regression analysis was used. The regression residual structure was evaluated graphically using appropriate diagnostic plots (Crawley, 2003). Adjusted R^2 was documented to measure the goodness of fit of the linear regression model.

For the purposes of data processing; the mean values for the parameters determined in the soils from each of the Main Study farms were incorporated into the Between-Farm soil dataset. This increased the number of soil results in the combined Between-Farm dataset to 44 (10 Main Study farm results and 34 Between-Farm soils). Similarly, the mean total Se concentrations in potatoes from the two Main Study farms were included in the Between-Farm dataset resulting in 36 values in total (18 from each Se area).

4. Results and discussion

4.1 Analytical data quality

To allow a direct comparison to be made of the quantitative Se concentrations reported in this paper against that from other sources, the quality assurance/control data obtained during this study are presented in Table 3. The results demonstrated good accuracy and precision of the analytical methods.

4.2 Overview of project results

4.2.1 Soil

In order to ensure that inferences being made from the Between-Farm soil-Se data had not been influenced by the choice of food commodity, the farmer questionnaires were interrogated for information regarding previous land usage. This exercise demonstrated that both calabrese and wheat had been grown in the fields, prior to the potato crop, so the same

geographical areas would have been included in the sampling exercise, had one of the other commodities been chosen for the Between-Farm study.

Total soil-Se concentrations in the dataset as a whole ranged from 0.115 to 0.877 mg kg^{-1} (Table 4). These values were within a similar range to those reported previously for Scottish soils (Ure et al., 1979; MacLeod et al., 1996; Shand and Hillier, 2009). However, the results were narrower in range, and lower in average concentration, than those reported by Fordyce et al. (2010) for rural soils on the periphery of Glasgow (Figure 1). This was expected given 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}, Fordyce, 2005) and to an extensive area of Eastern England (Humber-Trent; East Midlands and East Anglia) surveyed by the BGS (0.400 mg Se kg^{-1} in 19,500 soil samples; BGS, 2009). Although the present project aimed to target low and high-Se environments in Scotland; the range in total Se concentrations was very narrow. Indeed, the majority of soil total-Se values were below the suggested deficiency threshold in soils for the rearing of animals (0.6 mg kg^{-1}, Fordyce, 2005). These low values were 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. Water-soluble soil-Se concentrations ranged from 6.69 to 26.78 µg kg^{-1} (Table 4). These concentrations were much higher than those reported from Se-deficient areas of China (0.03-5 µg kg^{-1}; Fordyce, 2005) but markedly lower than values in Se-rich areas of England and Ireland (50-430 µg kg^{-1}; Nye and Peterson, 1975). Water-soluble soil-Se accounted for 1-3% of the soil total-Se concentration in most cases, which is comparable to ranges reported previously for UK soils (1-2% Williams and Thornton, 1973; Nye and Peterson, 1975) but much lower than the 50% water-soluble soil-Se reported from seleniferous areas of the USA (Nye and Peterson, 1975). 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 4). 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. Indeed the results for soil pH and the proportion of water-soluble soil-Se highlight the contrast in bioavailable-Se between the arid alkaline Se-rich environments of the Mid-West USA and the temperate acid-neutral Se-poor environments of much of the UK. Soil loss-on-ignition was measured as an indicator of organic matter content, and values ranged from 1.71 to 14.30%. These values are indicative of moderate to high organic matter contents, typical of Scottish soils (SEERAD, 2006).

4.2.2 Food and grass

The total Se concentrations in the food products and grass samples collected for the present study are outlined in Table 5 for information only since - with the exception of potatoes - the commodity samples were collected from pairs of farms only. Therefore, the results are not indicative of Se concentrations in these foodstuffs in Scotland. However, it should be noted that the Se concentrations in grass were below recommended levels for forage intake of 40 µg kg^{-1} (Levander, 1986) and, with the exception of milk, concentrations in food were comparable to or lower than those reported in the same foodstuffs as part of the UK TDS and in studies from the USA and elsewhere (Table 5) (Fordyce et al., 2009).

4.3 Can geology be used to predict the concentration of Se in the overlying soil?

Examination (two-sided t-test) of the combined Between-Farm dataset of 44 soils, showed that the total soil-Se concentrations from the predicted high-Se areas were significantly higher ($p < 0.05$) than those in the predicted low-Se areas as expected. However, the Se concentration range between the two was narrow, with much overlap between the two datasets (Figure 4a). Indeed, only 12.6% of the variance between the datasets was accounted for by the predicted Se area. Plotting the locations of these results confirmed the lack of a marked spatial association between the predicted high-Se areas and demonstrably higher total soil-Se concentrations than in the predicted low-Se areas (Figure 3). Indeed, the

average total soil-Se concentration in predicted high-Se areas (0.484 mg kg^{-1}) was only 1.3 times greater than that in predicted low-Se areas (0.372 mg kg^{-1}).

