REGULAR ARTICLE

# Soil factors affecting selenium concentration in wheat grain and the fate and speciation of Se fertilisers applied to soil

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**Abstract** UK crops have a low selenium (Se) status, therefore Se fertilisation of wheat (*Triticum aestivum* L.) at 10 field sites was investigated and the effect on the content and speciation of Se in soils determined. Soil characterisation was carried out at each field site to determine the soil factors that may influence wheat grain Se concentrations in unfertilised plots. Soil samples were taken after harvest from each treatment to determine the fate and speciation of selenate fertiliser applied to soil. Wheat grain Se concentrations could be predicted from soil Se concentration and soil extractable sulphur (S) using the following

Responsible Editor: Peter Christie.

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regression model: Grain Se=$a$+$b$(total soil Se)+$c$ (extractable soil Se) - $d$(extractable soil S), with 86 % of the variance being accounted for, suggesting that these properties control Se concentrations in grain from unfertilised plots. Extractable soil Se concentrations were low (2.4 – 12.4 µg kg$^{-1}$) and predominantly consisted of selenite (up to 70 % of extractable Se) and soluble organic forms, whereas selenate was below the detection limit. Little of the added Se, in either liquid or granular form was left in the soil after crop harvest. Se fertilisation up to 20 g ha$^{-1}$ did not lead to a significant Se accumulation in the soil, suggesting losses of Se unutilised by the crop.

## Introduction

Selenium (Se) is an essential micronutrient for humans and animals; needed for hormone regulation and the immune system. However, the intake of Se by the UK population is below the reference nutrient intake, which is a cause for concern (Broadley et al. 2006). An important source of Se in diets is wheat grain and its products, which contributes 22 % of dietary Se in the UK (Rayman 2000). However concentrations of Se in European wheats are generally low (Hawkesford and Zhao 2007), likely to be caused by low Se supply from the soil.

An important factor in soil Se supply to plants is the Se content of parent rocks (Spadoni et al. 2007), which controls soil Se concentrations. Excessive soil Se concentrations (>3 mg kg$^{-1}$) occur in areas of North America, China and Ireland, whereas deficient soil Se concentrations (<0.125 mg kg$^{-1}$) occur in Siberia, New Zealand and the Keshan area of China (Broadley et al. 2006). Total Se concentrations in UK soils range between 0.1 – 4 mg kg$^{-1}$ with 95% of the samples containing <1.0 mg kg$^{-1}$ (Broadley et al. 2006). Another important factor is plant availability as Se bioavailability generally decreases with decreasing pH and the increased content of organic matter, clay minerals and iron hydroxides (Gissel-Nielsen et al. 1984). Furthermore, the interactions of Se with soil components are dependent on Se speciation; in aerobic soils, inorganic Se exists as either selenite or selenate. Selenite, the dominant Se species under moderate redox soil conditions (Elrashidi et al. 1987), is strongly adsorbed by iron and aluminium oxides and hydroxides, resulting in limited plant availability and a low potential for leaching. Selenate is found in soils with high redox conditions (Elrashidi et al. 1987) and is weakly adsorbed by electrostatic forces of attraction (Jackson and Miller 1999), resulting in high plant availability and potential for leaching. It is therefore important to identify the factors controlling Se bioavailability in areas where concentrations of Se in plants are considered to be low for human and animal nutrition.

Se fertilisation of crops has been shown to significantly enhance Se concentrations in crops grown in soils with low plant available Se concentrations. Finland has successfully applied agronomic biofortification with Se since 1984, with the annual input of Se fertiliser dose now being 10 g Se ha$^{-1}$ (Hartikainen 2005). However, the fate of Se fertiliser in Finnish soils is not accounted for, considering <20 % of applied selenate is taken up by the crop (Keskinen et al. 2009). It is assumed that selenate is reduced and immobilised in the soil (Keskinen et al. 2009), which is supported by evidence of a limited residual effect. For example, the application of 10 g Se ha$^{-1}$ selenate to cereals grown in a Canadian soil was found to have a negligible residual effect after 1 year (Gupta 1995). However, to monitor the fate of Se fertiliser in soil directly, changes in the concentration and speciation of Se in soil should be determined.

The aim of this study was to investigate the role of soil properties in influencing Se concentration in wheat grain and the fate and speciation of Se fertilisers in the soils. Crop responses to Se fertilisation are reported in a companion paper (Broadley et al. 2010). The soils of the experimental sites had a wide range of physico-chemical properties, and different Se fertilisation regimes. Soil characterisation and changes in Se concentrations and speciation before and after Se fertilisation were determined.

## Materials and methods

### Treatments and experimental design

Full details of experimental design, treatments and wheat (*Triticum aesticum* L.) cultivation are described

in a companion paper (Broadley et al. 2010). Briefly, there were two types of experiments testing either liquid or granular Se fertilisation. For the liquid fertilisation experiment (LF series), Se was applied at doses 0 – 100 g Se ha$^{-1}$ as $Na_2SeO_4$ at four field-trial sites. For the granular fertilisation experiment (GF series), Se was applied in commercial granular fertiliser forms, using Selcote Ultra® ($BaSeO_4$: $Na_2SeO_4$) and Top Stock® at 10 g Se ha$^{-1}$ at six field-trials sites.

Soil sampling

Soil samples were collected from all of the field trials in 2006 and 2007 to investigate the influence of soil properties on the variation in crop Se uptake. Samples were collected at 2 time points during each year; just before Se addition to determine soil properties, and after wheat harvest to determine the fate of Se fertilisers in soil. The protocol for soil sampling before Se addition (late March – early April) was to collect 10 soil cores (in a W shape) and bulk into one sample from each block ($n=4$), to provide representative samples for characterisation of soil properties. To investigate changes down the soil profile, the soil cores were taken from three depths, 0–30, 30–60 and 60–90 cm. The protocol for soil sampling after wheat harvesting (August – September) was to collect five soil cores and bulk into one sample from each of selected plots ($n=24$). All plots were sampled from the granular trials, and in the liquid trials, the zero, intermediate and high Se doses were chosen. All soil samples were air dried at room temperature (20°C) in drying cabinets. Stones and roots were removed and air dried soils were sieved (<2 mm) using a roller mill. A portion of each soil was finely ground (< 0.45 mm) using an agate ball mill (Retch PM 400 mill). Sieved soils were stored in sealed polythene bags at room temperature and finely ground soils were stored in 10 ml screw-top glass vials at room temperature before analysis.

