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Selenium Uptake from Livestock Pasture Extremely Enriched in Selenium, Molybdenum and Uranium: A Field and X-ray Absorption Study

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Abstract: The agricultural soils of West Limerick, Ireland, contain very localised, extremely high natural Se concentrations that reach levels that are very toxic to grazing livestock. The Carboniferous shales that formed in anoxic deep-water marine environments are the source of the selenium, which, along with the other redox-sensitive elements of molybdenum, uranium, arsenic and vanadium, were mobilised and reprecipitated in post-glacial anoxic marshes. The result has been a history of selenosis and molybdenosis in livestock in this important dairy province. Soils collected at 10–20 cm from five different agricultural sites were analysed, and all yielded concentrations greatly in excess of the safe Se limits of 3–10 mg kg^{−1}; the highest value recorded was 1265.8 mg kg^{−1} Se. The highest recorded value for Mo in these soils was 1627.5 mg kg^{−1}, and for U, 658.8 mg kg^{−1}. There was a positive correlation between Se, Mo U and organic matter in the soils. Analysis of non-accumulator pasture grasses (*Lolium perenne* (perennial ryegrass), *Festuca arundinacea* (tall fescue), *Dactylis glomerata* (cocksfoot) and *Phleum pratense* (timothy grass)) revealed the shoot/leaf to contain up to 78.05 mg kg^{−1} Se while *Trifolium repens* (white clover) leaves contained 296.15 mg kg^{−1} Se. An in situ growing experiment using the Se accumulator species *Brassica oleracea* revealed 971.2 mg kg^{−1} Se in the leaves of premier kale, which also contained 1000.4 mg kg^{−1} Mo. Translocation factors (TFs) were generally higher for Mo than Se across all plant species. Combined X-ray absorption near edge spectroscopy (XANES) with micro-X-ray fluorescence (μ -XRF) showed the Se was present in the soil predominantly as the reduced immobile phase, elemental Se (Se⁰), but also as bioavailable organoselenium species, mainly selenomethionine (SeMet). SeMet was also the main species identified within both the Se non-accumulator and Se accumulator plants. The Se soil–plant system in West Limerick is dominated by SeMet, and uptake into the cattle pasture results in selenosis in the grazing dairy herds. The hyperaccumulating *Brassica oleracea* species could be used to extract both the Se and Mo to reduce the toxicity of the blighted fields.

Keywords: selenium; molybdenum; soil; Limerick; grass; brassica; XAS; μ -XRF; bioavailability



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1. Introduction

Selenium uptake and accumulation in plants is important to human and animal health as plants are the main dietary source of this vital micronutrient. Whereas selenium deficiency is a widespread problem, Se toxicity is a localised but serious issue in certain areas of the world [1]. The uptake and bioavailability of selenium in plants are determined by the concentration and selenium speciation in soil and water and controlled by physico-chemical conditions [2–5]. Selenium is not essential to plants but is assimilated into a number of organoselenium compounds in a similar manner to the sulfur uptake that increases plant growth and antioxidant activity [6]. The translocation and distribution

of selenium into different parts of the plant are influenced by the species of selenium in the soil [7]. When plants accumulate selenium, this can lead to human poisoning, resulting in acute selenium toxicity, characterised by loss of sight, paralysis, respiratory failure and death [8]. In livestock, chronic selenium toxicity leads to alkali disease, which is characterised by hair loss and the deformation of hooves and bones. Anthropogenic activity is a major cause of selenium toxicity, with mining-derived selenium causing a major poisoning in the Ensi District of China [4,9], whereas agricultural practices resulted in the catastrophic selenium poisoning of the Kesterson Reservoir in California [10]. Natural toxicity is often associated with selenium derived from mudstone/shale formations, such as in the Punjab area of India and western USA, although irrigation methods greatly enhanced the problem [11–13]. Naturally occurring selenium toxicity has been recognised in the soils of Ireland, associated with Carboniferous mudstone and shale [14–18]. In particular, high selenium and molybdenum have been identified in West Limerick, where there is a historical record of both selenium and molybdenum toxicity in cattle [14–18]; there is also a record of high uranium in the soils of the region [19]. This region is a major producer of dairy products ('The Golden Vale'), and the local dairy farmers are well aware of the localised fields where selenium poisoning occurs; however, detailed analyses of the soils and plants of the region have not been undertaken.

Much of the extensive research on selenium in the environment has focused on high redox potential, alkaline systems where Se-oxyanions prevail [6,20–22], especially using controlled, experimental conditions [23]. The current study focuses on the uptake and speciation of selenium in the 'natural' system found in these temperate livestock grasslands of western Ireland. The extreme naturally derived concentration of selenium in these soils allows the determination of selenium bioavailability in the pasture and the potential of Se accumulator plants (*Brassica* sp.) in the phytoremediation of these soils [24,25]. The concentrations of molybdenum and uranium in these Irish soils are also extremely high in places; data for these elements, as well as arsenic and vanadium, are also presented here.

As with selenium, molybdenum is an essential micronutrient for plants and higher life forms. It is also extensively documented that elevated molybdenum values in soils result in high concentrations in herbage, leading to molybdenosis in livestock. Uptake of molybdenum from soil is via molybdate, MoO_4^{2-} , and concentrations of 5 mg kg⁻¹ molybdenum in herbage can prove fatal in cattle due to copper deficiency resulting from the development of non-absorbable copper thiomolybdate complexes in the rumen [26–30]. Background values for soil worldwide are 0.5–5 mg kg⁻¹, but Irish soils are known to have elevated values (6.5 mg kg⁻¹) associated with the Carboniferous mudstones, shales and limestones [27]; values of 2 to 25 mg kg⁻¹ were recorded in herbage in Irish soils, where molybdenosis is a recorded problem [30]. The use of copper supplements is often employed to deal with molybdenosis [31], although phytoremediation strategies to deplete excess molybdenum in soils have been explored and the use of soil amendments have been successfully demonstrated [32,33].

Although it is not a focus of this work, we recorded high uranium values in the soils, and these data are also presented here. High accumulations of uranium can damage plant growth and enable uptake by livestock. Global average uranium values in soil are in the range of 1–4 mg/kg⁻¹, but the accepted toxicity level is contentious, with values of 5 to 1000 mg/kg⁻¹ being quoted [34]; the bioavailability of uranium is highly dependent on its speciation in soils, and this is, in turn, is dependent on a range of factors, namely, redox, pH, phosphate content, soil organic matter (SOM) and microbial populations [35,36].

Arsenic is a redox-sensitive element commanding great interest in toxicological investigations in soil and water (see [37] and references therein). We provide data on this element, but the data were relatively unremarkable and, therefore, are little discussed in this work.

2. Methodology

2.1. Field Sampling

Soils in pastures grazed by livestock were sampled from five Se-enriched sites (FF, MO, TL, TJ, PK; Figure 1) that were identified by the Irish Agricultural Food Development Authority, Teagasc, combined with information from previous studies [15,17,38] and local farmers; sites FF and MO are in adjacent fields separated by a small ditch. Further information on the sites is provided in Appendix A, Figures A1–A4. The sites are all relatively low-lying grassland in gentle topography (a maximum of 20 m elevation changes), with organic-rich soils overlying Carboniferous limestones, mudstones and shales. Soil samples (500 g) were collected over the sites from 10–20 cm depth for bulk chemical analysis, and soil cores were also collected from sampling locations TL and FF to determine the changes in Se concentration with depth. A first suite of samples analysed informed further sampling and the choice of coring locations (see Table A4 for sample site locations of the analyses presented herein). The soil cores were sampled using a Van Walt corer with 4.4 cm stainless steel diameter cylinders; a complete core to bedrock (approximately 50 cm) was recovered and divided into 10 cm sections, which were used for bulk chemical analysis. An adjacent core was collected and stored under oxygen-free nitrogen at the site to maintain an anaerobic environment suitable for speciation and microbial analysis. The cores were tightly capped and stored at 4 °C to attenuate microbial activity.



Figure 1. Map of part of West Limerick, Ireland, showing the patchwork of dairy livestock fields in this part of Ireland’s ‘Golden Vale’. Sampling locations are shown (FF, MO, PK, TL and TJ). Satellite image, Google Earth Pro, ©Google. Image© Maxar Technologies. See Figures A1–A4.

The pasture plants grazed by the livestock were also sampled from the 5 sites (Figures A1–A4 and A6), where the vegetation consisted of species of perennial grasses, namely, *Lolium perenne* (perennial ryegrass), *Festuca arundinacea* (tall fescue), *Dactylis glomerata* (cocksfoot) and *Phleum pratense* (timothy grass) as well as *Trifolium repens* (white clover). The sampling of plant material was undertaken at the locations identified in the soil analysis as high in selenium. An in situ growing experiment using three varieties of the Se hyperaccumulator species *Brassica oleracea* (red flare cabbage, hispi cabbage and premier kale) [39,40] was conducted in a plot adjacent to sites FF/MO. This plot had a soil

concentration of 276 mg kg^{-1} Se (and 91 mg kg^{-1} Mo, 23 mg kg^{-1} U; Table A1), and the *Brassica sp.* were grown in an area cleared of grass for 11 weeks (early May–late July) under natural conditions. To minimise stress, all the plant species analysed in this investigation were collected by digging them out, complete with soil, and placing the whole unit in $20 \times 20 \times 20 \text{ cm}$ pots (see Figures A7 and A8). These were transported to an experimental greenhouse and watered with local (Limerick) rainwater; sampling was undertaken within 2 days.

2.2. Soil Analysis

In preparation for chemical analysis, the 10 cm sections of core and the 500 g soil samples collected at 10–20 cm were dried at 50°C . Rock fragments and roots were removed by sieving (0.1 mm) before the samples were powdered ($<50 \mu\text{m}$). Wax-mounted pellets were prepared using 12 g of sample and 3 g of wax pressed under a 10 t load. The pellets were analysed by X-ray fluorescence (XRF) using a PANalytical Axios sequential X-ray fluorescence spectrometer with Omnia[®] and Pro-trace[®] standardisation. Then, 1 g of soil was separated for loss on ignition (LOI) by measuring the weight loss of the soil at 110 and 1000°C to calculate the moisture loss ($\text{H}_2\text{O wt}\%$) and carbon dioxide ($\text{CO}_2 \text{ wt}\%$) released by heating. Another 1 g was used to calculate the total organic carbon (TOC) content of the soils by measuring the weight loss of the soil at 110 and 400°C and applying a correction factor assuming 58% of organic matter contains organic carbon [41]. The Se, Mo, U and As concentrations measured at 10–20 cm depth were plotted using Golden LLC Surfer[®] V15 software to provide a visual indication of the localised nature of their enrichment. The selenium concentrations derived from XRF analysis of the 10–20 cm soil samples were used to determine the plant and core sampling locations. Eh and pH values for the soils were taken in both the field and laboratory using deionised water mixed with an equal volume of soil using a Denver Instrument UB-10 pH/mV benchtop meter calibrated in a redox buffer solution of pH 7 and Eh 220 mV. X-ray diffraction analysis using a Bruker D8 Advance diffractometer was used to determine the crystalline phases in the soils.

2.3. Plant Chemical Analysis

As the grass pasture species were intermingled, they were not separated but divided into shoot (blade) and root material. White clover (from site TJ) and the *Brassica oleracea* varieties were separated into leaf, stem and root material. Once separated, the plant material was rinsed in deionised water to remove any soil particles. To determine the bulk elemental concentrations, the plant material was frozen at -80°C and freeze-dried. Then, 0.1–0.2 g of powdered plant material was added to a PFA microwave vessel, and 6 mL nitric acid (HNO_3) and 3 mL hydrogen peroxide were added to digest the material overnight; 1 mL of hydrofluoric acid was added to the vessels, and after 1 h, they were sealed and digested at 170°C in a MARS microwave digestion unit for a total of 55 min at 800 W (12 samples per batch). Once the vessels depressurised, the acidified sample was poured into PTFE beakers and heated on a hot plate until the HF evaporated. The residue was dissolved in 2% ultra-pure HNO_3 and reheated to incipient dryness, and the residue washed and diluted to 50 mL with 2% ultra-pure HNO_3 . In each batch, samples were digested in triplicate with standard reference material CRM 402 (a white clover with Se $6.7 \pm 0.25 \text{ mg kg}^{-1}$), with an average recovery of 6.73 mg kg^{-1} and all blanks measuring $<0.1 \text{ mg kg}^{-1}$ Se. Then, 10 mL was filtered ($>0.45 \mu\text{m}$) and analysed for selenium (^{82}Se), molybdenum (^{95}Mo), arsenic (^{75}As), uranium (^{238}U), vanadium (^{51}V) and iron (^{56}Fe) using the inductively coupled plasma mass spectrometer (ICP-MS) Agilent 7500cx equipped with a pressurised octopole collision/reaction cell.

The translocation factor (TF) was calculated for the plant samples analysed as this demonstrates the plant's ability to translocate the trace elements from roots through to the shoots or leaves of the plant ($\text{TF} = C_{\text{shoot}}/C_{\text{root}}$) [42] as well as revealing phytoextraction capabilities and bioavailability. C_{shoot} in the white clover and *Brassica oleracea* was subdivided into leaf and stem material and the TF calculated separately; in the grass species,

the bulk shoot/leaf concentration was used. The bioaccumulation factor (BAF) was also calculated for the grass species; this is the ratio of the trace element concentration in the plant material to that in the soil ($BAF = C_{\text{plant}}/C_{\text{soil}}$), which is used to estimate a plant's ability to accumulate the trace elements from soil [43]. C_{plant} was subdivided into shoot and root material and the BAF calculated separately.

2.4. Synchrotron Analysis

X-ray absorption spectroscopy (XAS) analysis of the Se *K*-edge (12.654 keV) was undertaken at Diamond Light Source, Didcot, UK, to determine the selenium species and distribution in soil and plant material from seleniferous sites TL-1 and FF-2. For XAS analysis, 1 cm sub-sections of fresh leaf, stem and root material were flash-frozen in isopentane that was cooled in a container of liquid nitrogen to achieve a temperature of -150°C , which reduces/avoids ice crystal formation and sample damage. Sub-samples of soil were collected from the highly seleniferous horizons of two soil cores (at sites TL-C1 and FF-C2) and, in an anaerobic cabinet, sealed in 1 mL Eppendorfs. The soil samples were centrifuged at 6000 rpm for 15 min to remove pore fluid and make a robust sample for cryo-sectioning. These were flash-frozen in liquid nitrogen. All XAS samples were kept anaerobic and frozen, set in an optimum cutting compound (OCT) matrix to avoid element redistribution [44] and cryo-sectioned in a Leica CM3050 S Cryostat microtome at -20°C to a thickness of 30–50 μm for plant material and 100 μm for soils; the latter was the thinnest possible, with samples sieved to 50 μm . OCT is a water-soluble mix of glycols (polyvinyl alcohol, polyethylene glycol), resins and non-reactive ingredients. The sections were placed between two pieces of Kapton[®] tape and transported in dry ice (-78.5°C) to the I18 Microfocus Spectroscopy Beamline at Diamond Light Source [45].