Further interrogation (two-sided t-test) of the Main Study soil-Se dataset, on commodity-by-commodity basis, showed that the total soil-Se concentrations were significantly higher in the predicted high-Se potato and wheat farms than the predicted low-Se areas as expected ($p<0.01$ and $p<0.001$ respectively). Although total soil-Se concentrations from the predicted low-Se milk farm were generally lower than from the predicted high-Se milk farm; for milk and beef farm soils, there was no statistically significant difference in Se concentration ($p=0.18$ and $p=0.21$ respectively) between the two areas. This was despite the fact that Se-containing NPK fertiliser had been applied within the last four months to the field sampled at the predicted high-Se beef farm (2.82 g ha^{-1} sodium selenate; three times per year).

On the two farms from which the calabrese samples were collected, the total soil-Se concentration was significantly higher in the predicted low-Se area, than in the predicted high-Se area ($p<0.001$). An explanation for this disparity is the highly variable nature of the Carboniferous rock types in Fife where the predicted high-Se calabrese soils were collected (Figure 3). Carboniferous rock types underlie much of the Central Belt of Scotland from Ayrshire to Fife. These largely comprise cyclical sequences of narrow coals, shales, mudstones and siltstones (all likely to be higher in Se; Fordyce, 2005) interbedded with sandstones and limestones (likely to be lower in Se Fordyce, 2005). This made the categorisation of these rocks into potential low and high-Se areas difficult. Evidence from the G-BASE Glasgow soils dataset (Figure 1) was that soils developed over these Carboniferous cyclical sequences were likely to be higher in Se than some other rocks. Therefore, these rock types were classed as high-Se soil parent materials for the present study (Fordyce et al., 2008). Examination of the soil from the predicted high-Se calabrese farm showed that it was sandy in texture and contained the lowest organic matter content (mean 3.66%) reported on any of the farms surveyed in this study. This suggested that these soils were

collected on a sandstone-dominated part of the geological sequence, with the sandy nature of the soils and low organic matter content probably accounting for the lower-than-expected Se concentration in this sample. Therefore, using the additional information gained regarding the relationship between soil-Se and soil texture/organic matter, during the present study it should be possible to further refine the definitions of high and low-Se areas within the Carboniferous sequences, thereby improving the accuracy of the overall prediction strategy in the future.

These results highlighted the difficulties in trying to estimate soil-Se concentrations at any given location but none-the-less demonstrated that the selection of high and low-Se areas, on the basis of geological parent materials alone could, to a certain degree, be used to predict the total Se concentration of the soil. However, no striking contrast in total soil-Se concentration was observed between the two areas. Furthermore, even in the predicted high-Se area, the majority of soils could be classed as being Se-deficient, as they were below the 0.6 mg kg^{-1} recommended threshold for grazing livestock (Fordyce, 2005).

Many factors, not just the total soil-Se concentration control the amount of water-soluble Se present in soil. 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 (two-sided t-test). The results from the combined Between-Farm dataset of 44 soils showed that although water-soluble soil-Se concentrations were marginally higher in the predicted low-Se areas (Figure 4b); there was no statistically significant difference between the two Se areas (p=0.71).

One interesting observation from the Main Study dataset was that the water-soluble soil-Se concentrations from the predicted high and low-Se milk farms were notably higher (mean concentrations = 22.51 and 18.17 μg kg^{-1}, respectively) than reported for any of the other

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farms (mean concentrations 7.68 – 12.97 µg kg^{-1}). The reasons for this are uncertain and require further investigation.

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 geological parent materials alone. Similarly, soil pH and LOI showed no statistically significant difference in concentration between predicted high and low-Se areas (p=0.60 for pH and p=0.17 for LOI; two-sided t-test) (Figures 4c and 4d respectively).

The relationships between the four soil parameters in the combined dataset of 44 soils were evaluated, identifying significant correlations (Pearson correlation) between LOI and both total and water-soluble soil-Se (p<0.001; Table 6). These findings indicated that the soil organic matter content had a significant control over soil-Se concentrations as expected. Indeed, from adjusted R^2, 56.1% of the total Se and 48.0% of the water-soluble soil-Se variance in concentration was accounted for by the soil organic matter content. This also helped explain the apparent significant correlation between total soil-Se and water-soluble soil-Se concentrations (p=<0.01) evident in the dataset (Table 6). The results demonstrated that soil organic matter content had a greater control than geologically predicted high or low-Se parent material on the Se concentration of the soil. These results concur with those of Shand and Hillier (2009) who also found that soil organic matter content exerted a greater control on soil-Se concentrations than parent material type in Scottish agricultural soils.

No significant correlations were observed between pH and the other soil parameters, which may have been due to the very narrow range of pH values observed (Table 6).

