Understanding Selenium Toxicity in the Natural Environment

A thesis submitted to the University of Manchester for the degree of Doctor of Philosophy in the Faculty of Science and Engineering

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# Table of Contents

List of Figures .................................................. 4  
List of Tables ..................................................... 9  
Thesis Abstract ................................................... 12  
Declaration ......................................................... 13  
Copyright Statement ............................................ 14  
About the Author .................................................. 15  
Acknowledgements ................................................ 16

**Chapter 1 – Project Relevance and Thesis Structure**  
1.1. Project Context and Relevance ............................. 17  
1.2. Thesis Structure ............................................. 19  
1.3. References .................................................... 21

**Chapter 2 – Literature Review**  
2.1. Introduction .................................................. 22  
2.2. Selenium Toxicity .......................................... 22  
2.2. Selenium in Rocks ......................................... 24  
2.3. Selenium in Soils ............................................ 27  
2.4. Selenium in Plants ......................................... 33  
2.5. Sampling Site - Selenium Toxicity in West Limerick 38  
2.6. References .................................................... 40

**Chapter 3 – Research Methods**  
3.1. X-ray Techniques ........................................... 44  
3.2. Chemical Analysis ......................................... 49  
3.3. Organic Analysis ............................................ 52  
3.4. Microbiological Techniques .............................. 55  
3.5. References .................................................... 57

**Chapter 4 – Research Paper 1**  
Selenium Uptake from Soil to Plants: An XAS Study  
Abstract .......................................................... 59  
4.1. Introduction .................................................. 60  
4.2. Methodology ............................................... 62  
4.3. Results ....................................................... 67  
4.4. Discussion ................................................... 92  
4.5. Conclusions .................................................. 98  
4.6. References .................................................... 99
Chapter 5 – Research Paper 2
Microbial Transformations of Selenium Species in Toxic Irish Soils

Abstract 102
5.1. Introduction 103
5.2. Methodology 106
5.3. Results 110
5.4. Discussion 126
5.5. Conclusions 133
5.6. References 134

Chapter 6 – Research Paper 3
Selenium Fractionation and Speciation in Naturally Contaminated Agricultural Soils

Abstract 138
6.1. Introduction 139
6.2. Methodology 141
6.3. Results 147
6.4. Discussion 166
6.5. Conclusions 171
6.6. References 172

Chapter 7 – Conclusions and Further Work
7.1. Conclusions 176
7.2. Further Work 178
7.3. References 179

Chapter 8 – Supplementary Information
8.1. Report on investigations into the seleniferous soils of West Limerick Ireland 190

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## List of Figures

**Chapter 2 – Literature Review**

Figure 2.1. The cycling of selenium in soil showing the changes in species adapted from Dungan and Frankenberger (1999).

Figure 2.2. The positive trend of total organic carbon and negative trend of SiO$_2$ with selenium concentration in a soil core collected in County Meath, Ireland after Fellowes et al. (2013).

Figure 2.3. The Se K-edge XANES energy spectra for the hyperaccumulator plants *A. bisulcatus* and *S. pinnata* after Freeman et al. (2006).

Figure 2.4. Spatial distribution map of selenium which indicates selenium concentrations are in the low to deficient range in Limerick (black square), adapted from Fay et al. (2007).

**Chapter 3 – Research Methods**

Figure 3.1. Simplified diagram showing the three main interactions of X-rays with matter (left) and characteristic radiation showing the emission of an electron (right), after Brouwer (2003).

Figure 3.2. An illustration showing the Bragg equation after Borchert (2014).

Figure 3.3. Simple schematic of the I18 beamline at the Diamond Light Source facility after Mosselmans et al. (2009).

Figure 3.4. Example of an XAS spectra showing the XANES and EXAFS regions for a protein containing [2Fe-2S] after Penner-Hahn (2004).

Figure 3.5. Schematic diagram of an ICP-MS instrument after Agilent Technologies (2006).

Figure 3.6. Optical arrangement in a dual-beam spectrophotometer with optical paths shown in green after Hofmann (2010).

Figure 3.7. Diagram of a pyrolysis gas chromatography mass spectrometer unit, adapted from UCLA (2015).

Figure 3.8. An example protocol for PCR amplification of the 16S rRNA gene with primers targeting the V4 and V5 variable region after Lakshmanan et al. (2017).
Chapter 4 – Research Paper 1 ‘Selenium Uptake from Soil to Plants: An XAS Study’

Figure 4.1. Map showing soil and plant sampling locations in agricultural sites FF, MO, PK, TL and TJ in County Limerick, Ireland.