Soil properties

Soil properties were determined using the soil samples taken before Se addition. Available phosphorus (P) was determined by using a sodium bicarbonate extraction (Olsen P method). Soil pH was determined using a calibrated pH meter (ratio 10 g soil: 25 ml

deionised $H_2O$). Total nitrogen (N) and carbon (C) were measured using the combustion method (LECO CNS 2000). Particle size analysis was determined gravimetrically.

Total S and Se concentrations in soil

Total soil Se and S concentrations were determined in all soil samples taken before Se addition and after harvest. Finely ground soil (*ca*. 0.25 g) was digested with *aqua regia* in a heating block (McGrath and Cunliffe 1985). For quality assurance, analytical blanks, certified reference material (NIST 2711 Montana soil) and a sample repeat in every 10 samples were used. Concentrations of Se were determined using Inductively Coupled Plasma — Mass Spectrometry (ICP-MS) (Agilent7500ce Octopole Reaction System, Palo Alto, CA, USA). The sample introduction system consisted of a micromist glass concentric nebuliser, quartz scott type double pass spray chamber at 2°C, and nickel sample (1 mm) and skimmer (0.4 mm) cones. Operating parameters were optimised daily using a tuning solution containing 1 µg l$^{-1}$ Ce, Li, Te and Y. Other instrument conditions were RF forward power of 1550 W, sample depth 8.0 mm from load coil, carrier gas flow rate 0.89 1 min$^{-1}$, spray chamber 2°C. Hydrogen (4 ml min$^{-1}$) was used as the reaction gas to eliminate Ar polyatomic interferences on $^{78}Se$ and $^{80}Se$ which were used for Se quantification. An internal standard (500 µg l$^{-1}$ Ge) was used to correct for signal drift. External calibration was performed using external standards prepared from 1000 mg kg$^{-1}$ single element stock solutions in 5% $HNO_3$. Concentrations of S were determined by Inductively Coupled Plasma — Atomic Emission Spectrometry (ICP-AES) (ARL, Vallaire, Ecublens, Switzerland). The analytical procedures gave satisfactory values for the standard reference material NIST 2711, Montana soil; Se 1.42±0.03 mg Se kg$^{-1}$ (certified value 1.52 ± 0.14 mg Se kg$^{-1}$); S 0.418±0.02 mg S kg$^{-1}$ (certified value 0.42±0.01 g S kg$^{-1}$).

Extractable S and Se concentrations in soils

Extractable S and Se in all soil samples taken before Se application and after harvest were determined using $KH_2PO_4$ (0.016 m*M*, pH 4.8) extractions (ratio 10 g dry weight soil : 30 ml $KH_2PO_4$ w/v), (Zhao and

McGrath 1994), and the concentrations of Se and S were determined by ICP-MS or ICP-AES as described above.

## Speciation of Se in soil extracts

Selected $KH_2PO_4$ extracts were further analysed to determine Se speciation. A portion (1.5 ml) of $KH_2PO_4$ soil extract was filtered (0.45 μm) into a screwtop borosilicate glass vial for Se speciation analysis by LC-ICP-MS as described by Stroud et al. (2009). The LC-ICP-MS interface consisted of a minimal length of polyetheretherketone (PEEK) tubing. Chromatographic separation was carried out using an Agilent 1100 series HPLC system (Palo Alto, CA, USA). The anion exchange analytical column was a Dionex Ion Pack AS14 (9 μm particle size, 4 mm×250 mm id), fitted with a guard column (AG14). The mobile phase was 6 m$M$ $Na_2CO_3$ (pH 9.5) premixed with 2% (v/v) methanol to improve sensitivity. The injection volume was 50 μl and the mobile phase was delivered at 1 ml min$^{-1}$ isocritically. The identification of Se species was determined by comparison with retention times of standard compounds. Quantification of Se species was determined using external calibration curves and peak area measurements. Duplicate samples were measured with calibration, reagent blanks and calibration standards analysed at the start of the run were repeated at the end of the run for quality control.

## Selenium concentrations in wheat grains in unfertilised control plots

See the companion paper (Broadley et al. 2010) for grain sampling methodology and Se analysis. Samples from unfertilised control plots were used ($n$=4 per field trial).

## Data analysis

All soil Se and S concentrations are reported on a dry weight basis. Genstat (2007, 10th addition, VSN International Ltd., UK) was used for all statistical analysis. Analysis of variance was carried out to assess the significance of the treatments and multiple linear regression was used to analyse the relationship between soil properties and grain Se concentrations.

## Results

### Soil properties of the field sites

Soil characteristics from the field trials are shown in Table 1. The trials were conducted on field sites varying in soil textures, from silty clay to sandy loam. Topsoil properties varied at each site, with the range of extractable P 16.6 – 97.4 mg kg$^{-1}$; soil pH 5.56 – 8.0; total N 0.16 – 0.22 %, total C 1.93 – 4.28 %. One soil sample was taken to represent the field sites GF5 and LF3 as they had the same geographical location. Also included is the Rothamsted site which was used to investigate the impact of S and Se fertilisation on wheat grain Se concentrations (Stroud et al. 2010); the data for the control plots of this experiment were included for regression analysis.