These results suggest that inclusion of information on organic-rich superficial deposit soil parent materials such as peat or of soil organic matter content, for which there is a low

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resolution (5 km) national database for Scotland (MI, 2009), would improve the prediction of low and high-Se areas for future investigations. They also suggest that organic-rich soils typical of the upland areas of Scotland (SEERAD, 2006) are likely to contain more Se than soils in the main agricultural growing areas. Whether the Se in these soils would be readily available for plant/animal uptake is open to debate. Early work in Ireland showed some evidence that Se was more bioavailable to plants in seleniferous poorly drained peats than other soil types (Williams and Thornton, 1973). However, other studies have demonstrated that Se in organic-rich soils may be held in the soil and not readily phyto-bioavailable (Johnson et al., 2000). Further investigations would be required to establish the Se content of upland organic-rich Scottish soils and their relationship with the Se-status of foodstuffs produced from them such as lamb.

4.4   *Grass and foodstuff relationships with predicted high and low-Se areas*

The second aim of this study was to evaluate the validity of using geological soil parent material information to predict the likely Se concentrations in agricultural commodities grown in predicted low and high-Se areas.

In the Main Study dataset, the majority of food commodities demonstrated a significant difference (two-sided t-test) in relative total Se concentrations between the two predicted Se areas. Namely, beef ($p<0.001$); wheat ($p<0.001$); calabrese ($p<0.01$) and beef-farm grass ($p<0.05$). Indeed, 71.5%, 69.6% and 50.8% of the variance in Se concentration in the beef, wheat and calabrese samples, respectively, was explained by the predicted Se area. No statistically significant difference in Se concentration was observed in the potatoes ($p=0.36$), milk ($p=0.08$) and milk-farm grass ($p=0.18$).

These results presented some argument for pursuing the use of existing geological/geochemical information to predict the likely concentrations of Se in certain food commodities, and emphasised that refinement of the soil parent material classification

system and examination of the importance of crop types/varieties, were areas worthy of further effort.

4.5   Relationships between foodstuff-Se and grass-Se, and soil-Se concentrations

Another aim of this project was to assess whether differences in soil-Se concentrations were reflected in the foodstuffs produced on them. Although the food commodity and grass-Se concentrations generally reflected the predicted high and low-Se areas, the relationships with the measured soil-Se concentrations were more complex. Analysis of the direct relationships between the soil parameters and commodity Se concentrations was only truly possible for calabrese, potato, wheat and grass samples. For the milk and beef samples, the animals were in contact with relatively large areas of soil and grass, and were open to additional inputs, other than the grass on which they were feeding, e.g., mineral supplements, antibiotics, etc, making discussion of the relationships more tenuous.

Table 7 highlights those parameters where a significant correlation (ANOVA) was observed between a commodity and total soil-Se concentration. The table also presents the other soil parameters that exhibited a significant correlation with the Se concentration in a foodstuff.

In the Between-Farm dataset, potato Se concentrations showed a high correlation with total soil-Se (p<0.001). Similarly, in the Main Study dataset, wheat Se correlated significantly with total soil-Se (p<0.001). Indeed, 72.8% (R^2) of the variance in wheat concentration was accounted for by the total soil-Se concentration. In contrast, the calabrese and beef farm grass-Se results showed negative correlations with total soil-Se and water-soluble soil-Se, respectively. The reasons for these disparities are unclear but may relate either to marginally different ages in the grass/calabrese at the two sets of farms at the times of sampling (even though in the case of calabrese, sampling was carried out within a week of predicted harvest date), or differences in trace element fertiliser application noted in the questionnaires from the two sets of farms (the predicted low-Se calabrese farm soils received Cu and Mo

fertilisers and the predicted high-Se beef farm soils received Se fertilisers). It is also possible that the lower organic matter content of the predicted high-Se calabrese farm soil facilitated uptake of Se into the calabrese, despite lower soil-Se concentrations.

No significant correlations were observed between any of the parameters measured in the soil and the Se concentration in the milk-farm grass or Main Study potato samples. This may reflect the limited variation in soil-Se concentrations observed between the predicted high and low-Se areas in this dataset.

These results highlight the complexity of soil-to-plant element uptake, which is controlled by many environmental and physiological processes. Therefore, care must be taken if attempting to estimate 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. Further investigations would be required to establish the mechanisms controlling Se uptake into plants and animals in Scotland, as the results from the present study are based on two farms only for each commodity.