The samples were cryo-fixed onto the sample holder in liquid nitrogen vapour and mounted on an x-y-z stage inclined at 45° to the incident beam in a liquid helium cryostat and cooled to 20 K to prevent in situ beam damage reduction or oxidation. The data were acquired using a Si (III) monochromator, and energy calibration was achieved using Au foil (11.919 keV); the XAS data were collected over the range of 12.55 to 13.30 keV. A range of powdered Se standards was measured in the transmission mode; the standards chosen provided the range of coordination environments and Se-species expected in seleniferous soils and plants. These were sodium selenide (Na_2Se , Se^{II}), selenocystine ($\text{SeCys}_2\text{-C}_6\text{H}_{12}\text{N}_2\text{O}_4\text{Se}_2$, Se^{II}), selenomethionine ($\text{SeMet-C}_5\text{H}_{11}\text{NO}_2\text{Se}$, Se^{II}), elemental selenium (Se^0), sodium selenite (NaSeO_3 , Se^{IV}) and sodium selenate (NaSeO_4 , Se^{VI}).

Synchrotron $\mu\text{-XRF}$ maps of the samples were acquired with continuous scanning using two (one 6- and one 4-element) Si drift detectors with an incident beam energy of 13.0 keV and a fixed beam size of 5 μm , analyses every 10 μm and a 0.33 s acquisition time. Points of interest (POIs) displaying both high Se counts and representative concentrations of Se were selected for detailed X-ray absorption near edge structure (XANES) analysis for species identification using a step scanning mode and 1 s per point. Spectra from a range of points were screened to ensure the presented data were representative of the selenium species present. Two XANES scans were collected during the analysis. Even at liquid helium temperatures, the possibility of beam damage was a concern and was continually monitored. Tests showed no changes in each point in the first two scans presented here.

The $\mu\text{-XRF}$ spectra were processed into elemental maps by windowing the Se $K\alpha$ peak and using PyMCA 5.2.2 software [45]. The XANES spectra were normalised using the same optimal energy range for fitting the pre- and post-edge lines for all the spectra and processed using ATHENA 0.9.26 software. Linear combination fitting (LCF) was performed over the energy range -20 to $+50$ eV around the Se *K*-edge against 4–6 standard spectra, providing a goodness of fit (*R*) and a percentage of the main species present [46–48].

3. Results

3.1. Soil Characterisation

The trace and major element compositions of the soils sampled at 10–20 cm are presented in Tables A1–A3, and the Se, Mo, U, As and V concentrations are summarised in Table 1. The selenium distribution at all sites was characterised by zones of very high concentration surrounded by areas of very low values (Figures 2 and 3). The highest selenium concentration of 1265.8 mg kg^{−1} was observed in a sample at site MO (Table 1). The remaining four sites have maximum selenium concentrations ranging from 28.4 to 682 mg kg^{−1} (Table 1). Sites FF and MO are in adjacent fields (Figure 1) and contain particularly high concentrations of Se, Mo, U and As (Table 1; Figure 3). In general, the molybdenum concentrations have a similar distribution to that of selenium and are highest at site FF, with one sample containing 1627.5 mg kg^{−1} Mo. Uranium concentrations are also high at sites FF and MO, with a maximum value of 599.5 mg kg^{−1} at site FF and 407 mg kg^{−1} at site MO. The arsenic concentrations ranged from 0.0–99.8 mg kg^{−1} at site FF and 0.0–115.3 mg kg^{−1} at site MO. The vanadium concentrations for all sites have a low range of 175.7–195.2 mg kg^{−1}. pH values of the soils were in a narrow range, from 6.0 to 6.5 and Eh 126 to −90 mV.

Table 1. The maximum and minimum concentrations of key elements derived from bulk samples collected at 10–20 cm depth from five agricultural sites in West Limerick, determined by XRF.

Site		Se mg kg ^{−1}	Mo mg kg ^{−1}	As mg kg ^{−1}	U mg kg ^{−1}	V mg kg ^{−1}
TL	Min	0.0	0.2	7.5	5.2	54.3
	Max	88.2	1.9	39.8	13.0	85.9
TJ	Min	0.0	1.0	6.2	6.8	20.7
	Max	28.4	19.0	17.1	26.6	80.9
FF	Min	0.0	2.8	0.0	8.3	48.4
	Max	489.1	1627.5	99.8	599.5	189.7
MO	Min	1.0	3.2	0.0	17.0	77.8
	Max	1265.8	898.1	115.3	407.0	195.2
PK	Min	0.9	1.9	0.4	10.0	175.7
	Max	682.0	34.9	63.1	12.4	212.2

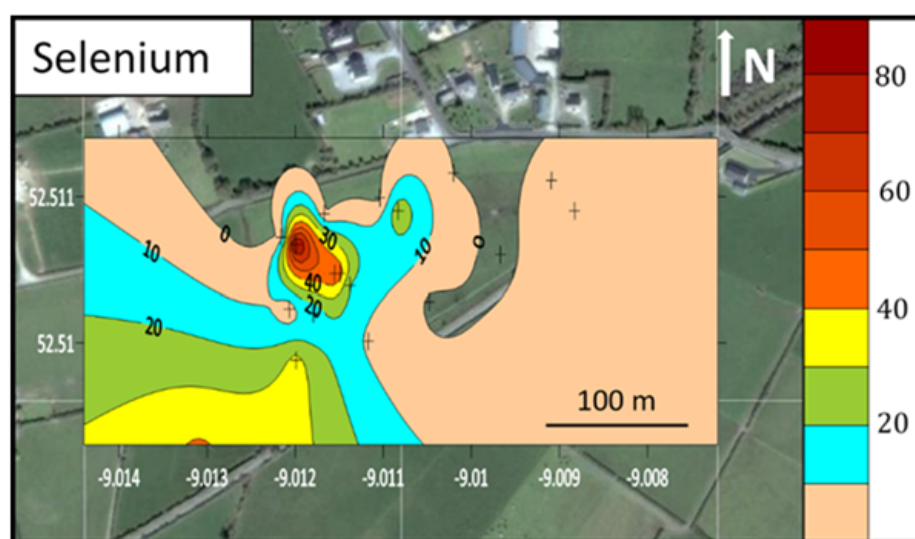


Figure 2. Map showing the spatial distribution of selenium in soil at 10–20 cm at site TL, produced using Golden LLC Surfer® V15 software and superimposed on a satellite image of Clonaugh, West Limerick (52°30′38″ N, 9°0′43″ W) (Google Earth Pro, ©Google. Image© Maxar Technologies, using Kriging gridding). Sample site marked with a+, scale in ppm, axes in longitude and latitude.

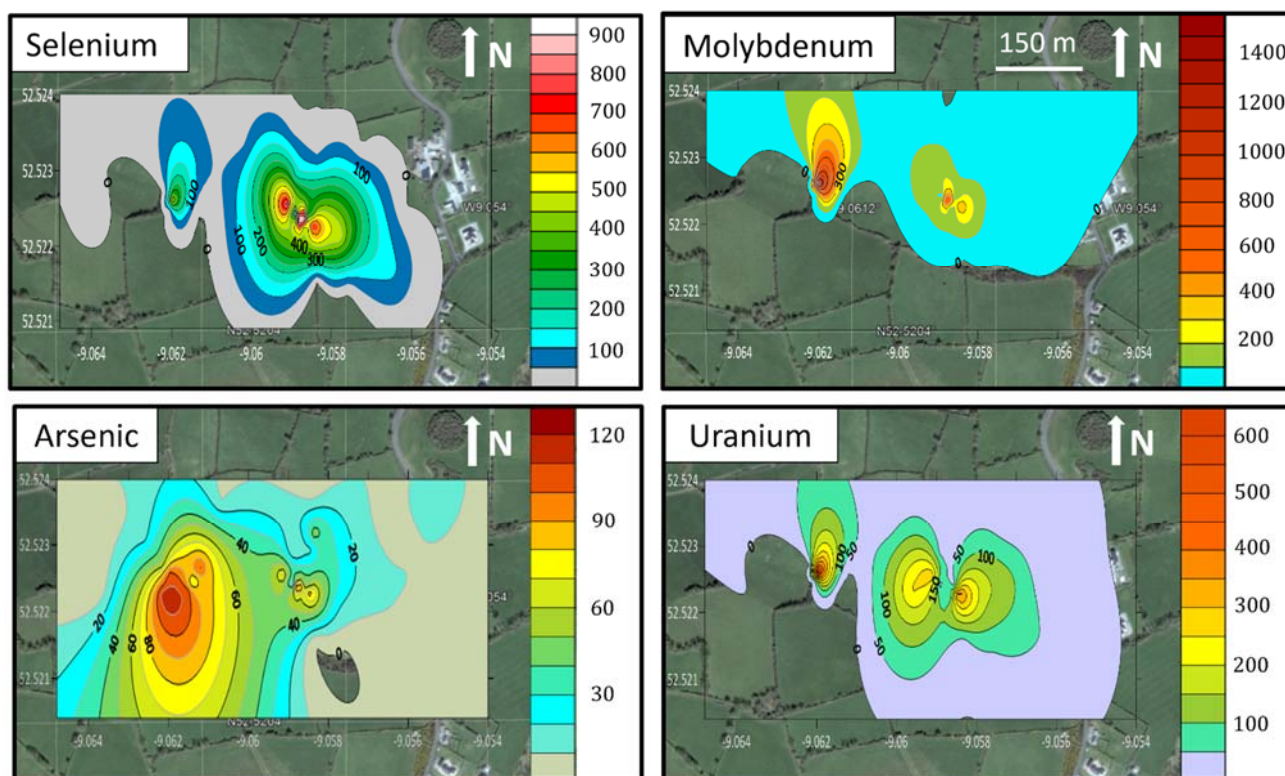


Figure 3. Maps showing the spatial distribution of selenium, molybdenum, arsenic and uranium concentrations in soils at 10–20 cm at site FF, produced using Golden LLC Surfer® V15 software and superimposed on a Google Earth image of Ballyegny Beg, West Limerick (52°31'22" N; 9°3'32" W) (Google Earth Pro, ©Google. Image© Maxar Technologies, using Kriging gridding). Values in ppm, axes longitude/latitude.

3.2. Soil Core Bulk Analysis

The variation in the trace and major elemental composition with depth was examined by analysing a complete soil core from two of the sites, FF and TL, selected from the selenium values in the 10–20 cm data. At both sites, the brown organic soil profiles were developed over a thick impermeable, grey clay encountered at a depth of 30–50 cm; the bedrock beneath was not reached. The results are shown in Table 2, and the Se values were plotted against TOC in Figure 4. The highest Se concentrations in both soil cores were at a depth horizon of 30–40 cm, with 171 mg kg^{−1} in core TL-C1 and 926 mg kg^{−1} in core FF-C2.

Table 2. XRF data showing the concentration of selected trace and major elements for two soil cores, TL-C1 and FF-C2. R² = the correlation with selenium. TOC = total organic carbon.

Sample	Depth	Se	Mo	As	U	V	Mn	Cu	CaO	Fe ₂ O ₃	SiO ₂	Al ₂ O ₃	TOC
	cm	mg kg ^{−1}	mg kg ^{−1}	mg kg ^{−1}	mg kg ^{−1}	mg kg ^{−1}	mg kg ^{−1}	mg kg ^{−1}	wt%	wt%	wt%	wt%	wt%
TL-C1	0–10	55.2	0.8	25.2	7.2	82.0	260.9	67.5	3.1	3.7	56.5	11.0	8.5
	10–20	45.1	1.0	22.5	6.9	81.1	312.8	64.9	4.0	3.8	57.1	11.2	7.1
	20–30	58.2	1.0	26.0	7.5	76.8	280.2	62.1	4.2	3.8	55.5	11.4	7.4
	30–40	171.7	2.4	54.6	13.0	75.8	274.6	51.3	3.7	4.3	40.4	10.6	15.1
	40–50	61.9	1.0	15.9	9.2	34.5	156.6	14.0	18.8	2.1	40.5	7.0	7.0
FF-C2	R ²	-	0.97	0.90	0.93	0.01	0.01	0.01	0.03	0.19	0.45	0.00	0.96
	0–10	99.1	22.9	12.9	88.7	119.9	613.5	107.4	3.2	3.9	28.6	5.96	25.7
	10–20	239.9	49.2	48.0	359.3	133.2	194.8	101.8	5.1	5.1	13.4	3.1	33.3
	20–30	163.8	120.2	117.4	658.8	100.4	194.1	72.5	6.7	4.8	1.2	0.5	37.3
	30–40	926.8	191.9	132.3	354.9	138.0	221.8	120.0	8.0	4.3	4.8	1.3	35.9
	40–50	268.6	163.3	94.2	78.7	86.9	214.4	17.4	32.7	2.3	8.3	1.4	11.2
	R ²	-	0.52	0.41	0.01	0.27	0.12	0.14	0.00	0.00	0.19	0.17	0.09

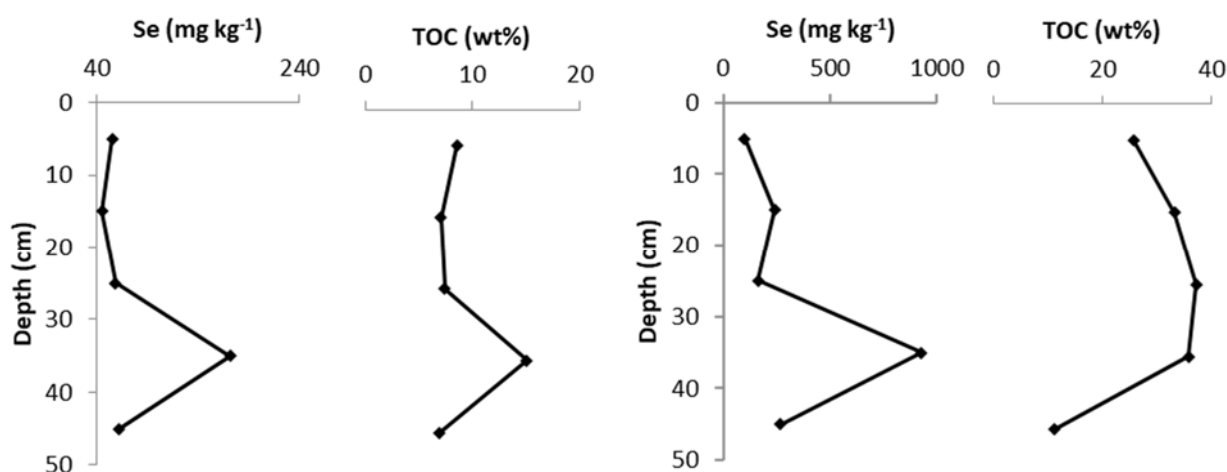


Figure 4. Se and total organic carbon (TOC) values measured in vertical cores at site TL-C1 (left) and site FF-C2 (right).

In core FF-C2, the most seleniferous horizon also contained 191.2 mg kg⁻¹ Mo, 94.2 mg kg⁻¹ As and 354.9 mg kg⁻¹ U. However, only molybdenum had a positive correlation (R^2 value of 0.52) with selenium through the core profile (Figure 4). The highest uranium concentration of 658.8 mg kg⁻¹ seen in this study was observed at a depth of 20–30 cm in core FF-C2.

In core TL-C1, the most seleniferous horizon contained the highest concentrations of arsenic (54.6 mg kg⁻¹) seen in this profile, with a highest uranium concentration of 13 mg kg⁻¹ but only 2.4 mg kg⁻¹ molybdenum. These elements had high positive correlations with selenium throughout the soil profile, with an R^2 value of 0.93 for uranium, 0.97 for molybdenum and 0.90 for arsenic. Sub-samples from the most seleniferous horizon of both soil cores were selected for further analysis by XAS to determine the selenium speciation (see Section 3.5).