Figure 4.2. Map showing the spatial distribution of Se, Mo, As and U concentrations (mg kg\(^{-1}\)) in site TL, produced using Surfer\textsuperscript{®} software. Contours based on sample sites chosen to represent the vegetation and slope variations in the field.

Figure 4.3. Map showing the spatial distribution of Se, Mo, As and U concentrations (mg kg\(^{-1}\)) in site FF, produced using Surfer\textsuperscript{®} software. Contours based on sample sites chosen to represent the vegetation and slope variations in the field.

Figure 4.4. Selenium and Total Organic Carbon concentration with depth of the soil profile in core TL-1.

Figure 4.5. Selenium and Total Organic Carbon concentration with depth of the soil profile in core FF-2.

Figure 4.6. Normalised XANES spectra for both inorganic and organic selenium standards (Se K-edge) in the oxidation states -II, 0, IV and VI (see text).

Figure 4.7. \(\mu\)-XRF maps showing selenium in a section through a soil pellet with the selected points of interest (POI) for Se K-edge XANES analysis labelled 1-4 in soil FF-1 (left) and labelled 1-3 in soil MO-1 (top). The \(\mu\)-XRF is on a ‘pseudo-temperature’ scale with high Se counts in red and areas with no counts in dark blue. Bottom: Normalised Se K-edge XANES spectra showing the selenium species in selected POI, the red elemental Se and SeMet Standards.

Figure 4.8. Top: \(\mu\)-XRF maps showing selenium in grass blades from sites TL-1 and FF-2 and the selected points of interest (POI) for Se K-edge XANES analysis labelled 1-3. The \(\mu\)-XRF is on a ‘pseudo-temperature’ scale with high Se counts in red and areas with no counts in dark blue. Bottom: Normalised Se K-edge XANES spectra showing the selenium species in selected POI and the SeMet Standard.

Figure 4.9. \(\mu\)-XRF maps showing selenium in a white clover leaf (right) with selected points of interest (POI) for Se K-edge XANES analysis labelled 1-4 and a cross section through a white clover stem (bottom) with selected POI labelled 1-3. The \(\mu\)-XRF is on a ‘pseudo-temperature’ scale with high Se counts in red and areas with no counts in dark blue. Left: Normalised Se K-edge XANES spectra showing the selenium species in selected POI and the SeMet Standard.
Figure 4.10. Left: µ-XRF maps showing selenium in a cross section of a
premier kale leaf vein, stem and root with selected points of interest (POI)
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 with no counts in
dark blue. Right: Normalised Se K-edge XANES spectra showing the
selenium species in selected POI and the SeMet Standard.

Figure 4.11. Top: µ-XRF maps showing selenium in a hispi cabbage leaf,
cross section through a stem and root with selected points of interest (POI)
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 with no counts in
dark blue. Bottom: Normalised Se K-edge XANES spectra showing selenium
species in the selected POI and the SeMet Standard.

Chapter 5 – Research Paper 2 ‘Microbial Transformations of Selenium
Species in Toxic Irish Soils’

Figure 5.1. Topographical map showing the location of soil samples taken in
the field sites FF-1 and MO-1 which contained elevated Se and Mo
concentrations. B-1 and B-2 were sampled upslope and contained <5 mg
kg\(^{-1}\) Se. Green area indicates field boundaries.

Figure 5.2. Graph showing the positive correlation between selenium (mg
kg\(^{-1}\)) and TOC (wt%) concentration in soil cores MO-1 and FF-1 (Combined
\(R^2 = 0.58\)).

Figure 5.3. µ-XRF maps showing selenium dispersed throughout soil MO-1
and selected points of interest (POI) S1-S3 for Se K-edge XANES analysis.
The µ-XRF is on a ‘pseudo temperature scale with high Se counts in red and
areas with no counts (spaces due to soil drying) in dark blue. The black
rectangle shows the area selected for µ-XRF maps of iron (Fe) and
molybdenum (Mo) where high counts (red) appear to correlate. Selected
POI for Mo K-edge XANES analysis are labelled M1-M3.

Figure 5.4. Normalised Se K-edge XANES spectra for reduced selenium
species and the 3 points of interest (POI) selected from the Se µ-XRF map of
soil MO-1.