None-the-less, the results of this study have demonstrated that soils in the main agricultural growing areas of Scotland and the foodstuffs produced from them are likely to be low in Se concentration. Therefore, further examinations to determine the Se-status of Scottish produce and the Scottish diet may be justified. Should these demonstrate an insufficiency of Se; methods to raise the Se content of foodstuffs by the application of Se-fertiliser to agricultural soils and crops may be worthy of consideration. These methods are currently under trial in the UK as part of the Biofortification through Agronomy and Genotypes to Elevate Levels of Selenium (BAGELS) project (Broadley et al, 2010; Stroud et al., 2010a; Stroud et al., 2010b) and have been used successfully in other Se-deficient countries such Finland (Eurola et al., 2003) and New Zealand (Oldfield, 1999) to raise Se dietary intake.

However, any attempts to increase the Se-status of Scottish foodstuffs should be mindful 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; Vinceti et al., 2009).

5.   Conclusions

This study has demonstrated that, in the absence of national soil-Se geochemical maps for Scotland, geological soil parent material information alone can, in part, be used to predict differences in the Se concentration of Scottish soils, and for three of the five staple food commodities chosen (wheat, beef and calabrese). However, the difference in total soil-Se concentration between predicted high and low-Se areas was marginal. From this, it is not unreasonable to consider the further development of the predictive strategy, to incorporate a more detailed approach to defining likely high and low-Se areas on the basis of existing geological/geochemical information, including soil organic matter content. Similarly, there are aspects of the foodstuff selection that would benefit from further investigation, e.g., food type, variety, age, etc.

The study also highlighted the fact that trends in soil-Se concentration will not always be reflected in the foodstuffs produced from them (only wheat and potato Se concentrations correlated significantly with soil-Se), suggesting that parameters, other than soil parent material and soil-Se, need to be considered in any future studies into the relationships between soil and foodstuffs in Scotland.

This study also confirmed previous investigations, indicating that the Se concentrations of Scottish soils are generally low i.e., below the 0.6 mg kg^{-1} recommended threshold for grazing livestock. Hence; even if the prediction of likely high and low-Se areas is refined in

the future; high-Se soils are unlikely to be common in the main agricultural growing areas of Scotland. Furthermore, the Se concentrations in grass from this study were below the 40 μg kg^{-1} recommended deficiency threshold for grazing livestock. Given the relatively low concentrations of Se also reported in the food commodities sampled, further investigations may be warranted in order to fully characterise the Se concentration of Scottish produce and, hence, in the Scottish diet. An outcome of this may be consideration of a national soil-Se supplementation strategy, via fertiliser applications, as has been performed in some other countries.