The XRF data revealed a high SiO₂ content in the top 20 cm of the profile, and this is indicative of relatively recent sediment input to the sites. CaO increases downwards towards the clay layer. A clear positive correlation of TOC with selenium is apparent at both sites (TL-C1 $R^2 = 0.96$, and the highest TOC in FF-C2 of 37.3 wt% at 30–40 cm coincides with the maximum selenium; Figure 4). XRD analysis showed that the dominant crystalline mineral phase in both cores was quartz. The XRD analysis also showed that site TL-C1 contained minor amounts of muscovite and clinocllore, whereas FF-C2 had minor amounts of dolomite, K-feldspar, muscovite and calcite.

3.3. Se, Mo, As U and V in Grass and White Clover (Non-Accumulators)

The concentrations of Se, along with those of Mo, U and As, determined from grass shoot and root material from the five sites, are presented in Table 3. In addition, at site TL, white clover was separated into leaf, stem and root material for analysis.

Selenium is most concentrated in the root material of all grass samples, with the exception of one sample, FF-P2. Despite the concentration of Se in the soils at site TL being lower than those at FF, MO, and PK, the highest Se concentrations in grasses are in the two samples from this site. Sample TL-P2 contained 112.2 mg kg⁻¹ Se in the roots and 78.1 mg kg⁻¹ in the shoots, whereas in adjacent site TL-P1, the shoots contained 67.9 mg kg⁻¹ in the roots and 44.9 mg kg⁻¹ in the shoots. The lowest Se in plant material is from site PK, with sample PK-P2 containing 4.28 mg kg⁻¹ in the roots and 1.32 mg kg⁻¹ in the shoots; in the four 10–20 cm soil samples taken from site PK, one sample contained 682 mg kg⁻¹ Se, but the other three had <5.4 mg kg⁻¹ Se.

Table 3. The concentration of Se, Mo, As, U and V in selenium ‘non-accumulator’ clover and grass from pasture in West Limerick. TF = the translocation factor, a value >1 indicates the plant can translocate the element effectively from the root to shoots, stem and leaves. Analysis by ICP-MS, all experiments performed in triplicate $\pm$ the standard error. Nearest soil sample sites are in brackets.

	Material	Se mg kg ⁻¹	Mo mg kg ⁻¹	As mg kg ⁻¹	U mg kg ⁻¹	V mg kg ⁻¹
TL-P1 (site TL-1)	Clover Leaf	296.2 $\pm$ 4.50	15.98 $\pm$ 0.25	0.27 $\pm$ 0.01	0.05 $\pm$ 0.00	0.70 $\pm$ 0.02
	Clover Stem	249.6 $\pm$ 7.20	17.27 $\pm$ 0.29	0.23 $\pm$ 0.00	0.03 $\pm$ 0.00	0.38 $\pm$ 0.02
	Clover Root	150.4 $\pm$ 16.19	4.89 $\pm$ 0.25	1.54 $\pm$ 0.05	0.34 $\pm$ 0.01	4.63 $\pm$ 0.16
	TF = Leaf/Root	1.97	3.27	0.18	0.14	0.15
	TF = Stem/Root	1.66	3.53	0.15	0.09	0.08
	Grass Shoot	44.9 $\pm$ 7.23	6.20 $\pm$ 0.05	0.39 $\pm$ 0.02	0.05 $\pm$ 0.00	0.92 $\pm$ 0.01
	Grass Root	67.9 $\pm$ 2.57	4.59 $\pm$ 0.49	30.1 $\pm$ 0.77	1.40 $\pm$ 0.04	37.69 $\pm$ 1.40
	TF = Shoot/Root	0.66	1.35	0.01	0.04	0.02
TL-P2 (site TL-1)	Grass Shoot	78.1 $\pm$ 7.81	3.19 $\pm$ 0.49	1.24 $\pm$ 0.08	0.18 $\pm$ 0.00	4.45 $\pm$ 0.13
	Grass Root	112.1 $\pm$ 1.72	1.80 $\pm$ 0.03	6.6 $\pm$ 0.28	0.79 $\pm$ 0.03	18.58 $\pm$ 2.24
	TF = Shoot/Root	0.70	1.77	0.19	0.23	0.24
TJ-P1 (site TJ-1)	Grass Shoot	5.8 $\pm$ 0.67	11.59 $\pm$ 0.95	0.66 $\pm$ 0.06	0.47 $\pm$ 0.04	2.29 $\pm$ 0.21
	Grass Root	17.27 $\pm$ 0.16	6.94 $\pm$ 0.05	10.46 $\pm$ 0.11	6.18 $\pm$ 0.04	30.89 $\pm$ 0.19
	TF = Shoot/Root	0.33	1.67	0.06	0.08	0.07
TJ-P2 (site TJ-2)	Grass Root	5.1 $\pm$ 0.06	4.14 $\pm$ 0.02	19.72 $\pm$ 0.15	3.97 $\pm$ 0.02	11.17 $\pm$ 0.07
	Grass Shoot	1.19 $\pm$ 0.07	13.68 $\pm$ 0.07	0.21 $\pm$ 0.00	0.12 $\pm$ 0.00	0.64 $\pm$ 0.01
	TF = Shoot/Root	0.23	3.30	0.01	0.03	0.06
FF-P1 (site FF-1)	Grass Shoot	4.13 $\pm$ 0.24	35.83 $\pm$ 0.47	0.30 $\pm$ 0.03	0.11 $\pm$ 0.00	1.40 $\pm$ 0.03
	Grass Root	22.3 $\pm$ 2.51	14.6 $\pm$ 1.17	179.7 $\pm$ 17.18	0.76 $\pm$ 0.07	50.19 $\pm$ 4.89
	TF = Shoot/Root	0.19	2.45	0.00	0.15	0.03
FF-P2 (site FF-2)	Grass Root	26.29 $\pm$ 0.13	15.02 $\pm$ 0.21	4.15 $\pm$ 0.06	4.10 $\pm$ 0.04	27.29 $\pm$ 0.28
	Grass Shoot	33.8 $\pm$ 2.15	79.6 $\pm$ 1.13	0.83 $\pm$ 0.01	4.41 $\pm$ 0.05	2.55 $\pm$ 0.01
	TF = Shoot/Root	1.29	5.30	0.20	1.07	0.09
PK-P1 (site PK-1)	Grass Shoot	3.93 $\pm$ 0.21	23.24 $\pm$ 0.13	0.63 $\pm$ 0.02	0.41 $\pm$ 0.00	1.84 $\pm$ 0.02
	Grass Root	31.10 $\pm$ 0.17	18.79 $\pm$ 0.11	26.98 $\pm$ 0.12	4.02 $\pm$ 0.02	132.91 $\pm$ 0.76
	TF = Shoot/Root	0.13	1.24	0.02	0.10	0.01
PK-P2 (site PK-2)	Grass Shoot	1.32 $\pm$ 0.12	14.76 $\pm$ 0.14	0.78 $\pm$ 0.02	0.42 $\pm$ 0.01	7.34 $\pm$ 0.08
	Grass Root	4.28 $\pm$ 0.22	5.52 $\pm$ 0.17	3.05 $\pm$ 0.12	0.95 $\pm$ 0.04	30.46 $\pm$ 1.03
	TF = Shoot/Root	0.31	2.67	0.26	0.45	0.24
MO-P1 (site MO-1)	Grass Shoot	48.3 $\pm$ 0.63	55.69 $\pm$ 0.47	0.30 $\pm$ 0.02	1.02 $\pm$ 0.04	0.84 $\pm$ 0.02
	Grass Root	84.3 $\pm$ 1.21	13.16 $\pm$ 0.55	1.17 $\pm$ 0.11	1.11 $\pm$ 0.02	1.50 $\pm$ 0.03
	TF = Shoot/Root	0.57	4.23	0.26	0.92	0.56

In all the grass samples, Mo was most concentrated in the shoot material compared to the root material, contrasting with Se. The highest Mo concentration was in the grass shoots of sample FF-P2 with 79.6 mg kg⁻¹ that also contained 15.02 mg kg⁻¹ in the roots. In general, uranium had a similar distribution to selenium and was more concentrated in the grass roots compared to the shoots, with the exception of sample FF-P2. The highest uranium concentration was in sample TJ-P1, with 6.18 mg kg⁻¹ in the roots but it contained only 0.47 mg kg⁻¹ in the shoots. In all grass samples, arsenic and vanadium were most concentrated in the root material compared to the shoots. The highest arsenic concentration was in sample FF-P1, with 179.67 mg kg⁻¹ in the roots but the shoots contained only 0.30 mg kg⁻¹ As. It was noted that the highest vanadium concentration was in sample PK-P1, with 132.91 mg kg⁻¹ in the roots and 1.84 mg kg⁻¹ in the shoots.

The ICP-MS data in Table 3 reveal that the highest selenium concentrations in the non-accumulator plants are in the white clover from TL-P1, with leaves containing 296.2 mg kg⁻¹ Se, the stems 249.5 mg kg⁻¹ Se and the roots 150.4 mg kg⁻¹ Se. These values were

significantly higher than the coexisting grasses. However, arsenic, uranium and vanadium concentrations were $<1 \text{ mg kg}^{-1}$ in the clover leaf and stem materials and $<5 \text{ mg kg}^{-1}$ in the root material.

The TFs for Se, Mo, U, As and V are presented in Table 3. The TF values for Se in grass samples were <1 , except for location FF-P2, which had a value of 1.29. The highest TFs for selenium were found in the white clover sample from site TL-P1 (Table 3), where the translocation from root to leaf was 1.97, and from root to stem it was 1.66. In all five sites, the TFs for molybdenum were greater than those for selenium, with the highest value of 5.30 in site FF-P2. The TFs for uranium were <0.5 , with the exception of locations FF-P2 (1.07) and MO-P1 (0.92). The TFs for arsenic and vanadium did not exceed 0.26 and 0.56, respectively. Samples of soil and plant material from TL-P1 and FF-P2 were selected for further analysis by XAS (below).

3.4. Se, Mo, U, As and V in Brassica (Se-Accumulators)

The concentrations of Se, Mo, U, As and V determined by ICP-MS in three *Brassica oleracea* varieties (premier kale, hispi cabbage and red flare cabbage), grown in a $4 \times 3 \text{ m}$ experimental plot adjacent to site FF, are shown in Table 4. In all three *Brassica* varieties, selenium was most concentrated in the leaves, followed by the roots, then stems. The selenium concentration in the leaves ranged from 971.7 mg kg^{-1} in the premier kale to 787.8 mg kg^{-1} in the red flare cabbage. The concentration in the roots ranged from 643 mg kg^{-1} in the hispi cabbage to 390 mg kg^{-1} in the red flare cabbage, and for the stems, from 539.9 mg kg^{-1} in the hispi cabbage to 336.2 mg kg^{-1} in the red flare cabbage. In all three *Brassica* varieties, molybdenum was most concentrated in the leaves, followed by the root material and then stem material, similar to selenium; the Mo concentration in the leaves ranged from $1000.4 \text{ mg kg}^{-1}$ in the premier kale to 271.6 mg kg^{-1} in the red flare cabbage. As with the non-accumulator plants, the uptake of arsenic, uranium and vanadium was low with the highest concentrations of arsenic and uranium in the roots of the hispi cabbage of 0.76 mg kg^{-1} and 9.38 mg kg^{-1} respectively; for vanadium, it was 1.11 mg kg^{-1} in the roots of premier kale. Premier kale proved the best accumulator of both selenium and molybdenum, demonstrating that the choice of plant variety is important in phytoextraction.

The TFs from the root to leaf ranged from 1.29–2.02 for selenium, and as with the non-accumulator species, the translocation of molybdenum was more efficient than selenium, with a TF from root to leaf of 1.45–3.24. The TFs for arsenic, uranium and vanadium were all below 1. These positive TFs for selenium and molybdenum are a further indication that *Brassica oleracea* will be effective in phytoextraction. Samples were selected from the premier kale and hispi cabbage for further analysis by XAS to determine the distribution of selenium and the selenium species present in the plants.

3.5. μ -XRF and XAS

Cryo-sectioned samples of selenium non-accumulator plants (tall fescue grass and white clover), Se accumulator plants (premier kale and hispi cabbage) and seleniferous soil from site TL-C1 and site FF-C2 at 30–40 cm depth were selected for XRF and speciation analysis using μ -XAS. Spectra of the Se standards (see above) were produced (Figure 5). A series of μ -XRF maps were then collected (Figures 6–9 and A6), revealing the selenium distribution, and from these XANES spectra were collected from representative points of interest (POIs) and compared to the Se standards. The Se K-edge energies for reference standards, based on the maximum of the first derivative of XANES spectra (e0), were 12665.0 eV for sodium selenate (NaSeO_4 , Se^{VI}), 12662 eV for sodium selenite (NaSeO_3 , Se^{IV}), 12658 eV for selenomethionine ($\text{C}_5\text{H}_{11}\text{NO}_2\text{Se}$, Se^{II}), 12657 eV for red elemental selenium (Se^0), 12657 eV for sodium selenide (Na_2Se , Se^{II}) and 12656 eV for selenocystine ($\text{C}_6\text{H}_{12}\text{N}_2\text{O}_4\text{Se}_2$, Se^{II}). Tables 5–8 show the results of the LCF of selenium species in plant and soil samples, undertaken on merged duplicates of the normalised XANES spectra using ATHENA 0.9.26 software. These fits are illustrated in Figure A5.

Table 4. The mean concentration of Se, Mo, As, U and V in selenium accumulator *Brassica oleracea* varieties from the experimental plot in a pasture in West Limerick. TF = the translocation factor; a value >1 indicates the plant can translocate the element effectively from the root to shoots, stem and leaves. Analysis by ICP-MS, all experiments performed in triplicate $\pm$ the standard error.