### Total and extractable S and Se concentrations in soils

Before application of Se fertilisers in GF or LF trials, total Se concentrations in the topsoil ranged between 245 – 590 μg Se kg$^{-1}$, with LF2 having the highest total soil Se concentration (Table 1). Among the 10 experimental sites, seven sites (LF1, LF2, LF4, GF1, GF2, GF3, GF5) showed a decreasing total Se concentration down the soil profiles (0 – 90 cm), whereas the other three sites (GF5, LF3 and Rothamsted) showed no clear trend. Extractable Se (by potassium dihydrogen phosphate) accounted for 1.1 – 3.4% of total Se. Generally, the GF trials had extractable Se concentrations that decreased down the soil profiles, whereas no change down the soil profile was found in LF trials.

Total S concentrations in the topsoil ranged from 313 to 816 mg S kg$^{-1}$, with GF5 and LF3 having the highest concentration. GF5 and LF3 also showed a significant increase in total S concentration down the soil profile to 2060 mg S kg$^{-1}$, whereas the other sites showed a decrease in total soil S down the soil profile. Extractable S (by potassium dihydrogen phosphate) generally decreased down the soil profile.

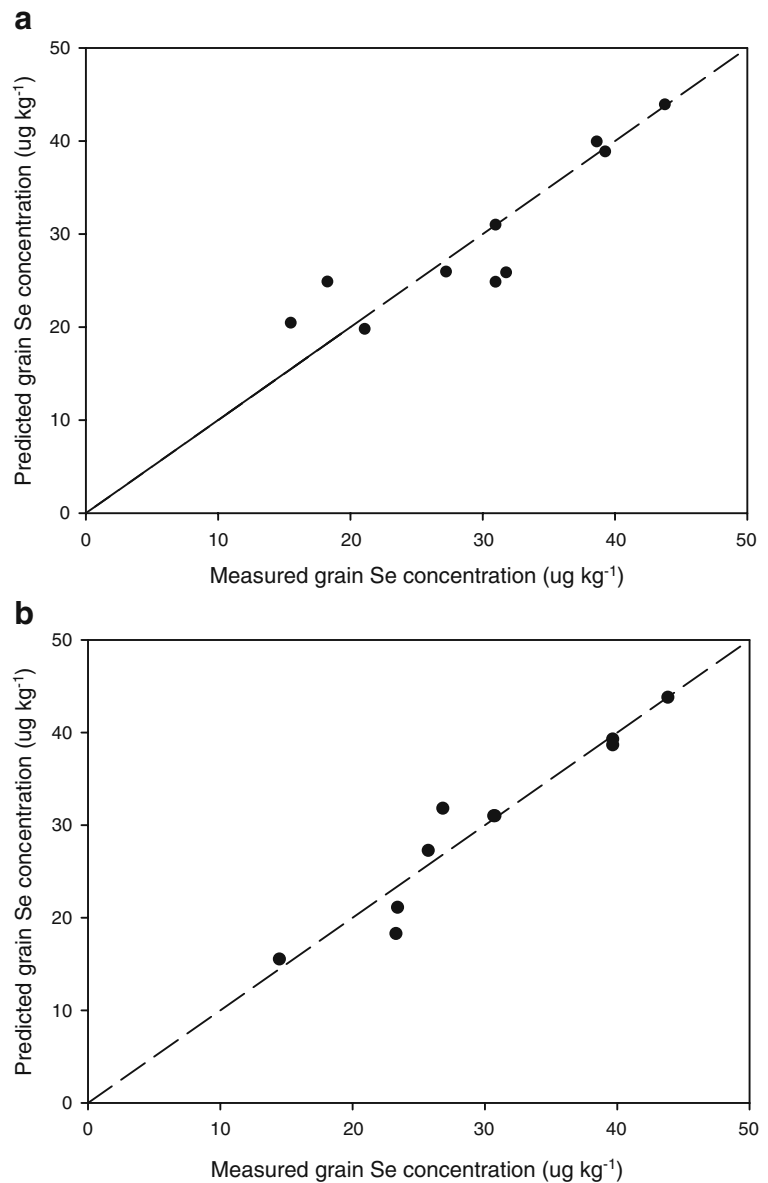### Influence of soil properties on grain Se concentrations from control plots

In all but one field trial (LF2), the control plots (i.e. not receiving Se fertiliser) yielded grain Se concentrations ranging between 15.5 – 43.8 μg kg$^{-1}$. These