6. Acknowledgements

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CM = Carboniferous Coal Measures: n = 38   CPV = Clyde Plateau Volcanic Formation: n = 66
CSG = Carboniferous Strathclyde Group sandstones: n = 30   DEV = Devonian Sandstones: n = 15
LUL = Carboniferous Limestone Groups: n = 92
Rural top soils (< 2 mm; 5 – 20 cm depth) analysed by X-ray Fluorescence Spectrometry.
Boxes show the median and interquartile range; whiskers show the 10th and 90th percentiles and points show outlying values in the data distribution. Data from Fordyce et al. (2010).
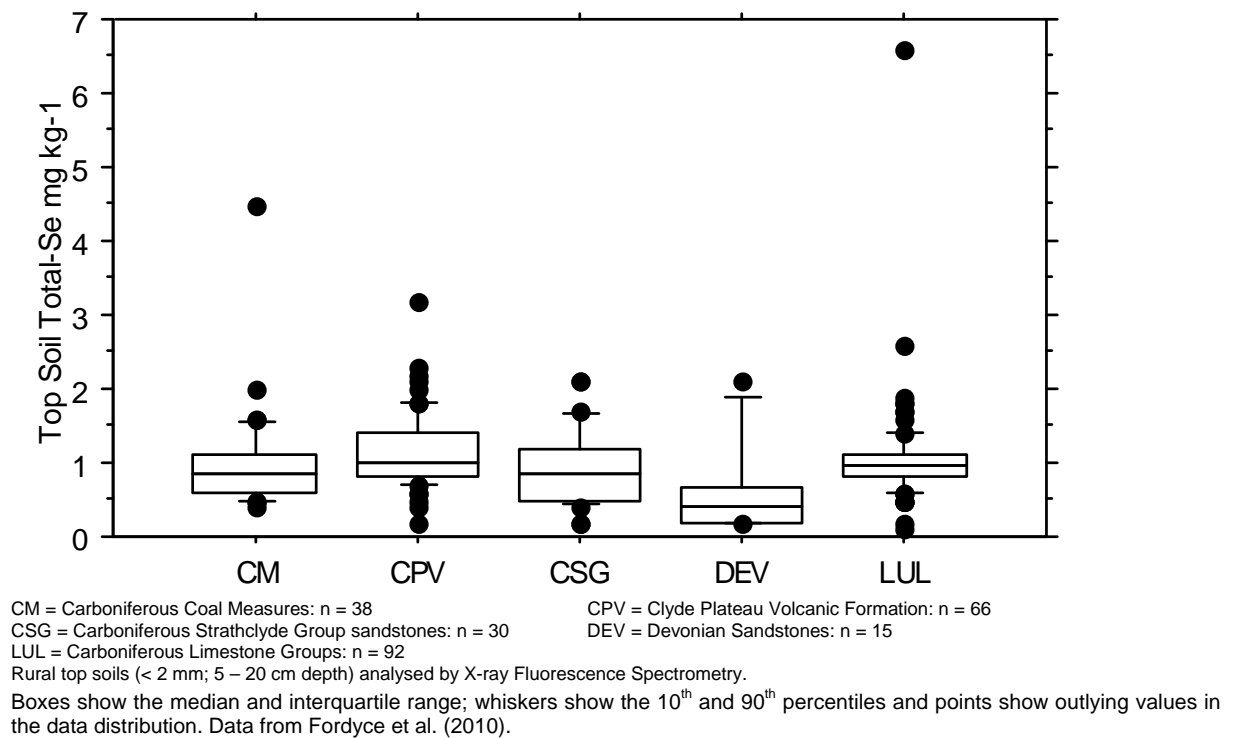
Figure 1. Box and whisker plots of total-Se concentrations over the main geological units in rural top soils around Glasgow.
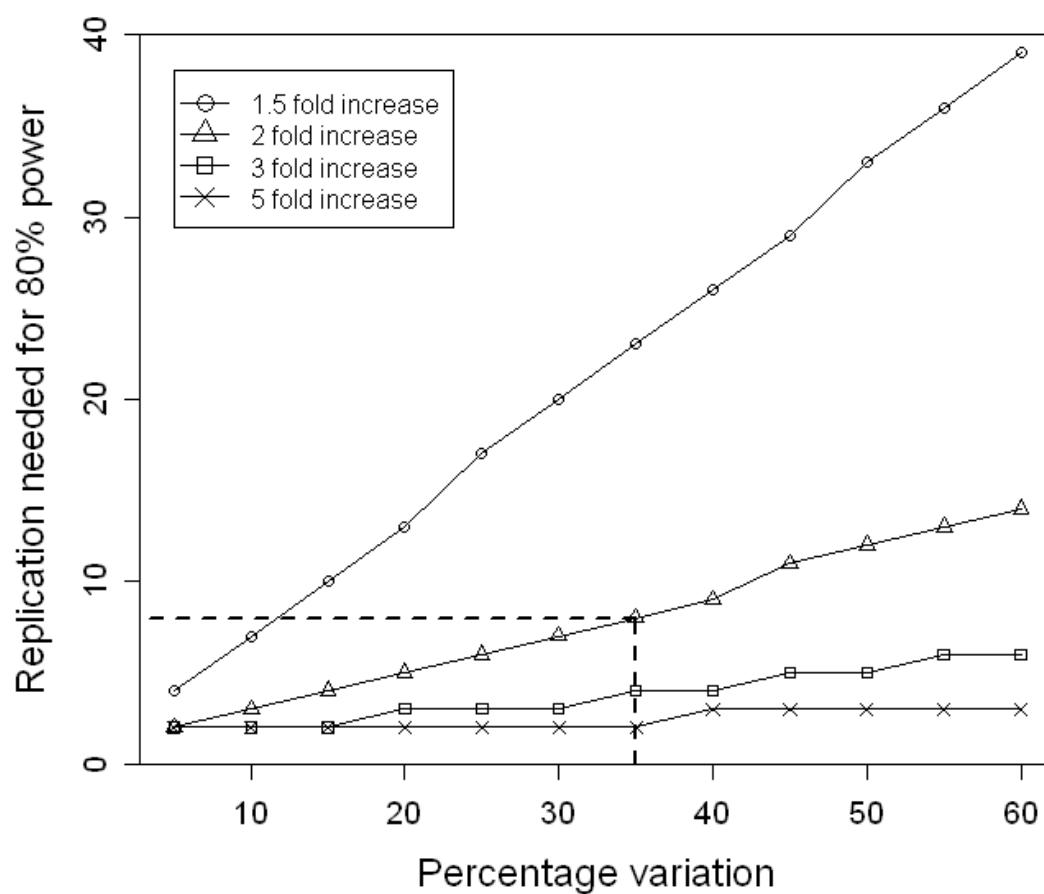
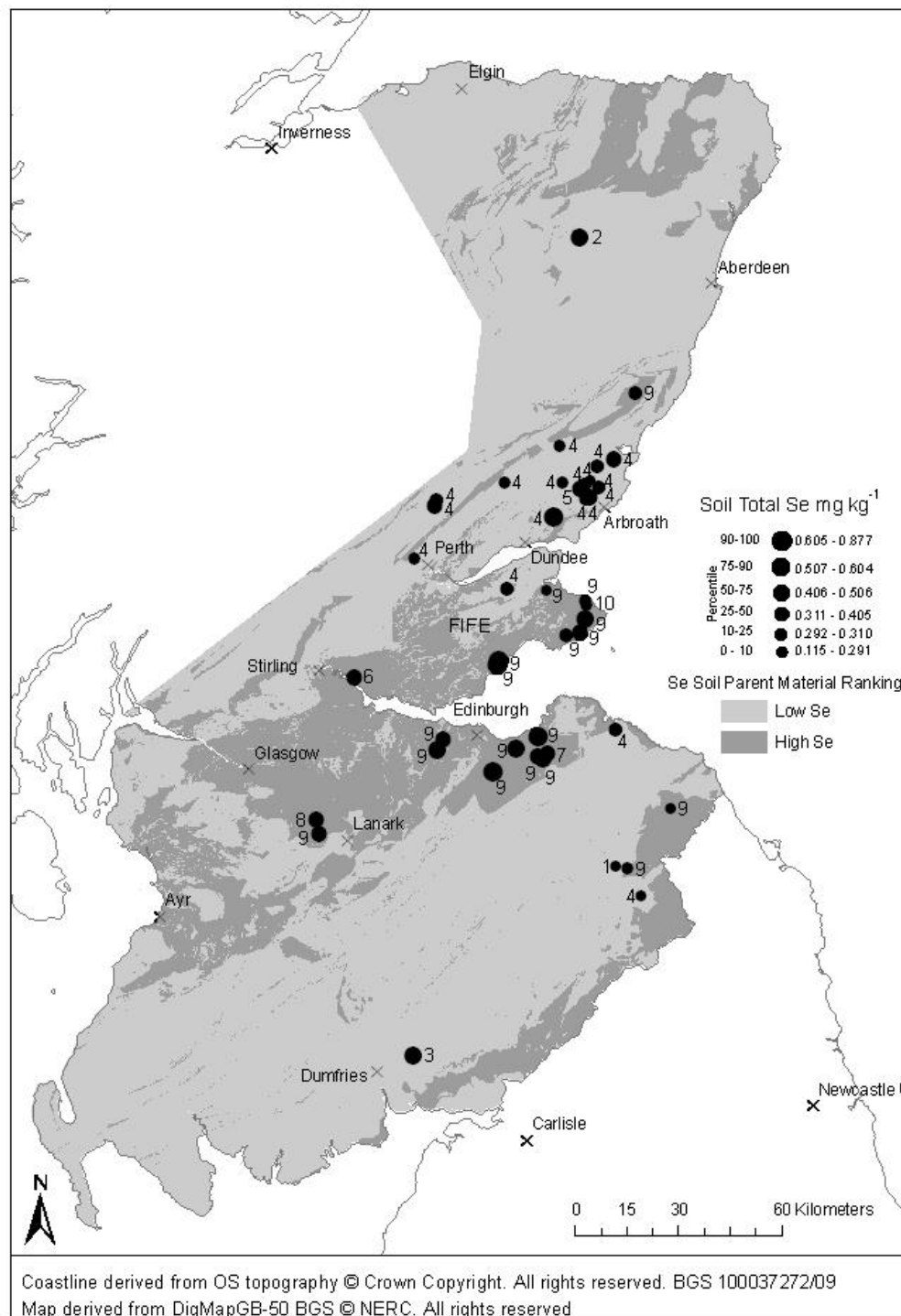Figure 2. Number of samples (replicates) required to achieve 80% statistical power per food commodity analyses based on 1.5 to 5 fold differences in total soil-Se concentration between high and low-Se areas.