Sample	Material	Se mg kg ⁻¹	Mo mg kg ⁻¹	As mg kg ⁻¹	U mg kg ⁻¹	V mg kg ⁻¹
Premier Kale	Kale Leaf	971.7 $\pm$ 18.08	1000.4 $\pm$ 14.91	0.20 $\pm$ 0.01	0.63 $\pm$ 0.04	0.37 $\pm$ 0.03
	Kale Stem	394.3 $\pm$ 2.04	181.2 $\pm$ 2.78	0.16 $\pm$ 0.02	0.29 $\pm$ 0.06	0.15 $\pm$ 0.03
	Kale Root	570.6 $\pm$ 6.38	309.2 $\pm$ 3.09	0.47 $\pm$ 0.01	4.74 $\pm$ 0.19	1.11 $\pm$ 0.05
	TF = Leaf/Root	1.70	3.24	0.43	0.13	0.34
	TF = Stem/Root	0.69	0.59	0.34	0.06	0.13
Hispi Cabbage	Hispi Cabbage Leaf	826.5 $\pm$ 10.72	443.2 $\pm$ 3.41	0.22 $\pm$ 0.01	0.93 $\pm$ 0.05	0.39 $\pm$ 0.02
	Hispi Cabbage Stem	539.9 $\pm$ 2.63	260.9 $\pm$ 2.53	0.36 $\pm$ 0.01	0.38 $\pm$ 0.02	0.13 $\pm$ 0.00
	Hispi Cabbage Root	643.0 $\pm$ 8.47	288.6 $\pm$ 1.78	0.76 $\pm$ 0.01	9.38 $\pm$ 0.29	0.83 $\pm$ 0.03
	TF = Leaf/Root	1.29	1.54	0.29	0.10	0.47
	TF = Stem/Root	0.84	0.90	0.48	0.04	0.15
Red Cabbage	Red Cabbage Leaf	787.8 $\pm$ 42.31	271.6 $\pm$ 13.45	0.03 $\pm$ 0.03	0.20 $\pm$ 0.02	0.10 $\pm$ 0.01
	Red Cabbage Stem	336.2 $\pm$ 8.38	162.8 $\pm$ 0.39	0.26 $\pm$ 0.01	0.32 $\pm$ 0.00	0.13 $\pm$ 0.00
	Red Cabbage Root	390.0 $\pm$ 6.45	187.6 $\pm$ 1.98	0.53 $\pm$ 0.01	4.60 $\pm$ 0.22	0.57 $\pm$ 0.04
	TF = Leaf/Root	2.02	1.45	0.06	0.04	0.17
	TF = Stem/Root	0.86	0.87	0.50	0.07	0.23

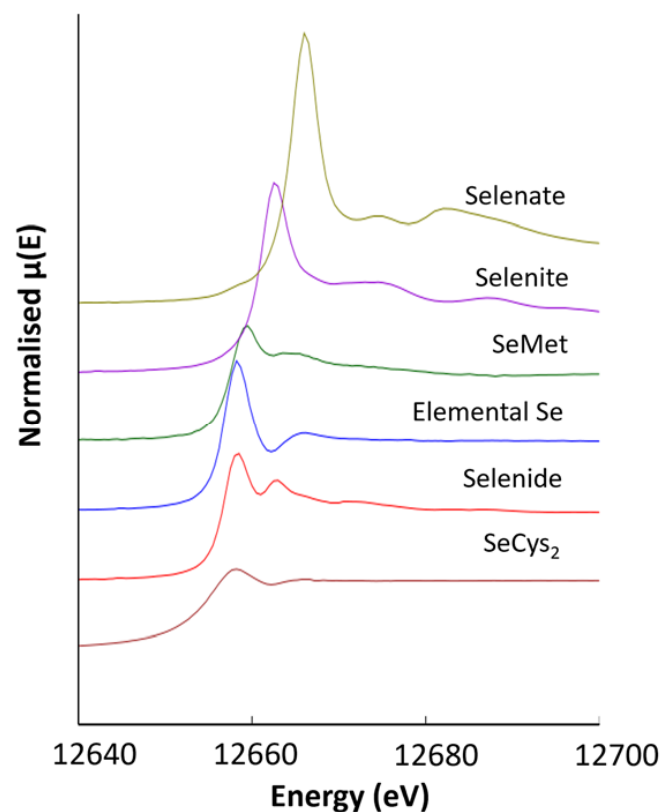


Figure 5. Normalised XANES spectra for inorganic and organic selenium standards in the oxidation states-II, 0, IV and VI.

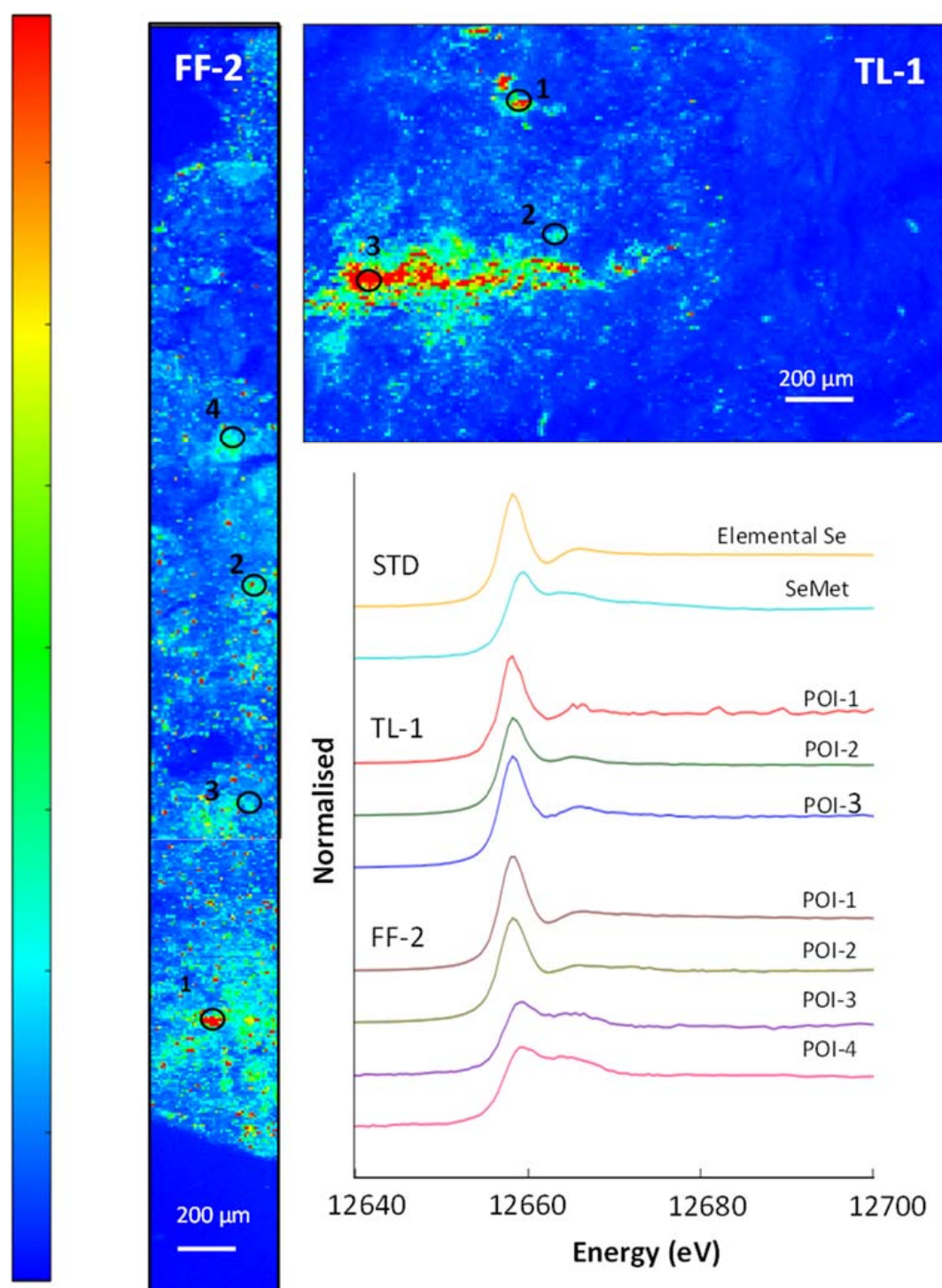


Figure 6. (Top) and (left): μ -XRF Se K-edge maps showing a section through a soil pellet. The colours are a 'pseudo-temperature' scale, with high selenium counts in red and areas with no counts in dark blue. (Right): Normalised XANES spectra showing selenium species in the selected points of interest (POIs) compared to elemental selenium and the SeMet standard.

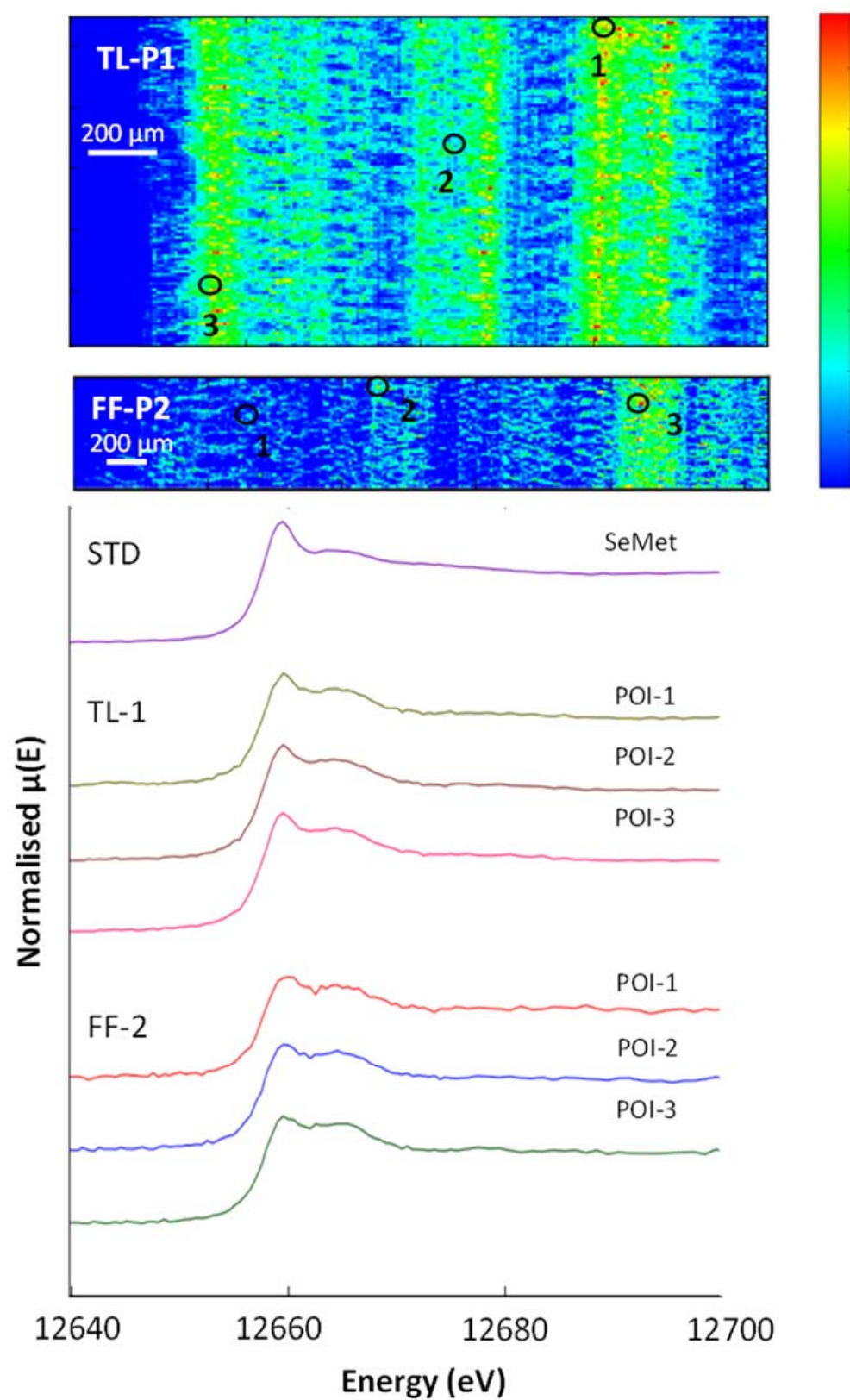


Figure 7. (Top): μ -XRF Se K-edge maps showing a grass leaf (shoot) from sites TL-P1 and FF-P2. The μ -XRF is on a temperature scale, with high selenium counts in red and areas with no counts in dark blue. (Bottom): normalised XANES spectra showing selenium species in selected POIs and the SeMet standard.

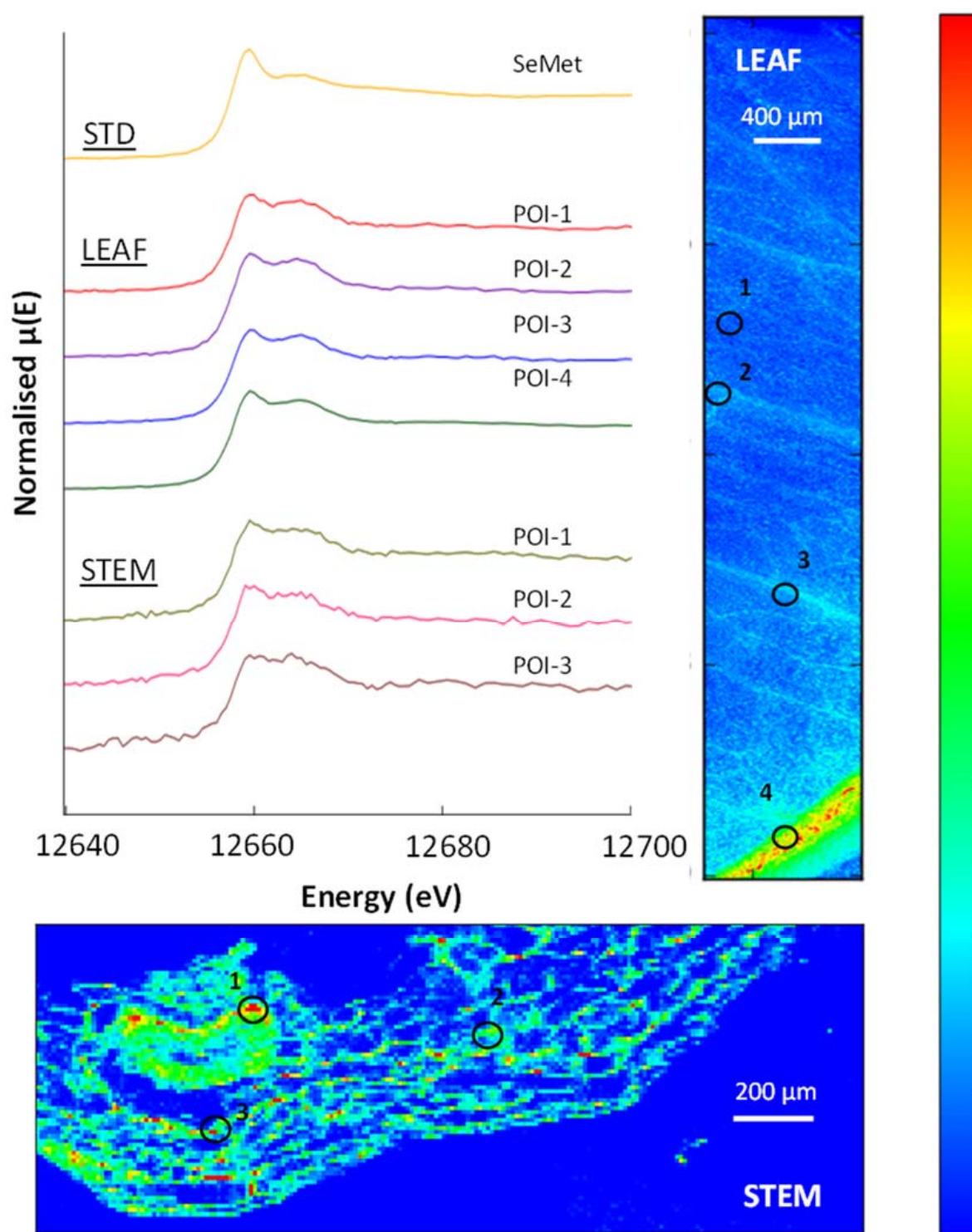


Figure 8. μ -XRF Se K-edge maps showing a white clover leaf and a cross-section through a stem and a leaf from the vein out towards the margin. The μ -XRF is on a pseudo-temperature scale, with high selenium counts in red and areas with no counts in dark blue. (**Top left**): normalised XANES spectra showing selenium species in selected POIs and the SeMet standard.