**Table 1** Soil properties of the field trial sites with granular (GF) or liquid (LF) Se application (mean±SE)

| Trial site and soil texture | Depth (cm) | Extractable P (mg kg$^{-1}$) | pH | Total N (%) | Total C (%) | Extractable Se (µg kg$^{-1}$) | Total Se (µg kg$^{-1}$) | Extractable S (mg kg$^{-1}$) | Total S (mg kg$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| GF1 Silty clay | 0 – 30 | 14.2±3.1 | 7.99±0.10 | 0.212±0.012 | 4.28±0.11 | 7.7±0.7 | 447±34.1 | 8.1±0.9 | 415±4.16 |
| | 30 – 60 | 4.6±0.4 | 8.27±0.10 | 0.064±0.016 | 4.89±1.18 | 4.2±0.7 | 220±57.1 | 7.1±2.8 | 220±13.2 |
| | 60 – 90 | 5.8±1.7 | 8.42±0.16 | 0.034±0.014 | 4.28±0.36 | 2.1±0.6 | 143±81.7 | 4.9±1.9 | 485±8.90 |
| GF2 Clay loam | 0 – 30 | 19.1±0.7 | 7.90±0.06 | 0.175±0.005 | 2.54±0.19 | 9.2±0.5 | 469±31.3 | 6.4±0.3 | 353±11.6 |
| | 30 – 60 | 4.9±0.2 | 8.28±0.09 | 0.072±0.012 | 3.17±0.81 | 6.8±1.3 | 315±41.8 | 4.1±0.2 | 225±18.4 |
| | 60 – 90 | 3.1±0.7 | 8.52±0.11 | 0.210±0.006 | 4.09±0.33 | 2.4±0.6 | 173±9.29 | 3.5±0.3 | 237±4.80 |
| GF3 Sandy loam | 0 – 30 | 86.0±4.6 | 6.72±0.21 | 0.216±0.029 | 2.90±0.59 | 5.9±0.5 | 382±69.42 | 8.3±1.0 | 459±78.3 |
| | 30 – 60 | 36.7±4.1 | 7.22±0.10 | 0.069±0.014 | 0.91±0.20 | 7.5±0.8 | 341±40.57 | 6.8±1.0 | 165±33.4 |
| | 60 – 90 | 6.1±0.9 | 7.50±0.18 | 0.023±0.007 | 0.21±0.12 | 5.1±1.5 | 205±19.26 | 9.1±1.5 | 69.1±17.8 |
| GF4[a] Silty clay | 0 – 30 | 23.0 | 7.69 | 0.26 | 2.61 | 12.4 | 363 | 9.6 | 409 |
| | 30 – 60 | 5.2 | 8.08 | 0.13 | 1.36 | 8.3 | 353 | 9.8 | 219 |
| | 60 – 90 | 7.8 | 8.05 | 0.08 | 1.40 | 6.7 | 221 | 5.4 | 132 |
| GF5, LF3[a] Clay loam | 0 – 30 | 76.2 | 6.38 | 0.22 | 2.48 | 6.8 | 527 | 9.6 | 816 |
| | 30 – 60 | 43.2 | 6.43 | 0.11 | 1.17 | 7.5 | 429 | 6.1 | 593 |
| | 60 – 90 | 22.2 | 7.55 | 0.05 | 0.49 | 2.7 | 531 | 6.0 | 2060 |
| GF6 Sandy loam | 0 – 30 | 95.8±1.8 | 7.04±0.1 | 0.16±0.00 | 1.97±0.03 | 5.4±0.2 | 300±35.0 | 4.0±0.3 | 313±7.23 |
| | 30 – 60 | 79.1±2.7 | 7.28±0.08 | 0.07±0.01 | 0.88±0.17 | 6.3±0.5 | 213±21.2 | 2.4±0.2 | 144±25.8 |
| | 60 – 90 | 33.7±5.1 | 7.44±0.18 | 0.03±0.00 | 0.25±0.02 | 7.2±0.9 | 209±13.6 | 2.8±0.3 | 59.5±3.61 |
| LF1 Sandy Loam | 0 – 30 | 72.5±8.9 | 6.77±0.13 | 0.173±0.007 | 2.07±0.14 | 6.7±0.8 | 352±37.3 | 8.1±0.6 | 329±14.6 |
| | 30 – 60 | 26.3±10.0 | 7.20±0.08 | 0.073±0.014 | 0.88±0.23 | 8.6±0.6 | 389±44.4 | 9.0±2.2 | 148±27.9 |
| | 60 – 90 | 8.7±5.5 | 7.38±0.05 | 0.028±0.001 | 0.26±0.03 | 6.3±0.4 | 285±28.1 | 11.2±3.3 | 68.8±8.50 |
| LF2 Sandy clay | 0 – 30 | 25.9±6.6 | 7.77±0.17 | 0.197±0.02 | 1.93±0.20 | 9.3±0.4 | 590±56.6 | 4.9±0.2 | 313±30.4 |
| | 30 – 60 | 11.2±3.6 | 8.02±0.15 | 0.114±0.008 | 1.48±0.23 | 10.6±1.5 | 558±25.0 | 6.5±0.7 | 231±12.8 |
| | 60 – 90 | 3.6±0.7 | 8.28±0.05 | 0.041±0.01 | 0.25±0.46 | 9.5±2.6 | 315±108 | 15.5±1.3 | 167±66.3 |
| LF4 Sandy loam | 0 – 30 | 97.4±1.3 | 7.12±0.10 | 0.16±0.00 | 2.16±0.06 | 7.3±0.7 | 245±6.93 | 5.0±0.3 | 318±12.9 |
| | 30 – 60 | 61.0±4.7 | 7.25±0.13 | 0.07±0.01 | 0.78±0.17 | 11.4±0.6 | 208±16.7 | 4.1±0.2 | 134±20.9 |
| | 60 – 90 | 11.2±1.2 | 7.51±0.10 | 0.10±0.04 | 0.27±0.01 | 6.2±0.7 | 169±7.77 | 5.9±0.4 | 67.2±0.94 |
| Rothamsted Clay loam | 0 – 30 | 16.6±1.4 | 5.56±0.06 | 0.225±0.003 | 2.43±0.05 | 5.9±0.4 | 556±13.4 | 6.4±0.9 | 340±3.60 |
| | 30 – 60 | 5.2±1.3 | 5.77±0.28 | 0.098±0.013 | 0.90±0.15 | 6.8±0.7 | 588±36.6 | 14.2±4.6 | 170±17.2 |
| | 60 – 90 | 6.0±0.3 | 5.98±0.24 | 0.071±0.007 | 0.66±0.06 | 6.2±1.1 | 540±36.2 | 21.3±4.5 | 138±14.6 |

[a] No standard errors for GF4 and GF5 due to the bulking of soil samples from different blocks

**Fig. 1** Relationship
between measured and fitted
values of Se concentration
in wheat grains using Eq.
[1] **a** and Eq. [2] **b**. The
dotted line represents a 1:1
relationship. LF2 was not
included in this regression
analysis



values reflect typical UK grain concentrations; in a
country-wide survey, Adams et al. (2002) showed that
88 % of UK wheat grain samples contain <50 μg Se
kg$^{-1}$. The influence of soil properties on grain Se
concentrations was investigated. Soil profile (0 –
90 cm) data were weighted with relation to the wheat
root distribution. The proportions used to calculate
weighted soil property data were based on the root
distribution of a mature wheat plant: 70 % in the
topsoil (0 – 30 cm), 25 % at 30 – 60 cm and 5 % at 60
– 90 cm (Hoad et al. 2001). Multiple linear regression

analysis was applied to identify soil properties that
could be used to fit a model to describe grain Se
concentrations. Initial multiple linear regression in-
cluded the soil properties measured in the spring
samples collected before Se additions: particle size
analysis, available P, pH, total N, total C, extractable
S and Se, and total S and Se. Homogeneity of
variances was checked and no log transformations
were required. Of all variables tested in the multiple
regression analysis, the combination of extractable S
and total Se resulted in a highly significant regression

model that explained 70.5 % of the variance in grain Se concentration. The model obtained was:

$$
\begin{aligned}
\text{Gain Se concentration } (\mu g\,kg^{-1}) \\
= 7.66 + 0.0954 \times (\text{total soil Se}(\mu g\,kg^{-1})) \\
- 1.530 \times \text{S} (\text{extractable soil S } (mg\,kg^{-1})); \quad (1)
\end{aligned}
$$

$R^2_{adj} = 0.70, p = 0.006, SE = 5.09, n = 10$

Figure 1a shows the fit between the observed and fitted grain Se values using regression model [1].