Figure 3. Map of predicted high and low-Se areas in the main agricultural growing regions of Scotland, overlain with the total soil-Se concentration data obtained at the various sampling locations.
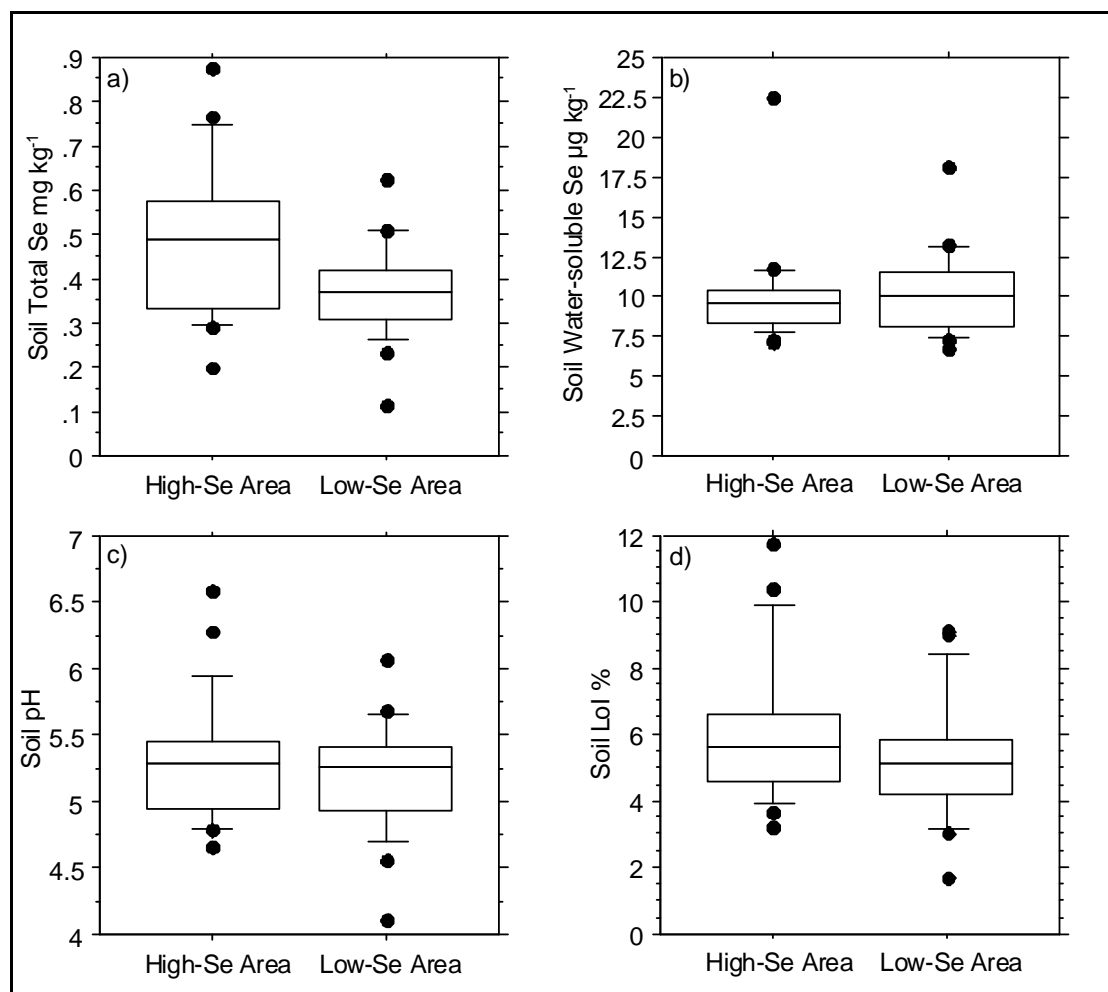
n each Se area = 22. Boxes show the median and interquartile range; whiskers show the 10th and 90th percentiles and points show outlying values in the data distribution