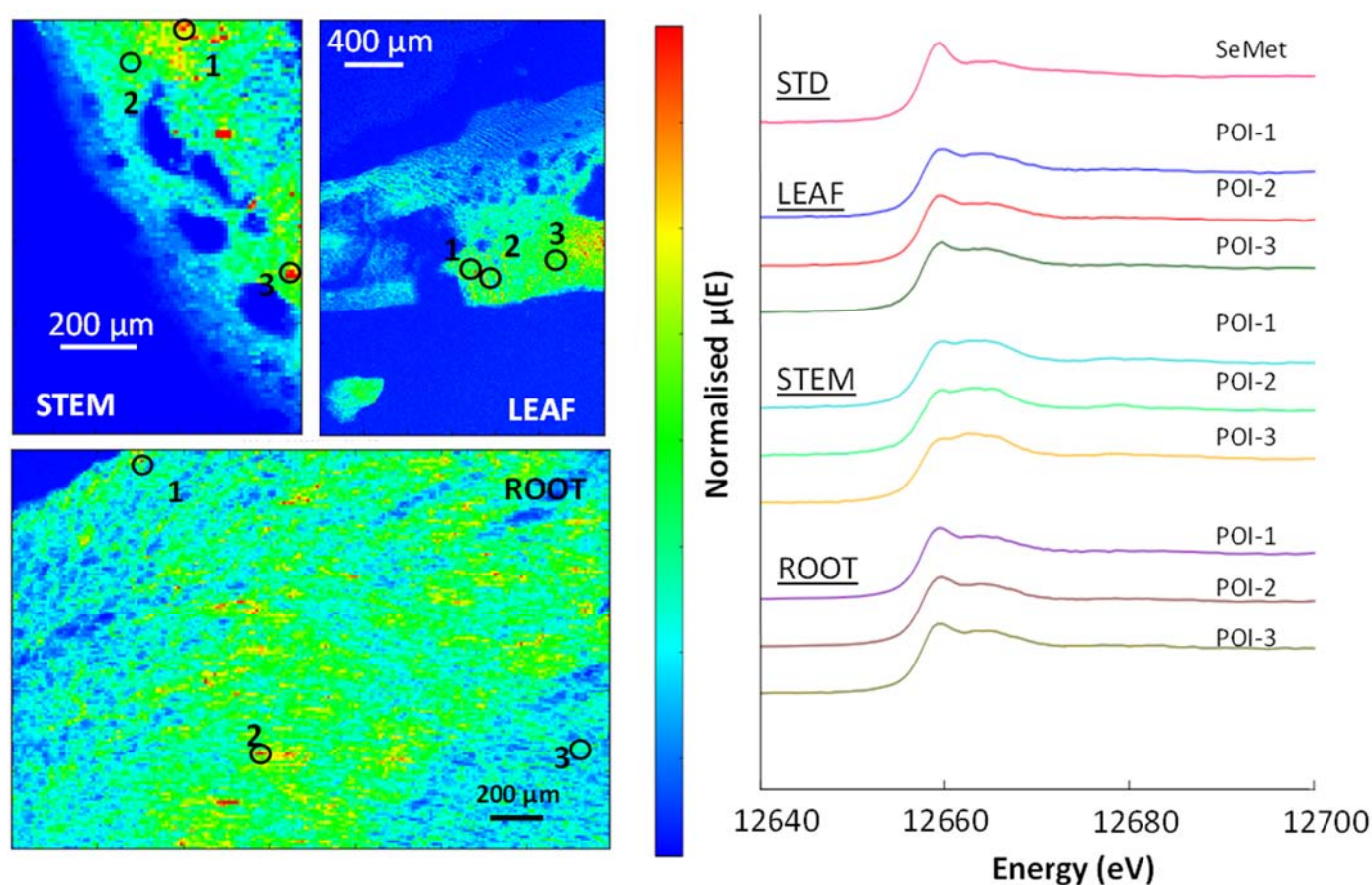


Figure 9. (Left): μ -XRF Se K-edge maps showing cross-sections through a premier kale leaf, stem and root. The μ -XRF is on a temperature scale, with high selenium counts in red and areas with no counts in dark blue. (Right): normalised XANES spectra showing selenium species in selected POIs and the SeMet standard.

Table 5. Linear combination fits for points of interest (POIs) selected in the seleniferous horizon (30–40 cm) of two soils from West Limerick. The goodness of fit is determined by the R-factor (approximate error $\pm 3\%$). (Note: there was no detectable selenate).

Sample	POI	SeCys ₂	SeMet	Selenide	Elemental Se	Selenite	R-Factor
		%	%	%	%	%	
TL-C1 Soil (core)	1	7	-	-	92	-	0.008
	2	6	34	-	65	-	0.003
	3	-	-	19	81	-	0.003
FF-C2 Soil (core)	1	-	-	7	93	-	0.008
	2	8	-	16	77	-	0.002
	3	24	76	-	-	-	0.020
	4	-	70	-	19	11	0.005

Table 6. Linear combination fits for selected selenium non-accumulator species measured in ATHENA. The goodness of fit is determined by the R-factor. The improvement in the fit for values <4% is marginal.

Sample	POI	SeCys ₂	SeMet	Selenite	Selenate	R-Factor
		%	%	%	%	
FF-P2 Grass blade	1	16	75	7	2	0.019
	2	8	80	9	4	0.005
	3	7	79	10	4	0.006
TL-P1 Grass blade	1	-	91	7	2	0.004
	2	-	90	8	2	0.004
	3	-	89	8	2	0.008
TL-P1 Grass roots	1	3	84	8	3	0.004
	2	3	81	10	3	0.005
	3	16	76	7	2	0.011
TL-P1 Clover Leaf	1	14	76	7	4	0.011
	2	7	82	7	3	0.005
	3	9	82	7	3	0.004
	4	8	84	11	4	0.007
TL-P1 Clover Stem	1	-	88	8	4	0.009
	2	-	89	8	2	0.013
	3	-	86	11	3	0.037

Table 7. Linear combination fits for selected *Brassica oleracea* (Se accumulator) species from site FF measured in ATHENA. The goodness of fit is determined by the R-factor. The improvement in the fit for the selenate values <4% was marginal.

Sample	POI	SeCys ₂	SeMet	Selenite	Selenate	R-Factor
		%	%	%	%	
Premier Kale Leaf	1	12	76	10	3	0.005
	2	7	84	7	2	0.004
	3	9	80	9	3	0.004
Premier Kale Stem	1	11	72	14	4	0.006
	2	14	67	15	4	0.005
	3	11	67	18	4	0.005
Premier Kale Root	1	9	83	6	2	0.003
	2	10	81	7	2	0.006
	3	9	82	7	2	0.004
Hispi Cabbage Leaf	1	16	73	10	1	0.015
	2	7	83	8	2	0.004
	3	8	84	6	2	0.003
Hispi Cabbage Stem	1	-	93	6	1	0.015
	2	-	97	2	1	0.001
	3	-	93	6	1	0.014
Hispi Cabbage Root	1	16	80	3	1	0.008
	2	17	82	7	-	0.007
	3	16	81	3	1	0.006

Table 8. Bioaccumulation factors (BAF) calculated for sites TL-P1 and FF-P2 (0–10 cm).

Site	BAF	Se	Mo	As	U	V
TL-P1	Grass Shoot/Soil	0.81	7.74	0.02	0.01	0.01
	Grass Root/Soil	1.23	5.74	1.19	0.19	0.46
	Clover Leaf	5.36	20.00	0.12	0.01	0.01
	Clover Stem	4.53	21.25	0.01	0.00	0.00
	Clover Root	2.72	6.25	0.08	0.04	0.06
FF-P2	Grass Shoot/Soil	0.34	3.48	0.06	0.05	0.02
	Grass Root/Soil	0.27	0.66	0.32	0.05	0.23

3.6. Soil Selenium Species

The μ -XRF map (Figure 6) from site TL-C1 shows an inhomogeneous distribution of selenium in the soil, with several points and a distinct layer of higher selenium counts. The linear combination fits in Table 5 show that elemental selenium (Se^0) was the most abundant selenium species, with 65–93% identified in the three selected POIs; the ‘layer’ represented by POI-3 contained 81% elemental selenium and 19% selenide. The μ -XRF map (Figure 6) also shows several areas of lower selenium concentration dispersed through the soil, represented by POI-2 and POI-4. The best fits for these areas are a combination of elemental selenium and organic selenium species, SeCys_2 in POI-4 and both SeCys_2 and SeMet in POI-2 (Table 5). In site FF-C2, elemental selenium was also the most abundant selenium species in POI-1 and POI-2 (93% and 77%), with 7% and 16% selenide, respectively, also present. However, SeMet was the most abundant selenium species in two areas (POI-3 and POI-4), with 70% and 76%, respectively. POI-3 also contains 24% SeCys_2 , while POI-4 has 19% Se^0 with 11% selenite; the latter is only seen as a small component of the soil selenium. In both soil samples, there was no positive correlation, at this scale, of selenium with other elements identified. For instance, the soil samples contained abundant iron, but the μ -XRF maps show that the areas of high iron did not correlate with selenium.

3.7. Selenium in Non-Accumulator Species

The μ -XRF maps in Figure 7 pick out the morphology of shoots (blades) of grass from TL-P1 and FF-P2 which are defined by their selenium content, with the highest selenium concentration associated with the leaf veins. The LCF data (Table 6) for the selenium non-accumulator plants showed SeMet was consistently the dominant selenium species in the shoot, stem and root material analysed. In the FF-P2 shoot and TL-P1 root, SeCys_2 was present, while in the grass blade (TL-P1), none was detected. Se-organic species comprise 86–92% of the selenium present in the grass. In all grass samples, the LCF revealed a small but significant component of selenite, typically 9%, but convincing evidence of the presence of selenate was lacking. The μ -XRF map in (Figure 8) shows the outer part of a white clover stem, with the cells of the cortex and phloem exposed by their selenium content. SeMet is the most abundant Se-species (86–88%), again with a component (~9%) of selenite. In the clover leaf, the mid-rib (POI-4; 82%), veins and blade, SeMet was again the dominant Se-species, with 8% SeCys_2 and 11% selenite.

3.8. Selenium in *Brassica oleracea*

The μ -XRF maps of the accumulator species indicate significant Se concentrations throughout the plants, with a particularly high concentration at the epidermis (Figures 8–10 and A6). Similar to the selenium non-accumulator plants, SeMet was the most abundant species in the leaf, stem and root material in both *Brassica* plants analysed; POIs revealed SeMet in the range 67–97%, and LCF revealed significant SeCys_2 to be present, with variable amounts (2–18%) of selenite. The two organic species accounted for 78% to 96% of Se-species present, although the stems of the premier kale contained significant (~16%) selenite (Table 7).

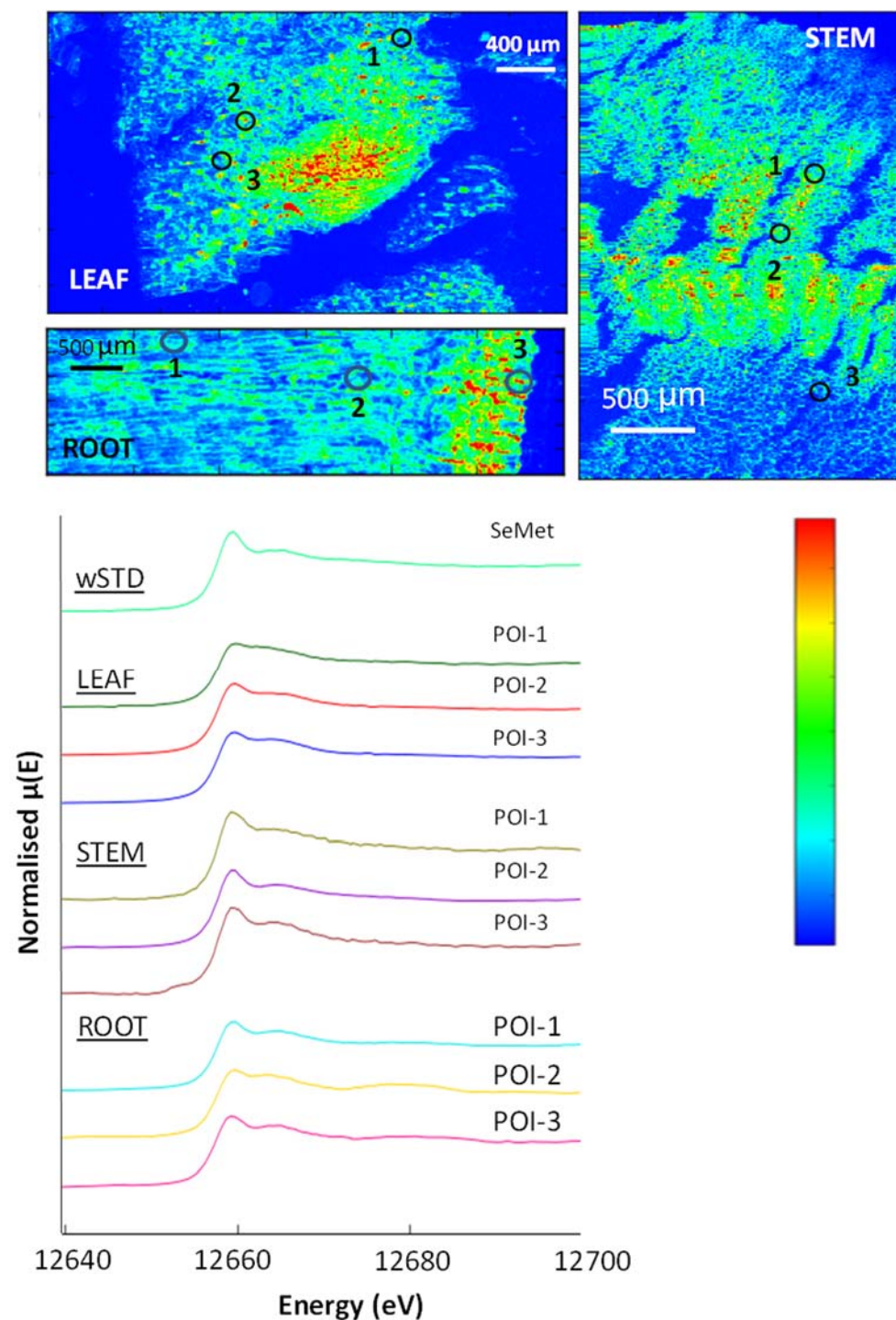


Figure 10. μ -XRF Se K -edge maps showing a hispi cabbage leaf cross-section through a stem and root (**top**). The μ -XRF is on a temperature scale, with high selenium counts in red and areas with no counts in dark blue. (**Bottom**): normalised XANES spectra showing selenium species in selected POIs and the SeMet standard.

4. Discussion

4.1. Selenium and Molybdenum in the West Limerick Soils

In localised zones, the selenium concentrations in the soils collected from the five dairy cattle pasture sites in West Limerick are extreme and far above the global soil average concentration of 0.44 mg kg^{-1} [1] and the trigger action value (TAV) for agricultural land of $3\text{--}10 \text{ mg kg}^{-1}$ (Tables 9 and A1).

Table 9. Trigger action values (TAVs) and upper safe limits for elemental concentrations in agricultural soils vary [1,49,50] and bioavailability is highly dependent on elemental species. Most publications quote the lower limits for Se, and Mo presented here [51,52].