Figure 5.5. Normalised Mo K-edge XANES spectra for reduced molybdenum
species and the 3 points of interest (POI) selected from the Mo µ-XRF map
of soil MO-1.

Figure 5.6. The changes in Se, Mo and U concentration in the soil solution
(µg L\(^{-1}\)) with different physiochemical conditions over a 42 day microcosm
experiment. Concentrations are determined by ICP-MS and the data plotted
are average values of triplicate samples with the standard error.
Figure 5.7. The concentration of Fe$^{II}$ (mM) on day 0 and day 42 of the microcosm experiments under anaerobic, anaerobic + 10 mM sodium sulfate, anaerobic + 10 mM sodium nitrate and microaerophilic conditions. Concentrations are averages of triplicate samples and the standard error from soil MO-1 and FF-1.

Figure 5.8. The relative abundance of observed phyla present in anaerobic samples B-1, B-2, anaerobic soil microcosm sample FF-1 (T = 0) and all soil microcosm conditions for sample MO-1 (T = 0 and T = 42) which exceeded 1% abundance in soils.

Figure 5.9. The relative abundance of the most abundant classes of phyla present in anaerobic samples B-1, B-2, anaerobic soil microcosm sample FF-1 (T = 0) and all soil microcosm conditions for sample MO-1 (T = 0 and T = 42) which exceeded 1% abundance in soils.

Chapter 6 – Research Paper 3 ‘Selenium Fractionation and Speciation in Naturally Contaminated Agricultural Soils’

Figure 6.1. Bedrock Geology map showing the location of soil cores FF-1, PK-1, TL-1 and TJ-1 (in red) and rock samples (in black), collected from outcrops of the Clare Shale Formation, the Rathkeale Formation, Shannon group and the Shanagolden Formation.

Figure 6.2. The relationship between selenium concentration (mg kg$^{-1}$) and the TOC (wt%) concentration with the depth of the soil profile in cores PK-1, FF-1, TL-1 and TJ-1.

Figure 6.3. The abundance of selenium (%) in the H$_2$O, phosphate, alkaline, sulfate, NaOCl and acid digestion extracts in soil samples PK-1, FF-1, TL-1 and TJ-1 calculated from the total amount of selenium in the extracts.

Figure 6.4. The abundance of selenite, selenate and unidentified selenium calculated from the total selenium concentration in the H$_2$O, phosphate and alkaline extracts in soil samples PK-1, FF-1, TL-1 and TJ-1.

Figure 6.5. The abundance of TDOC in the H$_2$O, phosphate, alkaline, sulfate and NaOCl extracts in soil samples PK-1, FF-1, TL-1 and TJ-1 calculated from the total amount of TDOC in the extracts.

Figure 6.6. The relative composition (%) of aromatics, nitrogen containing compounds, polysaccharides, phenols, lignin markers and lipids in the original soil material from cores PK-1, FF-1, TL-1 and TJ-1 and the dried alkaline extracts from step 3 of the sequential extraction protocol for each soil.
Figure 6.7. Chromatograms showing the peaks in pyrolysis moieties for soil samples PK-1 and FF-1 and the alkaline extract from both soil with the 8 largest peaks labelled. 1 - Toluene, 2 – Styrene, 3 – Phenol, 4 - 4-methoxyphenol (guaiacol), 5 - ¾ methylphenol, 6 - 4-vinylguaiacol, 7 - Prist-1-ene, 8 - Androstane Standard.

Figure 6.8. Chromatograms showing the peaks in pyrolysis moieties for soil samples PK-1 and FF-1 and the alkaline extract from both soil with the 8 largest peaks labelled. 1- Toluene, 2 – Styrene, 3 – Phenol, 4 - 4-methoxyphenol (guaiacol), 5 - ¾ methylphenol, 6 - 4-vinylguaiacol, 7 - Prist-1-ene, 8 - Androstane Standard.

Chapter 8 - Supplementary Information

Figure 8.1. µ-XRF maps showing iron in a section through a soil pellet with the selected points of interest (POI) for Fe K-edge XANES analysis labelled 1-4 in soil FF-1 (left) and labelled 1-3 in soil MO-1 (top). The µ-XRF is on a ‘pseudo-temperature’ scale with high Fe counts in red and areas with no counts in dark blue.

Figure 8.2. Normalised Se K-edge XANES spectra of the tall fescue root from site TL-1 showing selenium species in selected POI’s compared to the SeMet Standard.