The inclusion of extractable Se in the model [1] resulted in a highly significant regression model that explained 86.1% of the variance in grain Se concentration. The model obtained was:

$$
\begin{aligned}
\text{Grain Se concentration } (\mu g\,kg^{-1}) = -10.32 \quad (2) \\
+ 0.1085 \times (\text{total soil Se}(\mu g\,kg^{-1})) - 1.916 \\
\times (\text{Extractable soil S } (mg\,kg^{-1}) + 2.515 \\
\times (\text{Extractable soil Se } (\mu g\,kg^{-1}); R^2_{adj} = 0.86, \\
p = 0.004, SE = 3.46, n = 10
\end{aligned}
$$

Figure 1b shows the fit between the observed and fitted grain Se values using this regression model.

Soil and grain data from site LF2 were not included in regression analysis due to the very high grain Se concentration of $183\,\mu g\,Se\,kg^{-1}$, which is atypical of UK wheat (Adams et al. 2002). This particularly high grain Se concentration cannot be explained by the soil properties that were measured, indicating that unknown environmental factors or experimental error (such as liquid fertiliser spray drift) was the cause. It was considered to be appropriate to exclude this data point in order to investigate soil factors affecting typical UK grain Se concentrations.

Fate and speciation of Se fertiliser in soil

Soil samples were taken after harvest and extractable and total soil Se concentrations were compared between the control and fertilised plots in both the LF and GF trials, in order to determine the fate of Se fertiliser in soil. Figures 2 and 3 show the total soil Se concentrations in the control and Se-treated plots of each GF trial or LF trial respectively. No significant ($p > 0.05$) differences between the control and treated plots in total soil Se concentrations were found in
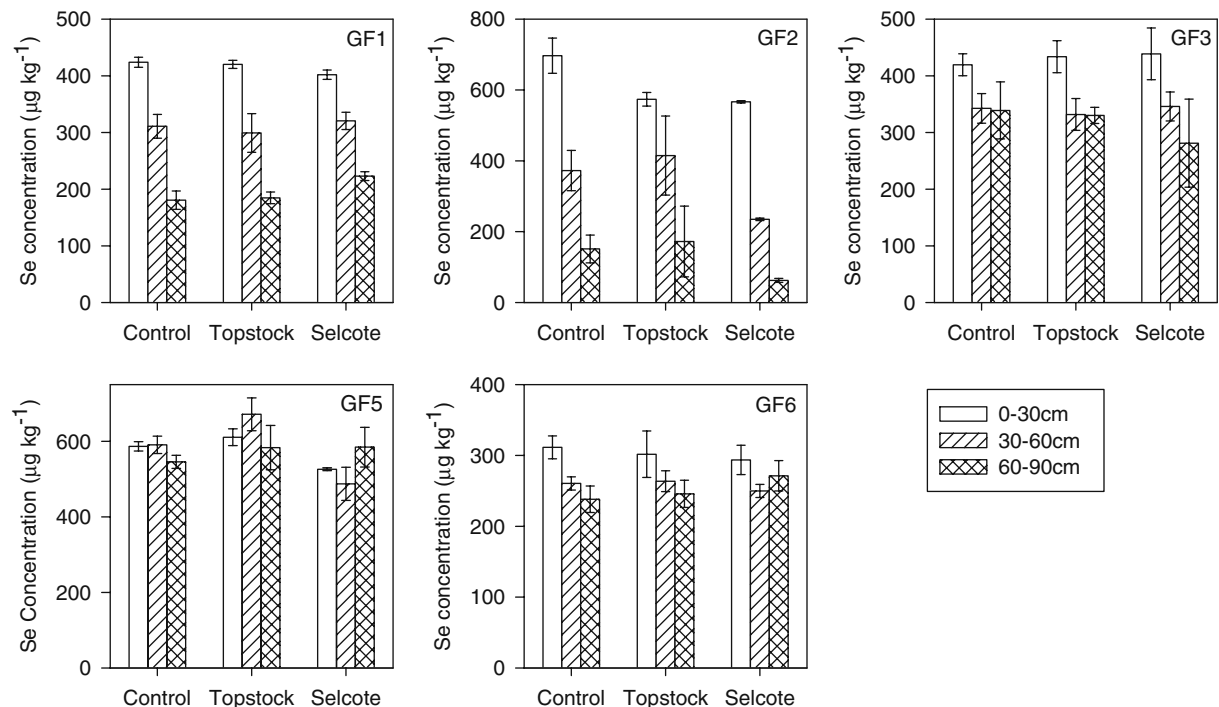


Fig. 2 Total Se concentrations in soil after harvest in field trials receiving granular Se fertiliser application (10 g Se ha$^{-1}$). GF4 has not been included because soil was not sampled after harvest. Data are means±SEs ($n=4$)

either the GF or LF trials down the soil profile (0 – 90 cm). Figures 4 and 5 show extractable soil Se concentrations in the control and treated plots in each GF and LF trial, respectively. In terms of extractable Se concentrations, no significant differences between the control and treatment plots were found for any GF or LF trials except for LF3. Only LF3 showed a statistically significant ($p=0.03$) increase in extractable Se concentrations, with an increase of 4 µg kg$^{-1}$ Se in the 50 g Se ha$^{-1}$ plots compared with the control plots.