Element	TAV
Se	5–20 mg/kg ^{−1}
Mo	3–10 mg/kg ^{−1}
As	10–65 mg/kg ^{−1}
V	100–350 mg/kg ^{−1}
U	5–500 mg/kg ^{−1}

The West Limerick soils in these zones are within the defined ‘seleniferous’ range of 1–1200 mg kg^{−1} [2,53,54], and the value of 1265.8 mg kg^{−1} at site MO is one of the highest naturally formed (unmineralised) soil selenium values recorded worldwide. These Se-hotspots occur in lower-lying areas surrounded by soils with low selenium values, many of the latter with concentrations below <0.1 mg kg^{−1} Se that would be considered ‘selenium deficient’ [12,55]. The boundary between the high and low selenium zones is sharp and defined by small (as small as 1 m) changes in elevation. The Se-rich zones in the soils are also high in molybdenum, and there is, generally, a positive correlation between selenium and molybdenum. The average world soil average of molybdenum is 1.1 mg kg^{−1}, and the TAV range is 5–10 mg kg^{−1}. Sites FF and MO have many values massively above the TAV, and these soils are defined as molybdeniferous to extremely molybdeniferous [17,49]. The soil sample collected from site FF-1, with a concentration of 1627.5 mg kg^{−1}, represents one of the highest naturally formed (unmineralised) concentrations recorded worldwide. The average world natural uranium soil concentration is 3 mg kg^{−1}, and many of the values in these Limerick soils are considerably above global averages, with some very high values for natural soils, up to 658.8 mg kg^{−1} in core FF-C2. In all sites studied, the arsenic concentrations (Tables A1–A3) also exceed the world soil average of 6.83 mg kg^{−1}, but most values were within the TAV range of 10–65 mg kg^{−1} [49] (Table 9).

In the bulk samples analysed by XRF, there is a close correlation between high selenium, molybdenum and uranium, but these positive correlations are not seen on the scale of the μ XRF. Although the arsenic concentrations in the bulk samples do not have the anomalously high values of selenium, molybdenum and uranium, the arsenic concentrations correlate with those of selenium, with the highest arsenic concentrations coincident with the highest selenium and molybdenum values.

Given that Se, Mo, U and As are all redox-sensitive elements that are mobilised in oxic weathering conditions and immobilised in reducing conditions, their co-incidence in these West Limerick soils is to be expected. The local source of these elements is the Carboniferous deep-water shales (the Clare Shales for sites FF, MO, PK, TJ; the Rathkeale Shales for site TL [56]), and local hydrology will have transported the elements as the oxyanions (SeO₄^{2−}, MoO₄^{2−}, AsO₄^{3−} and (UO₂(CO₃)₃)^{4−}) into the marshy areas that now form the high Se zones. High organic matter characterises these soils, and the thick clay layer observed in cores and trenches would have provided an excellent under-seal, leading to waterlogging and providing a perfect low Eh sink for the reduction, immobilisation and concentration of the Se, Mo, U and As. The West Limerick region was at the margins of the ice advance in the last glacial maximum, and periglacial conditions will have existed until ~11 ka BP [57]. From that time until the anthropogenic draining of the marshy areas, this redox system will have operated. At present, there is no addition of either selenium or other ‘redox’ elements to these soil systems.

The analysis of the soil at sites FF and TL reveals the presence of Se⁰ and selenide, the organic species SeMet and SeCys₂ and very limited selenite. The reduction of selenium in anoxic soils by microbial activity by metal/metalloid reducers is well recorded [58], and several strains of bacteria are able to reduce the selenate via selenite to Se⁰, producing

selenium nanoparticles [59–61]. This process will have been operating in the organic-rich West Limerick soils, where the natural microbial consortium will have managed the potentially toxic levels of selenium by converting it to the highly immobile Se^0 , and selenide, greatly reducing its bioavailability. Selenide can be absorbed into organic matter or occur as FeSe , and as there is no evidence of a positive Se-Fe correlation in the soils, it is likely to be associated with the extensive organic matter present in these soils [62,63].

Selenate is the most common selenium uptake route into plants [5,64], mimicking the sulfate pathway, but selenate does not play a significant role in these neutral to mildly acidic soils in West Limerick. However, the SeMet and SeCys_2 present in these soils are highly mobile and easily taken up by plants after being released from decaying Se-bearing organic matter [65]. It has been noted that these organo-selenium compounds can be taken up by wheat and *Brassicas* at rates over 20-fold higher than selenate or selenite [3,66], and plants have been shown to take up the SeCys and SeMet from soils via root amino acids [66]. The oxyanion selenite is a minor bioavailable component of the soils, and its uptake to roots has been associated with inorganic phosphate (Pi) transporters [67], although there is also evidence that the aquaporin NIP2;1 is able to take up selenite into rice [66,68–70].

The system that operated in West Limerick has similarities to that operating at Kesterson in California, where selenium derived from Cretaceous shales was concentrated by (in this case, human) irrigation processes and transported as selenate to the Kesterson wetlands. There, it was immobilised in the anoxic conditions as Se^0 and remobilised by microbial activity, taken up by plants and recycled via selenium in decaying organic matter [71,72], with disastrous effects on local wildlife.

4.2. Selenium in Pasture Plants

The pasture grasses take up both the Se and Mo in significant amounts, making it bioavailable for livestock (Tables 3 and 4). The concentration of Se in the roots and shoots of the grasses is variable, but values greatly exceed the recommended limit in pastures of 2–5 mg kg^{-1} Se [7,73,74]. The adjacent sites FF and MO have very high soil selenium, and this is reflected in the grass, with the shoot samples containing 34 and 48 mg kg^{-1} , respectively, and the roots 26 and 84 mg kg^{-1} . However, the highest concentrations (as high as 78.05 mg kg^{-1}) in the shoots were in grass samples from site TL-P1. Site TL, with ‘relatively’ low soil selenium (maximum 88 mg kg^{-1}), is not greatly different in character from the other sites; the site is well-grassed, with a grey clay layer at >60 cm depth, but it does have a lower TOC than other sites (Tables A1–A3). As a consequence, site TL-P1 has a higher BAF than FF-P2 (Table 8), demonstrating the selenium is relatively immobilised in the latter. As in many cases elsewhere [61,75], selenium in these West Limerick soils is strongly associated with the organic matter, and a study by [75] showed a decrease in selenium uptake when soils contained >11% organic matter, while soils with 5–11% organic matter showed optimum uptake (see Figure 4). Thus, the difference in uptake can be related to the amounts of organic matter present at these two sites, and the very high levels of organic matter at site FF-P2 will have enhanced stimulation of the microbial reduction of selenium to less bioavailable species such as Se^0 due to the increase in available electron donors [75–77]. The organic nature of the FF and MO soils may explain why the selenium content of the plants, although high, is not as high as might be expected given the soil selenium concentration. Root-to-shoot translocation in the grass is, with one exception, <1, consistent with experimental studies of wheat that showed restricted translocation in systems where the selenium was present as an organic selenium species in the roots; in selenate-dominated soil systems, translocation is much more efficient [78].

The white clover (*Trifolium repens*.) collected from site TL-P1 was high in selenium in all parts of the plant (296.15 mg kg^{-1} in leaf), representing a BAF factor of ~5 compared to ~1 for grass shoots from the same location (Table 8). This is in direct contrast to a previous comparative study [79] that, using selenate, showed a much higher amount of selenium in ryegrass than white clover, although another study [80] noted 79.39 mg kg^{-1} in white clover leaves grown in the seleniferous soils of the Enshi District, China. In West Limerick,

SeMet and SeCys₂ are the main soil species, and selenate is largely absent, revealing the importance of the bioavailable species in defining relative Se uptake [81]. In West Limerick, the white clover behaves as a secondary selenium accumulator (defined as containing >100 mg kg⁻¹ Se [7]), and, therefore, white clover should not be used to reseed pasture on selenium-bearing organic-rich soils.

There is very limited evidence for the presence of selenite in the West Limerick soils (Table 5), but there is a persistent amount of selenite seen in the plants (Tables 6 and 7). This will not have been produced in situ in the plants but must have been taken up from the soil. Selenite is the likely oxyanion to be present in anoxic soils and is taken up directly into plants and converted (both enzymatically or non-enzymatically) to selenide for incorporation into the amino acids SeMet and SeCys₂ [66]. Selenite uptake into plants has been linked to phosphate transporters [67,82], but competition with the poorly mobile phosphate would be reduced by the high organic content of these West Limerick soils. Thus, the uptake of selenite would be fast, leading to the fast depletion of selenite in the soil and incorporation in the plants. Grass shoots sampled from sites FF-P1/2, MO-P1, TJ-P1 and PK-P1 all contained molybdenum concentrations that exceed the maximum tolerable level for livestock of 5–10 mg kg⁻¹ [26–28], and sample FF-P2 contained 79.63 mg kg⁻¹ Mo. Compared to selenium, the translocation factor from root to shoot in grass is >1 and some values exceed 5; the low concentrations in samples from site TL-P1 reflected the low concentrations in the soil (Table 1), and this site was associated with the Rathkeale Formation, a different Carboniferous shale/mudstone unit to the likely source unit for the other areas. In molybdeniferous soils, molybdate is the mobile bioavailable Mo-species, and this will be stable at lower Eh values than selenate in mildly acidic soils. Molybdenum can be held in anoxic soils as sulfide or sorbed to organic matter but is not immobilised by dissimilatory metal-reducing bacteria in a similar way to selenium [77] and will thus be available as molybdate for uptake by plants. The translocation of the molybdate from roots to shoots is undertaken by sulfate transporters and will have been facilitated in these soils by the lack of competition from selenate [83]. Uranium, arsenic and vanadium pose less of a risk to livestock health as these elements have a poor translocation from roots to shoots. There are extensive studies on uranium uptake in plants, especially in phytoremediation studies [84,85]. Site FF had exceptionally high soil uranium in the top 30 cm, but uptake by plants at that site (and other sites) was limited to ~4.0 mg kg⁻¹. There is a high organic component in the soils, and in these humic-rich environments, bioavailable organic uranium complexes can form and persist as mobile U (VI). However, in anoxic environments, the uranium will tend to remain bound to the organic and mineral matter [86].

Selenium concentrations in plants largely reflect the concentrations in the soil environment [1], and the data presented here demonstrate that the pasture in these areas of former marshy ground has toxic levels of selenium and molybdenum. The main fodder for the cattle will be grass (15 kg/per day), and the recommended daily intake for dairy cows is 300 µg/kg DM Se [87]. Even though translocation from root to shoot in the grass is <1, the blades of grass provide a significant health hazard to the cattle, which would be taking in between 1 and 2 orders of magnitude of excess selenium. The predominant mobile Se-species identified in the root and shoot material of the grass and white clover was SeMet, which can also cause phytotoxicity in plant tissues in high concentrations due to the misincorporation of SeMet in place of methionine in protein synthesis [7,88,89]. However, it is selenocysteine that is thought to be the major source of problematic misincorporation into proteins [90], and the only evidence of phytotoxicity observed was the slight chlorosis (yellowing) of the tall fescue grass blades at site FF and in the hispi cabbage (see below); these samples had the highest concentration of plant SeCys₂ (Tables 3 and 4).

4.3. Selenium and Molybdenum Accumulator *Brassica oleracea*

All three *Brassica oleracea* varieties demonstrate the hyperaccumulation of selenium, with high selenium throughout the plants. Although selenate transporters were unlikely

to be active (see above), these samples show typical features of hyperaccumulators with a high SeMet content, effective translocation from roots to leaves (average 861 mg kg^{-1} in the leaves) and a concentration of selenium in the xylem; the latter is the main transport pathway [6,90,91]. The *Brassica* sp. also accumulated significant concentrations of molybdenum in the same pattern as selenium, with the highest concentrations in the leaves (Table 4; see also). Molybdenum toxicity and phytoremediation have been much less studied than selenium but has been successfully addressed by using soil amendments and *Brassica* sp. [92,93] and the ground cover species *Axonopus compressus*, grown on mine tailings [33]. The concentration of $\sim 800 \text{ mg kg}^{-1}$ Mo in the *Brassica* leaves observed in this study would pose a huge risk to livestock and human health; the intake limit to avoid toxicity for humans is $400 \mu\text{g}^{-1}$ per day [1,94]. However, the ability of the *Brassica* species to accumulate both selenium and molybdenum in concentrations throughout the plant demonstrates how effective they could be for the phytoremediation of seleniferous soils in West Limerick. Each 500 g plant would remove $400 \mu\text{g}$ of selenium if harvested. A *Brassica oleracea* root system can typically reach 50 cm, which means it would tap the whole seleniferous and molybdeniferous soil profile. In addition, the *Brassica* plants could be used for 'biofortification' to increase dietary intake in selenium- and molybdenum-deficient areas; most soils in the British Isles are selenium- and molybdenum-deficient [1,55,58,95].

5. Conclusions

In the highly restricted zones of the livestock pasture fields of West Limerick, there are extreme concentrations of Se, Mo and U in soils with the highest recorded concentrations of $1265.8 \text{ mg kg}^{-1}$ Se, $1627.5 \text{ mg kg}^{-1}$ Mo and 658.8 mg kg^{-1} U. The general concentrations in these elements are orders of magnitude above their TAVs. The soils enriched in these elements are in low-lying areas that were, historically, marshes or shallow lakes. The boundaries of the enriched zones are often sharp and marked by very small changes in elevation, representing the margins of the original marshy areas.

For soil analysis at a depth of 10–20 cm, 500 g samples within the enriched zones reveal that the concentrations of Se, Mo and U have a significant variation on a local scale, but there is a positive correlation between these three elements and with TOC. This positive correlation is confirmed in vertical cores. However, μ -XRF data reveals no positive correlation on a microscale. The main Se-species in the soil was non-bioavailable Se^0 , produced by microbial reduction, and organoselenium compounds were the most bioavailable source of selenium. Selenate is not a significant component of the soil Se in these anoxic, organic-rich soils.

The source of these elements was the Carboniferous shales/mudstones that were deposited in anoxic basins in the Carboniferous, where high concentrations of redox-sensitive trace metals accumulated. These elements were remobilised by oxidation into groundwater during post-glaciation weathering and transported into anoxic, organic-rich marshy areas and reprecipitated.

The grasses in the enriched zones have concentrations in the order of $20\text{--}80 \text{ mg kg}^{-1}$ Se in the roots, with $5\text{--}48 \text{ mg kg}^{-1}$ Se in the shoots with TF values of <1 . One site (TL) had the highest concentrations of Se (112.2 mg kg^{-1} Se in the roots and 78.1 mg kg^{-1} in the shoots) and a high BAF, which resulted from the relative lack of organic matter in the soil to retain the selenium at this site. White clover proved to be a strong accumulator of Se, with 296.2 mg kg^{-1} Se in the leaves, 249.5 mg kg^{-1} Se in the stems and 150.4 mg kg^{-1} Se in the roots. In sites containing significant soil Mo, the uptake of Mo was higher than Se, with TFs >1 . The uptake of U and As by the plants was restricted.

The hyperaccumulator *Brassica oleracea* grown over 11 weeks in a small experimental plot on the high Se and Mo soil recorded $971.72 \text{ mg kg}^{-1}$ Se and $1000.42 \text{ mg kg}^{-1}$ Mo in the leaves of premier kale, with TF factors of 1.70 and 3.24. There were significant differences in uptake between the three varieties examined.