Figure 8.3. Photographs of the Se non-accumulator plants (pasture species), tall fescue grass with necrosis of the leaves and white clover.

Figure 8.4. Photographs of the Se accumulator species *Brassica oleracea* (cabbage and kale). Note the hispi cabbage have necrosis of the leaves.

Figure 8.5. Photograph of an outcrop of the Clare Shale Formation at Inishcorker Island in the Shannon Estuary where selenium was up to 18 mg kg⁻¹.

Figure 8.6. HPLC-ICP-MS spectra showing the SeMet standard with an unknown peak caused by oxidation or reduction.

Figure 8.7. HPLC-ICP-MS spectra showing the SeCys, Se⁶, SeMet and Se⁶ standards. The signal for SeMet is poor due to the change in species during the sample run.
List of Tables

Chapter 2 – Literature Review

Table 2.1. Typical selenium concentrations found in different rock types. Sources: (Fordyce, 2013; Jacobs, 1989; Malisa, 2001; Plant et al., 2003).

Chapter 4 – Research Paper 1 ‘Selenium Uptake from Soil to Plants: An XAS Study’

Table 4.1. The minimum, maximum and mean chemical data for bulk soil samples collected at 10-20 cm depth from 5 agricultural sites determined by X-ray Fluorescence (values do not include soil core data).

Table 4.2 XRF data showing the concentration of selected trace and major elements for two soil cores TL-1 and FF-2 every 10 cm interval. \( R^2 \) = the correlation of selenium with other elements within the soil profile where a value closer to 1 is the better fit to the regression line. TOC = total organic carbon and is calculated by the Loss on Ignition method.

Table 4.3. ICP-MS data showing the mean concentration of Se, Mo, As, U and V for triplicate selenium non-accumulator samples ± the standard error for site TL and TJ. TF = the translocation factor, a value >1 indicates the plant can translocate the element effectively from the root to the shoots or leaf and stem of the plant.

Table 4.4 ICP-MS data showing the mean concentration of Se, Mo, As, U and V for triplicate selenium non-accumulator samples ± the standard error for site FF, PK and MO. TF = the translocation factor, a value >1 indicates the plant can translocate the element effectively from the root to the shoots of the plant.

Table 4.5. ICP-MS data showing the mean concentration of Se, Mo, As, U and V for triplicate Brassica samples ± the standard error from a growing experiment at site FF-2. TF = the translocation factor, a value >1 indicates the plant can translocate the element effectively from the root to the leaf and stem parts of the plant.

Table 4.6. Linear combination fits (%) of the Se K-edge XANES spectra for selected points of interest (POI) in the \( \mu \)-XRF maps of the seleniferous horizon (30-40 cm) in soil TL-1 and FF-1 measured using ATHENA software. The goodness of fit is determined by the R-Factor and Reduced Chi squared measured using ATHENA software and the lower the value the better the fit.

Table 4.7. Linear combination fits (%) of Se K-edge XANES spectra for selected points of interest (POI) in the \( \mu \)-XRF maps of the Se non-accumulator plants measured using ATHENA software. The goodness of fit is determined by the R-
Factor and Reduced Chi squared and the lower the value the better the fit.

Table 4.8. Linear combination fits (%) of the Se K-edge XANES spectra for selected points of interest (POI) in the µ-XRF maps of Brassica oleracea (Se accumulator plants) from site FF measured using ATHENA. The goodness of fit is determined by the R-Factor and Reduced Chi squared and the lower the value the better the fit.

Table 4.9. Bioaccumulation factor (BAF) calculated for site TL-1 and FF-2. BCF >1 indicates the grass is efficient in accumulating the trace element from the soil from 0-10 cm depth.

Chapter 5 – Research Paper 2 ‘Microbial Transformations of Selenium Species in Toxic Irish Soils’

Table 5.1. X-ray fluorescence data showing the concentration of major and trace elements in soil core MO-1 and FF-1. $R^2 =$ the correlation of selenium with other elements in the soil profile where a value closer to 1 is the better fit to the regression line. TOC was calculated by Loss on Ignition method.

Table 5.2. Linear combination fits (%) of the Se K-edge XANES spectra for selected points of interest (POI) in the µ-XRF map of soil sample MO-1 measured in ATHENA. The goodness of fit is determined by the R-Factor and Reduced Chi-squared and the lower the value the better the fit.