Selected potassium dihydrogen phosphate extracts were analysed to determine the concentration and speciation of inorganic Se (Table 2). Selenate was not detectable in the soil extracts at any sampling time (before fertilisation and after harvest). Selenite was the inorganic species found at all depths, at all sites in the LF and GF trials. The concentrations of selenite in the topsoil (0 – 30 cm) ranged between 0.94 – 5.36 µg Se kg$^{-1}$ soil, accounting for 13 % – 70 % extractable Se. The remainder of extractable Se was likely to be soluble organic Se (Stroud et al. 2009).

## Discussion

The total Se concentrations (0.2 – 0.6 mg kg$^{-1}$) in the topsoil (0–30 cm) at the 10 field sites are generally low and in line with the British Geological Survey which has identified that >95% of UK soils have < 1 mg kg$^{-1}$ Se (Broadley et al. 2006). Whilst these soils have total Se concentrations similar to the world average of 0.4 mg kg$^{-1}$ (Fordyce 2005), the concentrations of Se in wheat grain were below the 50 – 100 µg Se kg$^{-1}$ level required for adequate human nutrition (Gissel-Nielsen et al. 1984) in all but one field trial. It thus appears that soil Se bioavailability was an important factor controlling Se concentrations in wheat grains at these trial sites.

Martens and Suarez (1997) used phosphate ($KH_2PO_4$) extractions to estimate plant available Se in soil. The proportion of total Se which was extractable with $KH_2PO_4$ from topsoils in this study was low (1.1 – 3.4 %) and similar to other findings for low Se soils. Finnish agricultural soils are reported
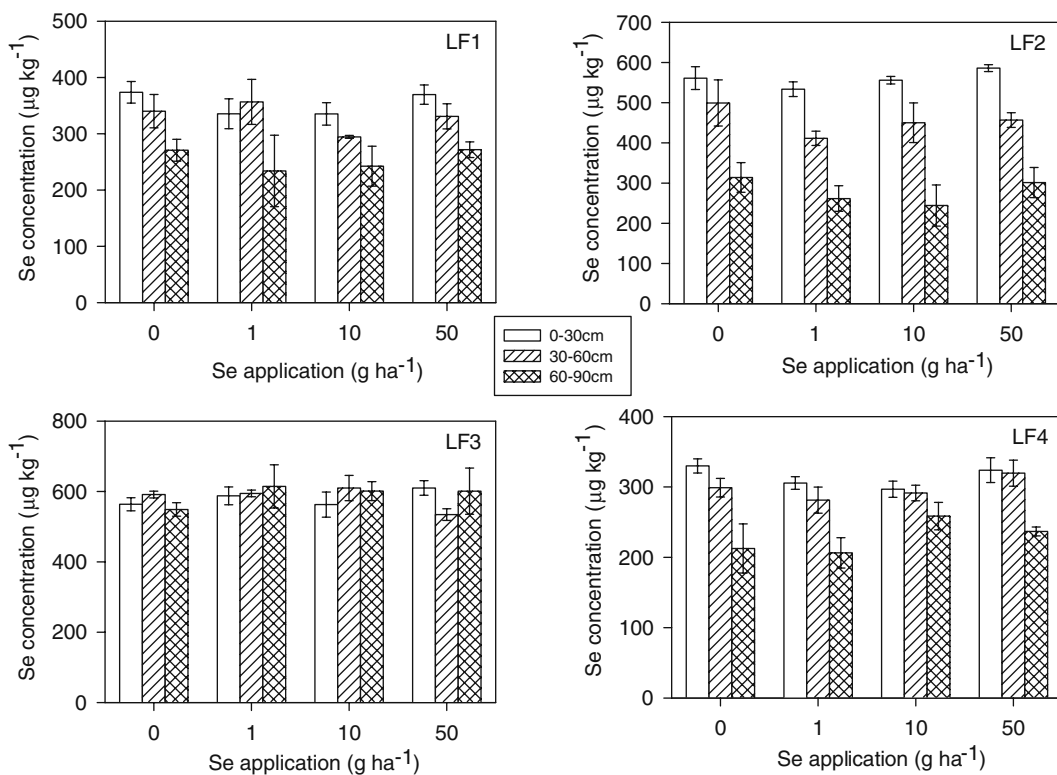


Fig. 3 Total Se concentrations in soil after harvest in field trials receiving liquid Se fertiliser application. Data are means±SEs ($n=4$)

to have an ammonium-disodium-EDTA- extractable Se concentration of up to 5 % total Se (Sippola 1979). Ylaranta (1983) found a 4.3 % extractable Se fraction in mineral soils using hot water. Further, Ylaranta (1983) reported extractable soil Se concentrations of 2 – 27 μg kg$^{-1}$, concentrations considered to be too low to produce crops with sufficient Se for human nutrition (Gissel-Nielsen et al. 1984). This agrees with the findings of this study both for extractable Se (<12.4 μg Se kg$^{-1}$) and grain Se (<50 μg Se kg$^{-1}$).