In all the plants examined in this study, selenomethionine is the major selenium species in the plants, with typically 70–90% of the Se budget and approximately 10% selenocysteine

and 10% selenite. Thus, the bioavailable selenium system in these West Limerick soils is one dominated by organoselenium species.

The concentrations in the grasses and clover of both Se and Mo are far above the recommended limits for livestock pasture and, as the local farmers well know, the cause of the selenosis and molybdenosis affecting the dairy cattle. The use of *Brassica oleracea* (var. premier kale) is a route to phytoremediation of the blighted areas, although the removal of the recalcitrant Se^0 would be an issue. However, this latter issue could be remedied by the oxidation of the soils and the organic matter they contain by ploughing. This would produce mobile Se-oxyanions and stimulate microbial activity that would also release volatile methylated Se-species [37]. The potential use of this Brassica foliage as a Se-supplement for livestock grazing Se-deficient soils is problematic due to the concomitant high uptake of Mo, although Mo-deficiency also causes health issues for both livestock and humans.

6. Postscript

The farmers of West Limerick are well aware of which fields or areas within fields cause Se and Mo poisoning. The fields often produce a rich crop of grass, but the farmers have to manage access of their dairy herds to these areas. Both sulfur and copper supplements are administered to avoid the toxic effects of selenosis and molybdenosis [95].

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Appendix A

Selenium Uptake from Livestock Pasture Extremely Enriched in Selenium, Molybdenum and Uranium: A Field and X-ray Absorption Study.



Figure A1. Site FF, looking west. The low ridge in the background is underlain by Clare Shales. The marshy ground to the left is the location of the soils with the highest selenium values. The grassed areas were much lower in selenium, and it decreased to background levels up the slope to the right, by the path.



Figure A2. Site TL, looking west. A field of rich grass pasture sloping gently right to left. The highest selenium values are in a zone in the centre of the field (Figure 2).



Figure A3. Site MO, looking east. The marshy foreground includes the highest selenium soil values recorded and high molybdenum. The drainage ditch to the left separates site MO from FF. See Figure 3 for contoured Se values.



Figure A4. Site PK, looking west. The soil in the rough ground in the foreground is high in selenium, but the soils in the field up the slight slope in the background are low in selenium.

Table A1. X-ray fluorescence data showing the concentration of selected major and trace elements in soil samples collected at 10–20 cm depth at sites MO and PK. TOC by loss on ignition.

Bulk Sample	Se mg kg ⁻¹	Mo mg kg ⁻¹	As mg kg ⁻¹	U mg kg ⁻¹	V mg kg ⁻¹	Mn mg kg ⁻¹	Cu mg kg ⁻¹	CaO wt%	Fe ₂ O ₃ wt%	SiO ₂ wt%	Al ₂ O ₃ wt%	TOC wt%
MO-1	227.2	59.3	39.4	26.0	104.6	45.4	871.8	5.4	6.1	31.5	4.9	41.7
MO-2	763.5	431.1	89.6	407.0	77.8	236.3	111.8	4.9	12.4	21.2	3.4	48.4
MO-3	404.3	71.5	57.5	33.5	93.1	488.2	165.8	5.7	8.1	35.9	5.1	36.3
MO-4	145.9	37.6	18.6	17.0	105.7	563.5	90.0	6.5	4.8	43.7	6.4	30.3
MO-5	840.2	193.7	72.5	295.2	94.4	152.6	144.5	5.1	9.5	10.4	1.8	61.9
MO-6	115.7	20.8	8.6	53.3	146.0	208.5	149.0	2.2	4.6	50.5	6.9	28.8
MO-7	14.7	3.2	0.0	51.8	152.8	54.4	397.9	1.4	2.3	58.2	8.3	23.5
MO-8	1.0	6.1	0.0	17.4	195.2	210.0	924.6	1.3	3.1	62.7	9.8	17.8
MO-9	482.1	80.0	23.4	155.0	170.7	518.1	191.5	7.7	7.2	35.4	5.7	37.2
MO-10	1265.8	898.1	115.3	131.3	190.2	232.9	197.8	6.2	11.5	20.0	3.4	52.9
PK-1	682.0	34.9	63.1	12.4	208.6	732.2	222.8	19.1	5.7	4.5	1.1	56.5
PK-2	5.4	1.9	0.4	10.0	184.2	78.9	51.4	1.5	1.6	65.3	8.7	15.7
PK-3	0.9	13.6	11.6	11.1	175.7	1101.2	28.2	0.2	3.4	72.9	9.2	8.5
PK-4	2.7	17.7	11.6	10.7	212.2	920.2	32.0	0.2	3.3	69.5	8.9	10.9

Table A2. X-ray fluorescence data showing the concentration of selected major and trace elements in soil samples collected at 10–20 cm depth at site FF TOC by loss on ignition.

Bulk Sample	Se mg kg ⁻¹	Mo mg kg ⁻¹	As mg kg ⁻¹	U mg kg ⁻¹	V mg kg ⁻¹	Mn mg kg ⁻¹	Cu mg kg ⁻¹	CaO wt%	Fe ₂ O ₃ wt%	SiO ₂ wt%	Al ₂ O ₃ wt%	TOC wt%
FF-1	489.1	1627.5	99.8	599.5	163.6	113.7	762.8	4.4	5.1	46.2	7.3	28.2
FF-2	276.3	90.7	40.6	253.6	82.7	430.0	236.0	7.3	4.5	26.7	5.0	48.6
FF-3	13.4	2.8	9.9	24.0	184.9	95.6	78.0	1.3	2.6	65.2	10.7	14.8
FF-4	56.4	9.6	47.1	37.2	115.3	262.9	46.1	2.5	4.0	46.5	7.7	32.5
FF-5	178.2	24.0	17.9	53.0	156.2	595.4	56.2	4.5	4.8	29.1	4.8	47.9
FF-6	362.2	81.6	21.9	61.8	95.4	121.0	79.8	5.5	3.5	28.4	4.6	47.8
FF-7	31.5	30.1	20.2	24.6	109.1	466.3	74.2	1.0	4.6	67.2	10.7	10.3
FF-8	1.8	10.2	13.3	9.8	54.4	367.4	28.9	0.3	4.2	72.3	12.2	5.8
FF-9	4.4	21.4	16.4	12.5	74.3	420.5	41.3	0.3	4.1	69.8	10.8	9.1
FF-10	19.1	32.1	21.0	12.8	52.4	518.7	54.3	1.9	4.8	62.1	9.4	14.6
FF-11	8.4	8.6	9.4	19.8	48.4	113.5	45.7	2.0	3.6	64.9	9.0	13.0
FF-12	473.5	53.3	25.8	43.9	65.3	98.7	137.5	12.8	3.7	40.9	7.0	28.1
FF-13	0.0	10.5	5.3	10.2	79.7	2043.8	32.2	0.6	5.0	68.9	11.3	7.8
FF-14	0.0	11.9	11.9	9.2	89.1	1914.9	38.2	0.9	5.4	66.4	11.6	8.9
FF-15	0.0	10.0	14.0	12.9	84.8	1771.9	30.9	0.5	5.6	68.1	12.5	7.1
FF-16	246.0	43.2	18.4	127.9	189.7	1151.0	85.5	2.8	6.2	47.9	6.8	29.6
FF-17	0.0	9.4	10.3	9.1	109.7	1728.1	42.9	0.6	4.9	68.9	11.2	8.0
FF-18	26.3	4.6	15.2	8.8	118.4	402.4	54.8	21.8	2.8	31.1	6.2	31.5
FF-19	0.0	3.6	0.0	14.6	93.6	381.0	17.3	1.2	3.8	67.5	11.3	10.4
FF-20	0.0	7.2	10.6	8.3	97.9	2051.5	40.4	0.7	5.2	67.8	12.7	7.1

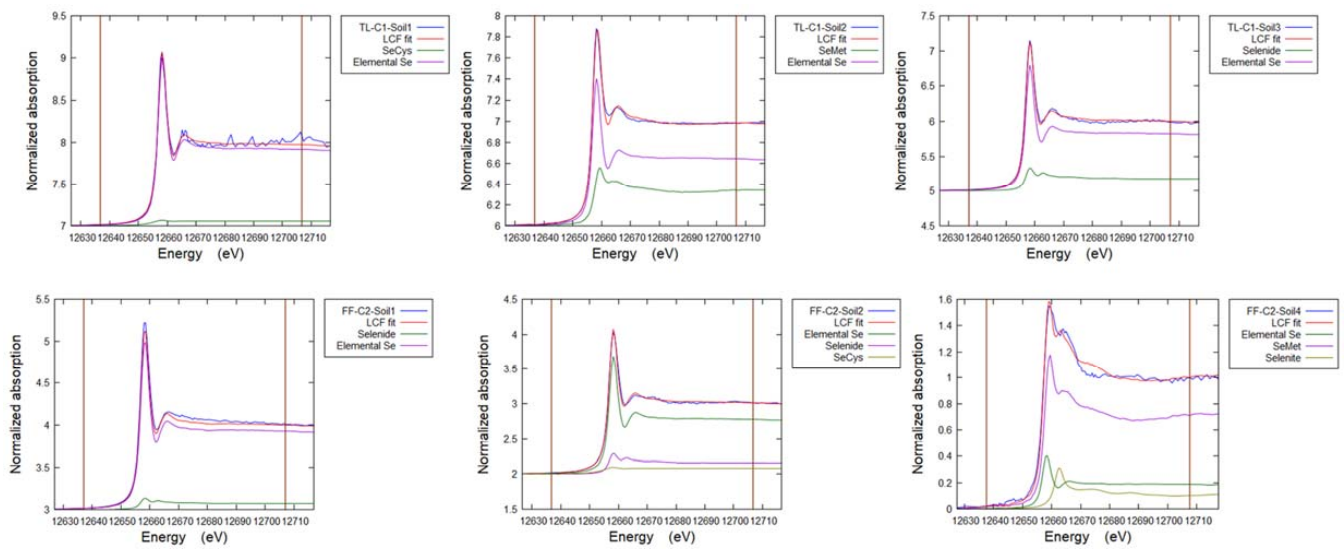
Table A3. X-ray fluorescence data showing the concentration of selected major and trace elements in soil samples collected at 10–20 cm depth at sites TL and TJ. TOC by loss on ignition.

Bulk Sample	Se mg kg ⁻¹	Mo mg kg ⁻¹	As mg kg ⁻¹	U mg kg ⁻¹	V mg kg ⁻¹	Mn mg kg ⁻¹	Cu mg kg ⁻¹	CaO wt%	Fe ₂ O ₃ wt%	SiO ₂ wt%	Al ₂ O ₃ wt%	TOC wt%
TL-1	1.9	0.9	13.9	6.4	79.3	602.2	54.7	1.9	3.7	62.3	11.0	13.5
TL-2	0.0	0.7	7.5	7.2	72.1	320.4	44.3	8.2	2.9	53.1	10.3	17.6
TL-3	0.0	0.4	12.2	6.5	68.5	248.0	30.1	2.5	3.5	64.6	10.7	11.8
TL-4	0.0	0.5	12.5	7.2	85.9	498.4	43.4	2.6	3.9	65.0	12.5	9.6
TL-5	1.9	0.5	11.1	6.6	60.9	257.4	42.0	5.9	3.1	57.4	9.4	17.7
TL-6	0.0	0.4	12.5	5.2	54.3	425.4	26.4	11.1	3.4	51.6	8.4	18.8
TL-7	3.3	0.2	13.0	6.7	77.7	240.5	42.3	2.6	4.5	54.3	12.1	18.2
TL-8	8.3	0.4	14.1	7.4	75.3	293.1	48.9	2.1	3.6	55.8	12.1	18.7
TL-9	34.2	1.9	39.8	13.0	82.1	435.8	51.4	1.9	4.8	48.1	12.1	25.4
TL-10	88.2	1.3	32.6	6.6	78.6	436.9	56.5	7.2	3.3	54.2	11.9	16.0
TL-11	28.0	0.2	11.2	6.5	76.5	318.1	53.3	5.5	3.8	58.9	10.7	14.4
TL-12	3.4	0.6	18.8	6.0	71.2	349.2	44.8	6.6	3.6	59.2	9.3	14.3
TL-13	0.0	0.4	9.4	5.4	58.4	477.3	31.2	2.0	3.5	55.0	12.7	18.4
TJ-1	0.0	1.0	15.7	6.8	65.6	1344.3	39.8	1.6	4.6	65.8	10.9	10.0
TJ-2	24.9	3.7	12.1	17.0	39.7	602.7	26.7	8.9	4.7	10.2	2.2	61.9
TJ-3	28.4	19.0	16.5	26.6	20.7	988.5	29.2	6.3	3.3	4.5	1.2	57.2
TJ-4	24.1	5.8	17.1	12.6	80.9	816.4	39.1	6.9	2.9	11.5	2.5	48.5
TJ-5	12.7	3.5	6.2	11.3	31.1	436.3	27.0	16.2	2.2	9.6	2.3	52.8

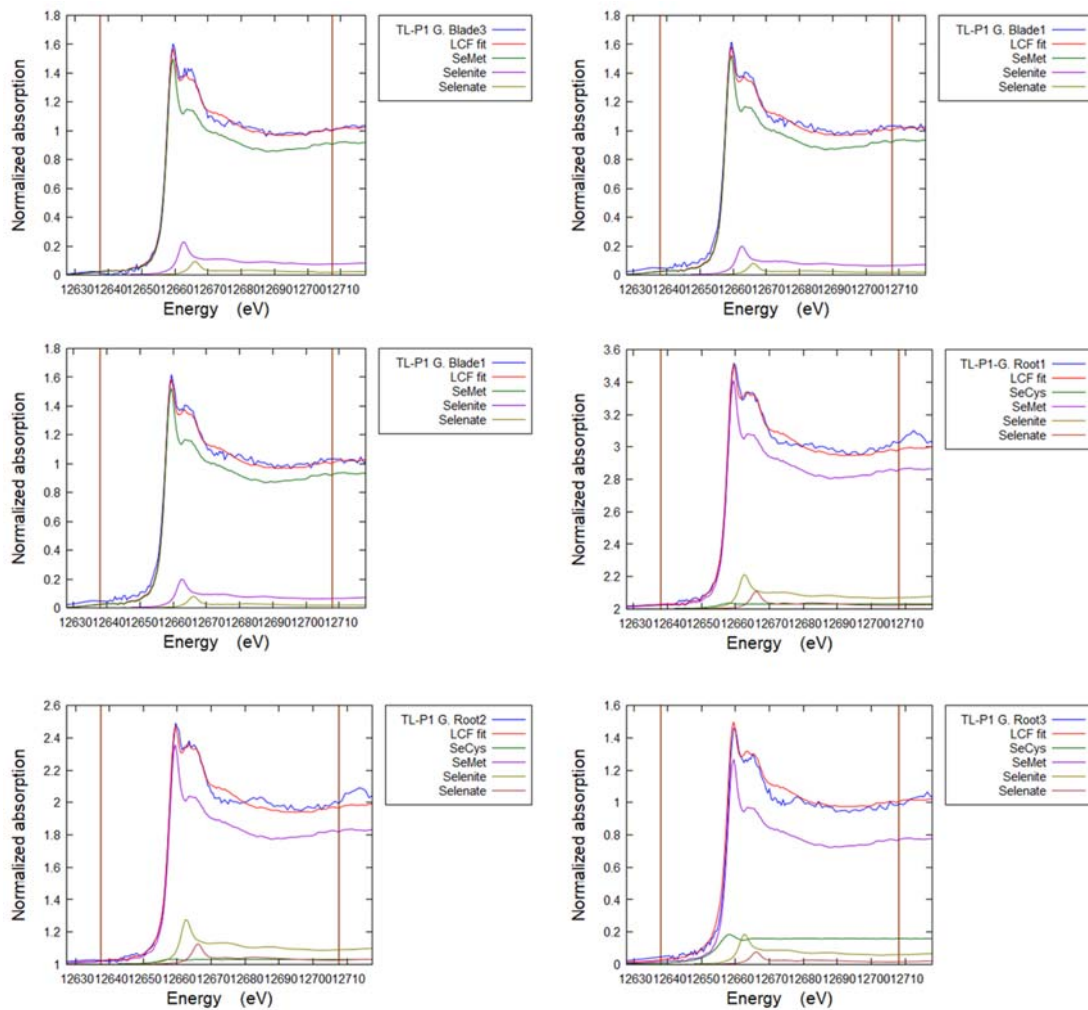
Table A4. Sample locations, West Limerick. Latitude and longitude.