Table 5.3. Linear combination fits (%) of the Mo K-edge XANES spectra for selected points of interest (POI) in the µ-XRF map of soil sample MO-1 measured in ATHENA. The goodness of fit is determined by the R-Factor and Reduced Chi-squared and the lower the value the better the fit.

Chapter 6 – Research Paper 3 ‘Selenium Fractionation and Speciation in Naturally Contaminated Agricultural Soils’

Table 6.1. X-ray fluorescence data showing the concentration of major and trace elements in soil core PK-1 and FF-1. $R^2 =$ the correlation of selenium with other elements within the soil profile where a value closer to 1 is the better fit to the regression line. TOC was calculated by the loss on ignition method.

Table 6.2. X-ray fluorescence data showing the concentration of major and trace elements in soil core TL-1 and TJ-1. $R^2 =$ the correlation of selenium with other elements within the soil profile where a value closer to 1 is the better fit to the regression line. TOC was calculated by the loss on ignition method.

Table 6.3. X-ray fluorescence data showing the concentration of major and trace elements of rock samples collected from outcrops of the Clare Shale Formation from locations I, F, O, D and M. TOC was calculated by the loss on ignition method.
Table 6.4. X-ray fluorescence data showing the concentration of major and trace elements in rock sample collected from the Rathkeale Formation (R and P) The Shannon Group (B) and the Shanagolden formation (Q). TOC is calculated by the loss on ignition method.

Table 6.5. The average concentration of selenium measured in triplicate ± the standard error in the sequential extracts for soil samples PK-1, FF-1, TL-1 and TJ-1 determined by ICP-MS.

Table 6.6. The average concentration of selenite and selenate measured in duplicate ± the standard error in the sequential extracts for soil samples PK-1, FF-1, TL-1 and TJ-1 determined by ICP-MS.

Table 6.7. The average concentration of TDIC measured in triplicate ± the standard error in the sequential extracts for soil samples PK-1, FF-1, TL-1 and TJ-1.

Chapter 8 - Supplementary Information

Table 8.1. X-ray fluorescence data showing the concentration of major and trace elements in soil samples collected at 10-20 cm depth at sites MO and PK. LOI = loss on ignition.

Table 8.2. X-ray fluorescence data showing the concentration of major and trace elements in soil samples collected at 10-20 cm depth at site FF. LOI = loss on ignition.

Table 8.3. X-ray fluorescence data showing the concentration of major and trace elements in soil samples collected at 10-20 cm depth at site TL and TJ. LOI = loss on ignition.

Table 8.4 X-ray fluorescence data showing the concentration of major and trace elements in soil core TL-2. TOC was calculated by the Loss on Ignition method.

Table 8.5. X-ray fluorescence data showing the concentration of major and trace elements in soil core TJ-2. TOC was calculated by the Loss on Ignition method.

Table 8.6. The pyrolysis moieties identified in the seleniferous soil horizon from core PK-1, FF-1, TL-1 and TJ-1 with approximate retention time and mass. Label A = aromatics, N = nitrogen containing compound, Ps = polysaccharide, Ph = Phenol, L = Lignin marker, Lp = Lipids.

Table 8.7. The pyrolysis moieties identified in the seleniferous soil horizon from core PK-1, FF-1, TL-1 and TJ-1 with approximate retention time and mass. Lp = Lipids (alkenes and alkanes).
Thesis Abstract

The biogeochemical cycling of the essential micronutrient selenium (Se) is explored in a naturally seleniferous area of West Limerick, Ireland where selenium toxicity in livestock is a historical problem. Plant, soil and rock samples were collected to determine the distribution of selenium and other deleterious elements. Seleniferous soil horizons were selected for speciation, microbial, organic and sequential extraction analysis. The selenium distribution in soils was inhomogeneous with highly seleniferous concentrations of up to 1266 mg kg\(^{-1}\) identified in close proximity to deficient values. Overlying pastures contained concentrations toxic to livestock and translocation was highest in *Trifolium repens* (white clover) with 296 mg kg\(^{-1}\) Se in the leaves. The *Brassica oleracea* species (cabbage and kale) accumulated up to 972 mg kg\(^{-1}\) Se in the leaves showing phytoextraction potential. Selenium was present in the soil predominantly in a reduced phase as elemental Se (Se\(^{0}\)) and the organic species SeMet (Se\(^{II}\)) which is bioavailable. SeMet was also the main species in the pastures and Se accumulator plants. Microcosm experiments showed anaerobic conditions decreased selenium solubility and bioavailability but the opposite occurred with molybdenum due to the reductive dissolution of Fe\(^{III}\)(hydroxy)oxides. The microbial community remained relatively stable to changing physiochemical conditions which was dominated by *Proteobacteria*, *Actinobacteria* and *Acidobacteria*. Selenium positively correlated with the total organic carbon except for the soil profile overlying the Clare Shale Formation. Selenium was fractionated mainly as elemental selenium, in fulvic and humic acids and also recalcitrant organic matter. Soils were abundant in lipid moieties, in particular prist-1-ene which originates from chlorophyll and suggests selenium is associated with the burial of plant material. The Clare Shale samples contained up to 18 mg kg\(^{-1}\) Se and weathering and transport has caused enrichment in the low lying soils and pastures. The findings in this thesis show how variations in plant species, redox conditions, soil organic carbon content, and geology influence the distribution of selenium species in soils and thus the bioavailability and toxicity to livestock and human health.
Declaration