Figure 4. Box and whisker plots of soil a) total-Se, b) water-soluble Se, c) pH and d) LOI in the predicted high and low-Se areas.

Table 1. 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 2. Sampling design adopted for the project

Predicted Low (L) Se Soils	Predicted High (H) Se Soils
Main Study:	
Farm-1 (L): Wheat (x 8) + soil (x 8)	Farm-6 (H): Wheat (x 8) + soil (x 8)
Farm-2 (L): Beef (x 8) + soil (x 8) + grass (x 8)	Farm-7 (H): Beef (x 8) + soil (x 8) + grass (x 8)
Farm-3 (L): Milk (x 8) + soil (x 8) + grass (x 8)	Farm-8 (H): Milk (x 8) + soil (x 8) + grass (x 8)
Farm-4 (L): Potato (x 8) + soil (x 8)	Farm-9 (H): Potato (x 8) + soil (x 8)
Farm-5 (L): Calabrese (x 8) + soil (x 8)	Farm-10 (H): Calabrese (x 8) + soil (x 8)
Between-Farm Study:	
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 3. Quality assurance and quality control data obtained during the study

Soil Analysis	Certified Reference Material	Measured Value	Certified Value	Units	N
Total Se	SRM 1643e (CRM Solution)	11.29 ± 0.36	11.97 ± 0.14	µg L^{-1}	4
	GSS-4 (Soil)	0.60 ± 0.03	0.64 ± 0.14	mg kg^{-1}	8
Water-soluble Se	SRM 1643e (CRM Solution)	11.23 ± 0.24	11.97 ± 0.14	µg L^{-1}	4
	GSS-4 (Soil)	12.54 ± 0.32	nd	µg kg^{-1}	6
Soil pH	QC-1	6.98 ± 0.01	7.03 ± 0.11	pH	7
Soil LOI	LLC	2.82 ± 0.44	2.85 ± 0.90	%	3
	QC-1	8.21 ± 0.09	8.39 ± 0.33	%	3
Foodstuff Analysis					
Total Se	NIST 1548a (Typical diet)	252 ± 13	248 ± 28	µg kg^{-1}	4
	NIST 8435 (Whole milk powder)	130 ± 11	131 ± 14	µg kg^{-1}	2
	BCR 184 (Bovine muscle)	184	183 ± 12	µg kg^{-1}	1
	NIST 8414 (Bovine muscle)	78	76 ± 10	µg kg^{-1}	1
	NIST 8436 (Durum wheat)	1314	1230 ± 90	µg kg^{-1}	1
	NIST 1577a (Bovine liver)	741 ± 40	710 ± 70	µg kg^{-1}	2
	NIST 1515 (Apple leaves)	56	50 ± 9	µg kg^{-1}	1
	NIST1549 (Non-fat milk powder)	127 ± 3	110 ± 10	µg kg^{-1}	2
	Spike (1 µg)	91% ± 8	na	µg	7

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   na = not applicable

Table 4. Summary of the project soil results

Sample Type	Mean	Median	Range	Count
Total Soil-Se (mg kg^{-1})	0.444	0.433	0.115 – 0.877	114 from 44 farms
Water-soluble Soil-Se (µg kg^{-1})	11.59	10.51	6.69 – 26.78	114 from 44 farms
Soil pH	5.22	5.23	4.11 – 6.59	114 from 44 farms
Soil LOI (%)	6.47	5.66	1.71 – 14.30	114 from 44 farms

Table 5. Summary of the project commodity results

Sample Type	Low-Se Farm median (range)	High-Se Farm median (range)	Food Studies Elsewhere	Reference
Main Study:	n = 8 from 1 farm	n = 8 from 1 farm		
Calabrese Total Se (µg kg^{-1})	2.39 (1.51-2.65)	4.12 (2.65-7.45)	7^	TDS
			49.3-84.7	USA Farnham
Wheat Total Se (µg kg^{-1})	9.68 (3.57-14.43)	32.95 (25.01-62.70)	40 - > 60	EA Adams
			760	Canada Adams
Beef Total Se (µg kg^{-1})	94.96 (81.09-117.68)	135.69 (115.18-151.08)	140¬	TDS
Milk Total Se (µg kg^{-1})	16.07 (14.69-19.26)	19.56 (12.92-22.02)	14	TDS
Grass Total Se (µg kg^{-1})	6.58 (3.74-11.18)*	8.61 (3.42-22.24)*		
Between-Farm Study:	n = 18 from 18 farms	n = 18 from 18 farms		
Potatoes Total Se (µg kg^{-1})	1.49 (0.00-2.96)#	1.90 (1.25-9.71)#	< 10	TDS
			16	Barclay

Foodstuff and grass data from the present study are reported as fresh weight

* n = 16 from two farms; grass collected from one beef and one milk farm in each Se area

includes average Se concentration from the Main Study potato farm

Barclay = Mean Se concentrations in 18 samples of potatoes imported to the UK (Barclay et al., 1995)

Canada Adams = Mean Se concentration in wheat from 12 locations collected over 3 years, Manitoba, Canada (Adams et al., 2002)

EA Adams = Mean Se concentrations (dry weight) in bread making wheat from East Anglia (Adams et al., 2002)

TDS = UK Total Diet Survey 2006 (FSA, 2009) ^ Green vegetables   ¬ Carcass meat/meat product

USA Farnham = Range of Se concentrations in 30 calabrese varieties in the USA (Farnham et al., 2007)

Table 6. Pearson correlation coefficients for relationships between soil parameters in the combined Main Study and Between-Farm dataset

Comparison	Correlation Coefficient	p Value
Total Soil-Se vs Water-soluble Soil-Se	**0.46**	**<0.01**
Total Soil-Se vs Soil pH	0.13	0.38
Total Soil-Se vs Soil LOI	**0.76**	**<0.001**
Water-soluble Soil-Se vs Soil pH	-0.06	0.70
Water-soluble Soil-Se vs Soil LOI	**0.70**	**<0.001**
Soil pH vs Soil LOI	-0.08	0.61

n = 44; significant correlations are show in bold

Table 7. Regression results (ANOVA) between soil parameters and commodity Se concentrations.

Commodity Type	Soil Parameter	Correlation	Percentage Variance (R^2)	p Value	Number of Samples
Main Study					
Wheat	Total Se	0.85	72.8	<0.001	16
	LOI	0.64	15.8	<0.001	16
Calabrese	Total Se	-0.69 *	47.5	<0.01	16
Beef-farm grass	pH	0.81	47.7	<0.001	16
	Water-soluble Se	-0.55*	30.1	<0.01	16
Between-Farm Study					
Potato	Total Se	0.58	33.5	<0.001	36

* = inverse correlation between soil-Se and commodity-Se, but positive correlation with Se area