Soil properties are an important factor controlling Se bioavailability to plants; therefore, multiple regression analysis was applied to help identify soil factors that may influence grain Se concentrations in control plots of both GF and LF trials. Total soil Se concentrations and extractable soil S concentrations together could predict grain Se concentrations in the field trials reasonably well (Fig. 1a). Inclusion of soil extractable Se further improved the regression model (Fig. 1b). These findings are based on our field trials and may not be applicable to other regions in the world where Se bioavailability may be influenced by other soil factors. The coefficients for both total and

extractable Se were positive, indicating their positive influence on the Se supply to plants. In contrast, the coefficient for extractable S was negative, which is consistent with the antagonistic effect of sulphate on selenate uptake by plants (Adams et al. 2002; Bell et al. 1992; Broadley et al. 2006; Hopper and Parker 1999; Terry et al. 2000). This effect also implies that the main form of Se taken up by wheat is selenate, because uptake of selenite is not inhibited by sulphate (Hopper and Parker 1999; Li et al. 2008). Yet, surprisingly (considering the high soil pH), selenate was not detectable in the phosphate extracts from any of the soils in our trials. Selenite was detectable in all soils, and it is likely that some soluble organic Se was also present in the extracts (Stroud et al. 2009), as the concentration of total soluble Se was greater than selenite (Tables 1 and 2). It is known that selenite is strongly adsorbed by soil minerals and thus has a low bioavailability to plants, whereas selenate is much more bioavailable (Gissel-Nielsen et al. 1984). It is possible that selenate present at below the detection level of LC-ICP-MS may be sufficient to explain the uptake by plants in the control plot. Total Se uptake
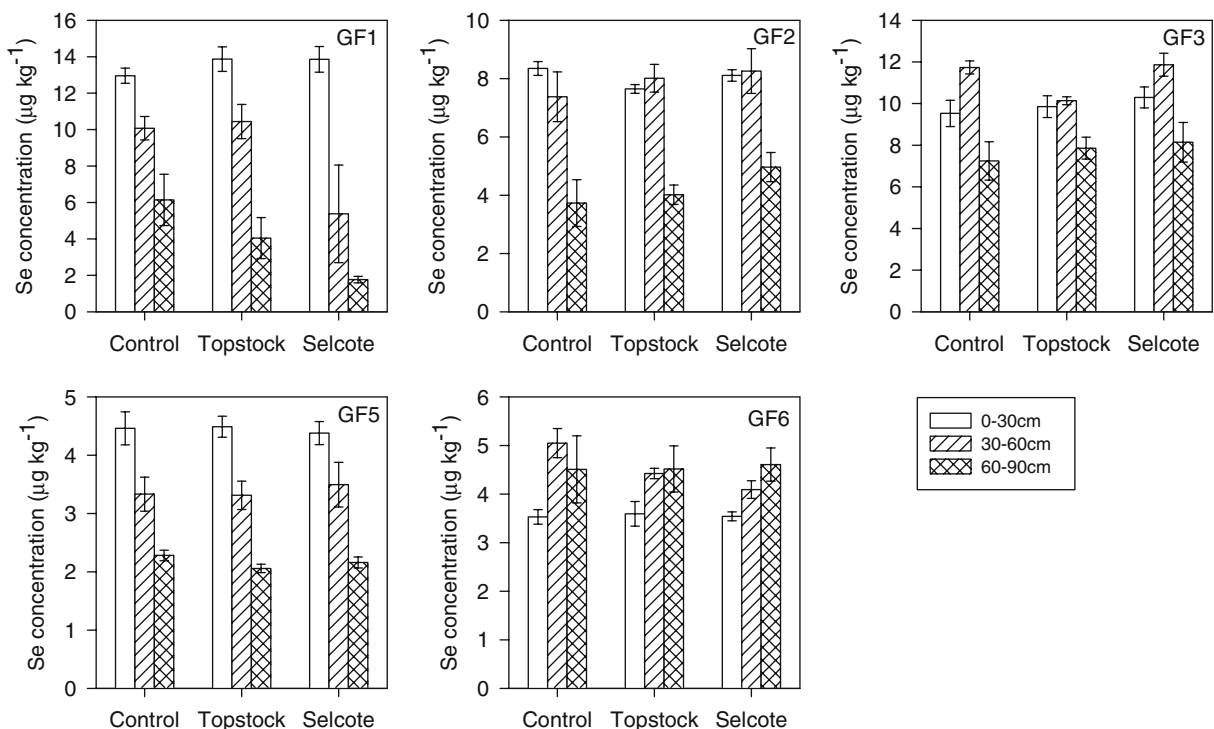


Fig. 4 Extractable Se concentrations in soil after harvest in field trials receiving granular Se fertiliser application (10 g Se ha$^{-1}$). GF4 has not been included because soil was not sampled after harvest. Data are means±SEs (*n*=4)
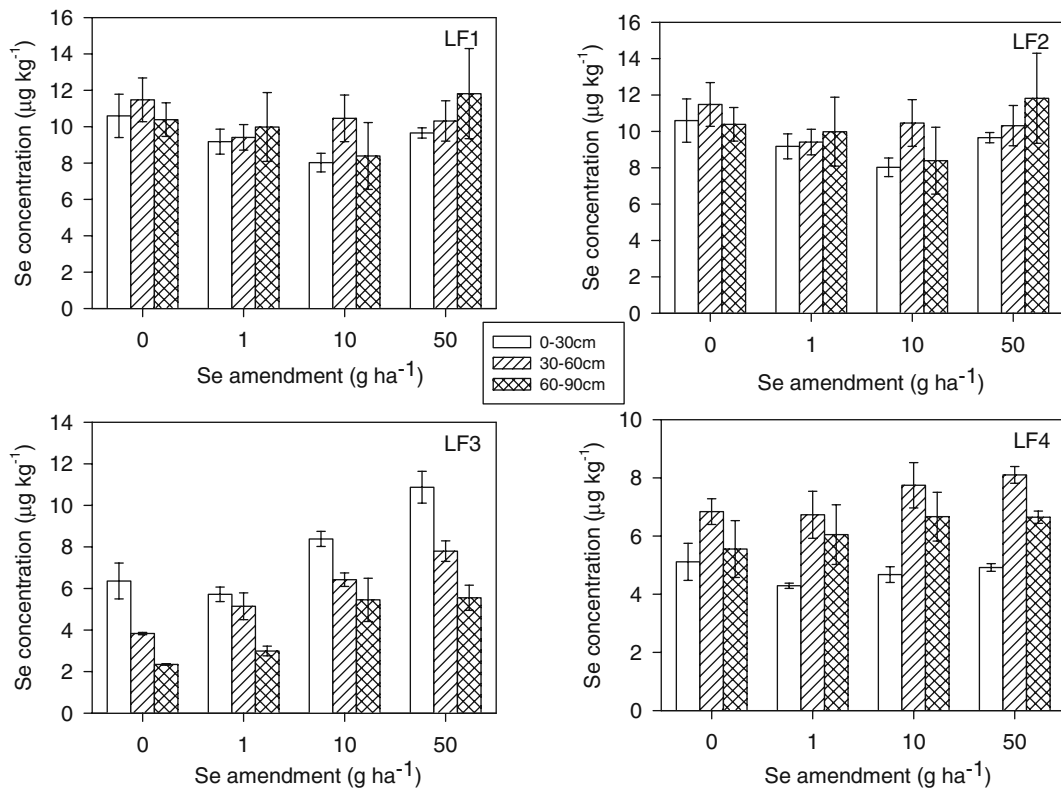
**Fig. 5** Extractable Se concentrations in soil after harvest in field trials receiving liquid Se fertiliser application. Data are means±SEs (n=4)