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About the Author

The Author of the thesis graduated from The University of Manchester in 2014 with an undergraduate degree in Environmental Sciences (BSc Hons). The final dissertation project was titled ‘Selenium Concentrations in Toxic Irish Soils’ which was awarded best dissertation project for the Environmental Science Programme. The Author then joined both the Organic Geochemistry group and Geomicrobiology Group in the School of Earth and Environmental Sciences to conduct the research reported in this thesis.
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Chapter 1 – Project Relevance and Thesis Structure

1.1. Project Context and Relevance

In recent years there has been a growing interest in selenium (Se) as it is a potentially toxic trace element in the natural environment, however it is also essential for human and animal health (Fordyce, 2013). In most global countries the major dietary source of selenium is plant foods and the nutritional value depends on the soil concentration and bioavailability (Winkel et al., 2012). However, selenium distribution in soils is inhomogeneous and is influenced mainly by the weathering of Se-rich rocks and the transport by surface or groundwater (Dhillon and Dhillon, 2003; Fernández-Martínez and Charlet, 2009). Therefore, understanding the biogeochemical cycling of selenium between plants, soils and rocks is critical to avoid health problems associated with selenium toxicity and deficiency.

Although selenium deficient environments are far more widespread (Fordyce, 2013), there is more environmental interest in selenium toxic environments as they often require remediation and phytoextraction methods offer the potential to supplement areas of the globe deficient in selenium (Wu et al., 2015). Selenium toxicity in livestock is a historical problem in areas of West Limerick, Ireland as a result of grazing pastures overlying highly seleniferous soils (Fleming and Walsh, 1956; Rogers et al., 1990). Therefore, this thesis project aims to understand the terrestrial cycling of selenium from the source rock, soil, to the uptake in plants using this unique field site to examine the biotic and abiotic processes involved. The project is funded by the Natural Environment Research Council (NERC), and is a collaboration with the University of Manchester and The Irish Agriculture and Food Development Authority (TEAGASC) in Newcastle West, Ireland.
1.1. Aims

The aims of the research project were to:

- Examine the distribution and bioavailability of selenium in agricultural soils from West Limerick and the uptake in the overlying vegetation to understand the terrestrial cycling of selenium and the risk to livestock health.
- Characterise the behaviour of selenium and molybdenum in horizons of soil where the levels of these elements were very high, focusing on the relationship between soil geochemical conditions, Se speciation and the microbial community.
- Understand the fractionation of selenium species in these agricultural soils, focusing on the influence of organic matter and the underlying geology on the distribution.

In order to achieve this aim the objectives were to:

(i) Examine the bulk inorganic chemistry, organic chemistry and mineralogy of soil samples and cores collected from 5 different agricultural sites to determine selenium concentration and distribution.

(ii) Analyse the bulk chemistry of Se non-accumulator plants (grass species and white clover) overlying seleniferous soils and Se accumulator species (Brassicas) following growing experiments to determine selenium uptake.

(iii) Use X-Ray Absorption Spectroscopy (µ-XAS) techniques to identify the Se and Mo species present in the most seleniferous soil horizons and analyse the distribution of Se species in non-accumulator and accumulator plants.

(iv) Conduct microcosm experiments to observe Se and Mo mobility in soils with changing bio-physicochemical conditions.

(v) Use DNA sequencing techniques to analyse the microbial community present in seleniferous soils and the microbial response to changing geochemical conditions.
Sequential extractions of soil horizons high in selenium to determine the fractionation of selenium according to the redox activity, solubility and exchangeability.