Sample	Lat.	Long.	Sample	Lat.	Long.	Sample	Lat.	Long.
FF-1	52.52260	−9.06199	MO-1	52.52242	−9.05856	TL-1	52.51072	−9.01216
FF-2	52.52233	−9.05963	MO-2	52.52228	−9.05845	TL-2	52.51088	−9.01167
FF-3	52.52366	−9.06071	MO-3	52.52225	−9.05867	TL-3	52.51099	−9.01104
FF-4	52.52317	−9.05835	MO-4	52.52242	−9.05890	TL-4	52.51116	−9.01020
FF-5	52.52293	−9.05842	MO-5	52.52255	−9.05912	TL-5	52.51111	−9.00909
FF-6	52.52271	−9.06215	MO-6	52.52167	−9.05648	TL-6	52.51060	−9.00967
FF-7	52.52247	−9.06204	MO-7	52.52143	−9.05757	TL-7	52.51028	−9.01048
FF-8	52.52238	−9.06208	MO-8	52.52153	−9.05835	TL-8	52.50988	−9.01199
FF-9	52.52229	−9.06212	MO-9	52.52212	−9.05778	TL-9	52.51023	−9.01207
FF-10	52.52230	−9.06152	MO-10	52.52236	−9.05875	TL-10	52.51067	−9.01199
FF-11	52.52245	−9.06139				TL-11	52.51090	−9.01083
FF-12	52.52265	−9.06124	PK-1	52.51888	−9.06054	TL-12	52.51090	−9.00883
FF-13	52.52334	−9.05645	PK-2	52.51911	−9.06244	TL-13	52.51090	−9.00883
FF-14	52.52364	−9.05577	PK-3	52.51934	−9.06318			
FF-15	52.52334	−9.05725	PK-4	52.51940	−9.06367	TJ-1	52.51633	−9.00081
FF-16	52.52244	−9.05725				TJ-2	52.51663	−8.99919
FF-17	52.52254	−9.05615	TL-C1	52.51047	−9.01149	TJ-3	52.51786	−9.00004
FF-18	52.52357	−9.05893	FF-C2	52.52311	−9.06078	TJ-4	52.51702	−9.00111
FF-19	52.52387	−9.05840				TJ-5	52.51663	−8.99919
FF-20	52.52397	−9.05722						

Figure A5a–e. Representative Linear Combination Fits from Se K-edge XANES for selenium speciation in soils and plants (Tables 5–7).

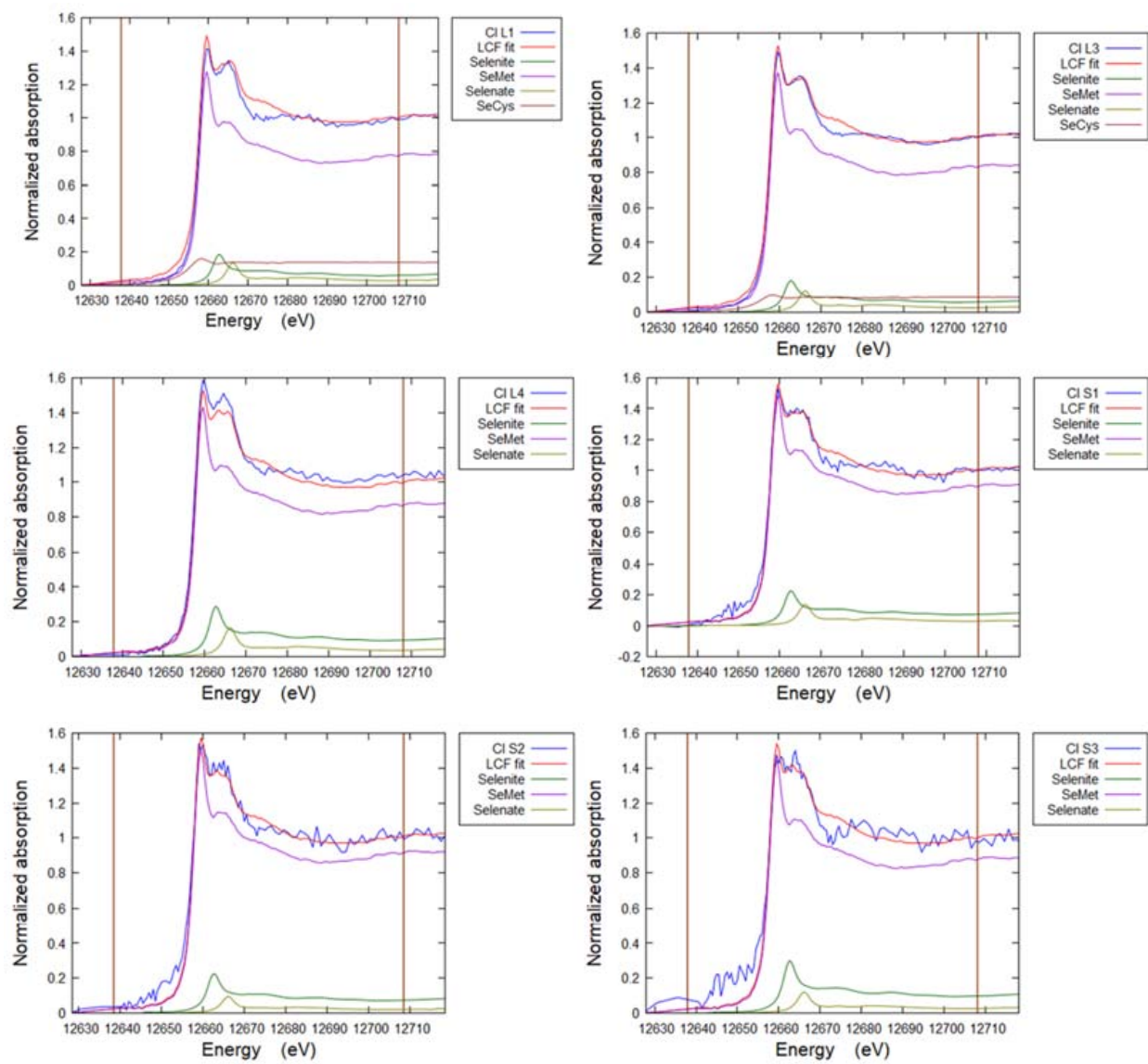


(a)



(b)

Figure A5. Cont.



(c)

Figure A5. Cont.

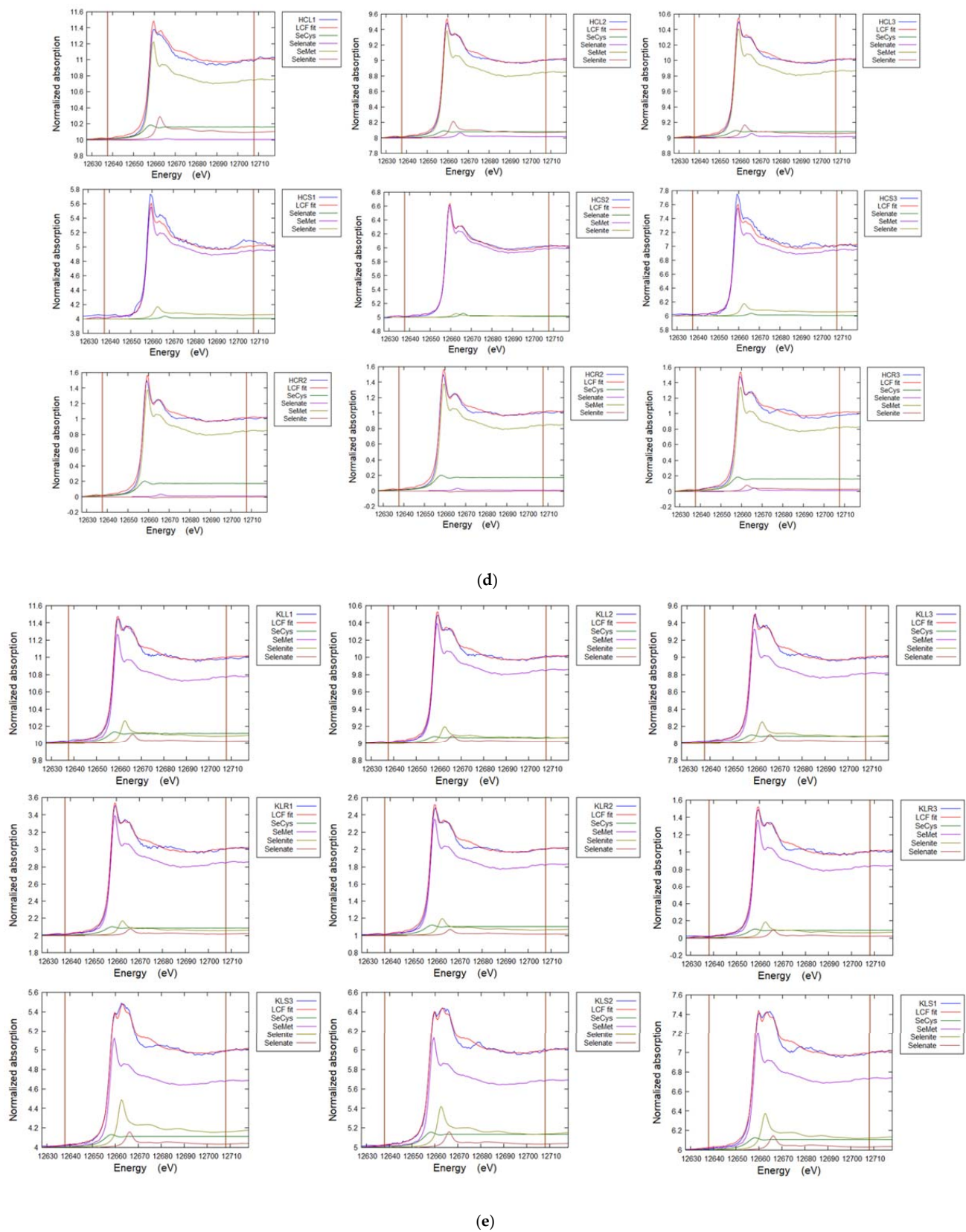


Figure A5. (a) Linear combination fit spectra for soil cores sites TL (TL-C1-3) and FF (FF-C1-3). (b) Linear combination fit spectra for grass from site TL. TL-P1 G Blade 1-3, TL-P1 Root 1-3. (c) Linear combination fit spectra for white clover. CL1,3,4 Leaf, CS1-3 Stem. (d) Linear combination fit spectra for hispi cabbage. HCL1-3 Leaf, HCS1-3 Stem, HCR1-3 Root. (e) Linear combination fit spectra for premier kale. KLL1-3 Leaf, KLR1-3 Root, KLS1-3 Stem.

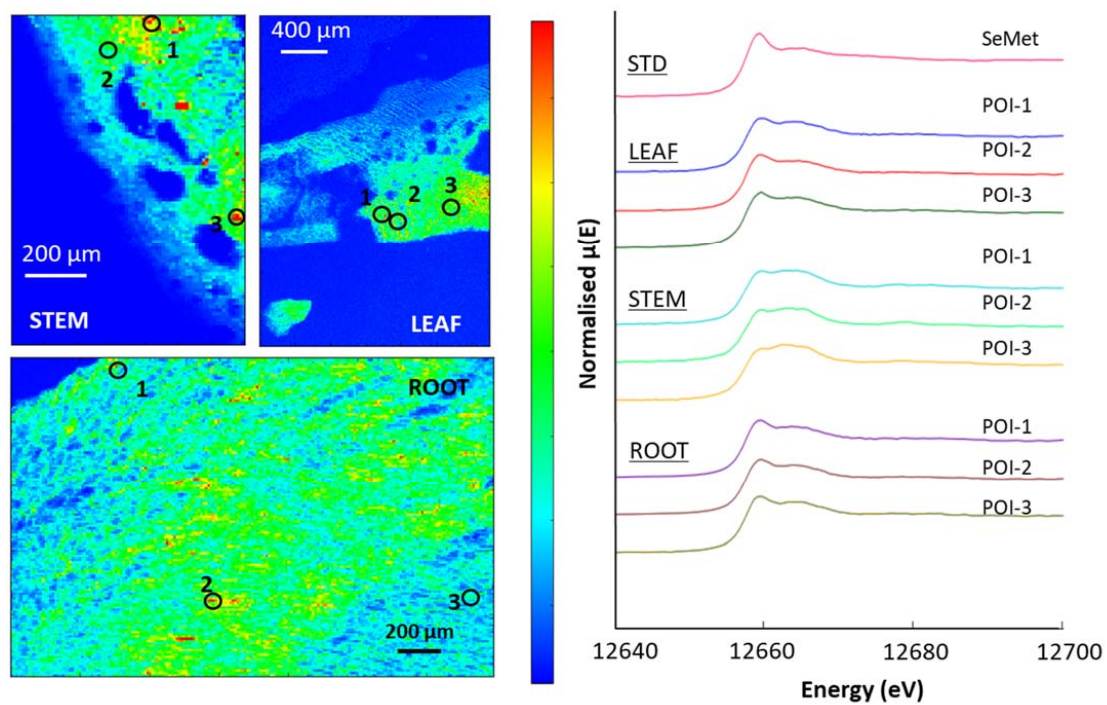


Figure A6. (Left): μ -XRF maps showing selenium in a cross-section of a premier kale leaf vein, stem and root, with selected points of interest (POIs) for Se K-edge XANES analysis, labelled 1–3. The μ -XRF is on a ‘pseudo-temperature’ scale, with high Se counts in red and areas and no counts in dark blue. (Right): normalised Se K-edge XANES spectra showing the selenium species in selected POIs and the SeMet.



Figure A7. Photographs of the selenium non-accumulator plants (pasture species) collected for sampling for chemical and XAS analyses. Samples of tall fescue grass with minor necrosis of the leaves and white clover.



Figure A8. Photographs of the Se accumulator species *Brassica oleracea* (hispi cabbage, red cabbage (left) and premier kale (right)). Note that the hispi cabbage has minor necrosis of the leaves.

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