by wheat crops from the control plots was very small (0.5 – 1.0 g ha$^{-1}$), which is equivalent to concentrations of Se in the topsoil (0–30 cm) of 0.13 – 0.25 µg kg$^{-1}$ (assuming a bulk density of 1.3 g cm$^{-3}$),

or 0.04 – 0.08 µg kg$^{-1}$ if roots access available Se from the 0–90 cm depth. Soil extractions result in a 3-fold dilution, and low concentrations (<0.05 µg l$^{-1}$) are below or bordering the detectable limit of the LC-

**Table 2** Se speciation in 0.016 M KH$_2$PO$_4$ soil extracts of selected samples (mean±SE)

| Trial | Sampling time | Treatment | Soil depth (cm) | Selenite (µg kg$^{-1}$) |
|-------|---------------|-----------|-----------------|-------------------------|
| GF1 | After harvest | Control | 0 – 30 | 5.36±1.31 |
| GF1 | After harvest | Control | 30 – 60 | 5.43±0.63 |
| GF1 | After harvest | Control | 60 – 90 | 3.79±10.2 |
| GF1 | After harvest | Selcote Ultra® | 0 – 30 | 1.88±1.04 |
| GF1 | After harvest | Top Stock® | 0 – 30 | 2.21±0.52 |
| LF1 | After harvest | 0 g Se ha$^{-1}$ | 0 – 30 | 3.57±0.92 |
| LF1 | After harvest | 1 g Se ha$^{-1}$ | 0 – 30 | 3.71±0.94 |
| LF1 | After harvest | 10 g Se ha$^{-1}$ | 0 – 30 | 3.67±0.27 |
| LF1 | After harvest | 50 g Se ha$^{-1}$ | 0 – 30 | 4.32±0.67 |
| GF5 | Before fertilisation | Site average | 0 – 30 | 2.21±0.00 |
| GF5 | After harvest | Top Stock® | 0 – 30 | 1.1±0.02 |
| LF4 | Before fertilisation | Site average | 0 – 30 | 0.94±0.00 |
| LF4 | After harvest | 50 g Se ha$^{-1}$ | 0 – 30 | 1.30±0.40 |

ICP-MS method used. If the above hypothesis is correct, it also means that the main reason for low Se in UK crops is due to the main forms of Se in soil being largely unavailable to plant uptake.

Fertilisation of wheat crops with Se is a proposed solution to the low Se status of UK crops (Broadley et al. 2006), with crop recovery of selenate fertiliser ranging between 20 – 35 % (Broadley et al. 2010). This means that a large proportion of Se fertiliser was not utilised by the current crop. Therefore, total and extractable Se, and Se speciation in soil was determined at each field site to determine the fate of selenate fertilisation. The fertiliser addition caused no significant increase in the total Se concentration in soil at any of the field sites. However, this is readily explained because this level of increase would be difficult to detect in the analysis of 200 – 600 $\mu g\ kg^{-1}$ total Se in soil. An addition of 10 g Se $ha^{-1}$ is equivalent to adding 2.6 $\mu g$ Se $kg^{-1}$ in the topsoil (0 – 30 cm, assuming soil bulk density of 1.3 g $cm^{-3}$), of which 30 % was taken up by crop, leaving only 1.8 $\mu g\ kg^{-1}$ in soil. Changes in soil Se concentrations would be more detectable in the extractable Se fraction (5 – 12 $\mu g\ kg^{-1}$) if most of the Se fertiliser remained in this fraction. In 9 out of 10 experiments, no increase in extractable Se was detected in the field trials. This is likely to be caused by the relatively small addition of Se through fertilisation and that some of the 1.8 $\mu g\ kg^{-1}$ Se input could be leached down the soil profile. This, together with soil heterogeneity makes it difficult to detect any increase in soil Se. No significant increase in extractable Se was detected in the 50 g Se $ha^{-1}$ treatment in trials LF1, LF2 or LF4, a treatment level which is estimated to leave 9.1 $\mu g$ Se $kg^{-1}$ in the topsoil. This result suggests that selenate was leached out of the soil profile, volatilised and/or converted to the unextractable Se fraction. Plants and the associated rhizosphere microorganisms may have volatilised some of the Se (Lin et al. 2000). The LF3 trial was an exception, showing a significant increase in extractable Se, with an increase of 4 $\mu g$ Se $kg^{-1}$ in the topsoil at an application rate of 50 g Se $ha^{-1}$. The soil at LF3 has a high proportion of clay (33 %), acidic pH and moderate carbon content; these properties favour the adsorption of Se (Fordyce et al. 2000). The fertiliser formulation (liquid application) and dose may also influence the fate of Se, as GF5, carried out on the same soil as LF3, showed no retention of Se at the

10 g Se $ha^{-1}$ rate. This indicates that monitoring of extractable Se concentrations in soils may be relevant under some conditions. These results suggest that there would be little residual effects of Se fertiliser, applied as selenate, to the subsequent crops at most field sites, which was confirmed in a residual trial (Stroud et al. 2010).

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