Analyse the bulk chemistry of rock samples collected from 4 different geological formations in the Limerick area to determine the source of selenium in soils.

1.2. Thesis Structure

This thesis has been written using the alternative format guidelines and consists of 7 chapters:

Chapter 1 - This chapter consists of an overview of the thesis project and structure including the aims, objectives and author contributions to individual papers.

Chapter 2 - Provides a detailed literature review of the current scientific knowledge in the research area.

Chapter 3 - Includes the analytical techniques used to obtain data for the project.

Chapter 4 - Research paper 1 titled ‘Selenium Uptake from Soil to Plants: An XAS Study’.

Author Contributions: S. L. McLoughlin – Principle author involved in all aspects of the research project, concept development, field sampling, experimental work and writing. R. A. D. Pattrick – Primary supervisor involved in the initial concept, concept development, field sampling, XAS data collection, and paper review. B. E. van Dongen – Supervisor involved in concept development, field sampling, XAS data collection and supervision. J. F. W. Mosselmans – Assisted in XAS data collection and analysis. J. Kelleher – Assisted in selecting field sampling locations.

Possible journal publication: Environmental Science and Technology or Nature Geoscience
Chapter 5 – Research paper 2 titled ‘Microbial Transformations of Selenium Species in Toxic Irish Soils’.

Author Contributions: S. L. McLoughlin – Principle author involved in all aspects of the research project, concept development, field sampling, experimental work and writing. R. A. D. Patrick – Primary supervisor involved in the initial concept, concept development, field sampling, XAS data collection and paper review. J. R. Lloyd – Supervisor involved in concept development and paper review. B. E. van Dongen – Supervisor involved in concept development and field sampling. C. Boothman – Assisted in microbial community analysis. J. F. W. Mosselmans – Assisted in XAS data collection and analysis.

Possible journal publication: Soil Biology and Biochemistry or Geobiology

Chapter 6 – Research paper 3 titled ‘Selenium Fractionation and Speciation in Naturally Contaminated Irish Soils’.

Author Contributions: S. L. McLoughlin – Principle author involved in all aspects of the project, concept development, field sampling, experimental work and writing. R. A. D. Patrick – Primary Supervisor involved in the initial concept, concept development, field sampling, and paper review. B. E. van Dongen – Supervisor involved in concept development, field sampling, organic analysis and paper review. J. Kelleher – Assisted in selecting field sampling locations.

Possible journal publication: European Journal of Soil Science

Chapter 7 – Includes a summary and conclusion of the entire PhD project drawn from the aims and objectives of the three research papers. This chapter includes a section on potential future research directions to increase the knowledge of selenium toxicity in the natural environment.

Chapter 8 – Provides supplementary information for the research papers in chapters 4, 5 and 6.
1.3. References

Chapter 2 - Literature Review

This chapter provides a detailed review of the current scientific literature relating to selenium in the natural environment.

2.1. Introduction

Selenium (Se) is a trace element present in the natural environment which is vital for animal and human health (Fordyce, 2007; Fordyce, 2013). It is a group VI-A chalcogen element with an atomic number of 34 and an atomic mass of 78.96 (Fernández-Martínez and Charlet, 2009). Therefore, selenium shares similar chemical properties to the element sulphur (S), although selenium atoms are much larger (Sors et al., 2005; White et al., 2007). Selenium is classified as a non-metal, however this is disputed as chemical and physical properties are in-between that of a metal and non-metal (Fordyce, 2013). In the environment, inorganic selenium exists in four main oxidation states (IV, VI, 0, -II). The oxyanions selenite (SeIV) and selenate (SeVI) are commonly found in soil, water and plants whereas elemental selenium (Se0) and selenide (Se-II) are commonly found in reducing soil conditions and rocks (Jacobs, 1989; Neal, 1995). Transformations between these selenium species is controlled largely by the activity of microorganisms in oxidation and reduction reactions (Chasteen and Bentley, 2002). However, selenium also exists in the environment as organic selenium species such as selenomethionine (SeMet) and selenocysteine (SeCys) (Jacobs, 1989; Neal, 1995), although many organic selenium compounds still remain unidentified (Fordyce, 2007). Understanding the mobility and bioavailability of these selenium species is crucial to assess the fate of selenium in the natural environment and to predict health exposure.

2.2. Selenium Toxicity

Selenium is an important micronutrient for humans and animals as it incorporates into proteins as the amino-acid selenocysteine, the analogue of cysteine which is critical for enzyme and metabolic function in the body. Selenium acts through essential selenoproteins as an antioxidant, contributes to immune system function and also the production of thyroid hormones (Rayman, 2000; Winkel et al., 2012).
However, in humans a daily intake of <40 µg d⁻¹ selenium in food is considered deficient and the intake of >400 µg d⁻¹ is considered toxic, both of which can lead to a host of health problems (Fordyce, 2007; WHO, 1996). In livestock, to avoid selenium deficiency the minimum concentration required in forage is 0.1-0.05 mg kg⁻¹ and to avoid toxicity the intake should not exceed 2-5 mg kg⁻¹ (Terry et al., 2000; Wu, 2004; Wu et al., 1996). The recommended limits for both human and livestock intake demonstrate the very small range between toxicity and deficiency (Fordyce, 2013). Therefore, to ensure the concentration of selenium in agricultural land is safe there is a trigger action value (TAV) of 3-10 mg kg⁻¹ (Kabata-Pendias, 2011).

Selenosis which is the term used for poisoning caused by high selenium intake in humans and animals has been documented in many countries including Australia, Brazil, China, Ireland, Israel, Russia, South Africa and the United States (Fordyce, 2007). In humans, symptoms of selenosis include skin lesions, hair loss, nail deformities, loss of cognitive function and paralysis. Selenosis was a huge endemic in the Enshi district of China in the 1960’s caused by consumption of vegetables and cereals growing on soils containing up to 2000 mg kg⁻¹ selenium which was derived from weathered coal (Fordyce, 2013; Yang et al., 1983). In livestock, chronic selenium toxicity leads to alkali disease which is characterised by hair loss and deformation of hooves and bones. Acute selenium toxicity is less common and causes blind staggers which is characterised by loss of sight and eventually leads to paralysis, respiratory failure and death of the animal (Rogers et al., 1990). Alkali disease has occurred in areas of grassland in County Meath and Limerick in Ireland where livestock are restricted to grazing small fields containing seleniferous soils (Crinion, 1980; Fleming and Walsh, 1956). It is therefore important to understand the biogeochemical cycling of selenium to understand the risk to human and livestock health and to prevent selenium toxicity related diseases.
2.2. Selenium in Rocks

2.2.1. Selenium Distribution in Rocks

The underlying geology of an area is thought to be the main control on selenium distribution in soils (Jacobs, 1989) as selenium concentration varies widely in different geological formations (Dhillon and Dhillon, 2003; Wen and Carignan, 2011). However, the average crustal abundance of selenium is only 0.05 mg kg\(^{-1}\) (table 2.1) and rarely exceeds 100 mg kg\(^{-1}\) (Dhillon and Dhillon, 2003). Generally, sedimentary rocks contain greater concentrations of selenium than igneous rocks (Neal, 1995) and metamorphic rocks (Malisa, 2001). The concentration of selenium in geological formations can vary in different beds and often in different areas of the same bed (Dhillon and Dhillon, 2003). Therefore, it is difficult to quantify average selenium values in geological formations and identify the source of selenium in rocks.

In igneous rocks, selenium concentrations are low and granites rarely surpass 0.05 mg kg\(^{-1}\) (table 2.1). The highest concentrations occur in the igneous rocks of volcanic origin such as basalts. The concentration in metamorphic rocks is low as selenium declines with the level of metamorphism (Malisa, 2001). In sedimentary rocks, the high abundance of selenium is thought to originate partially from gases expelled during volcanic activity. Another theory is mid-oceanic ridges where hydrothermal reactions between lava and sea water occur (Alloway, 2013). Selenium is usually enriched in the uppermost beds of sedimentary rocks because of selenium abundance in weathered material at the surface (Malisa, 2001). Table 2.1 shows that out of the sedimentary rocks, the highest selenium concentrations occur in Chinese stone-coal (6500 mg kg\(^{-1}\)), mudstone (1500 mg kg\(^{-1}\)) and USA shale (675 mg kg\(^{-1}\)). Whereas the concentration in limestone and sandstone rarely exceeds 0.1 mg kg\(^{-1}\) (Neal, 1995). However, according to Shamberger (1983) the highest selenium concentrations in the United States are mainly found in uranium ores of sandstones.
Table 2.1. Typical selenium concentrations found in different rock types.

<table>
<thead>
<tr>
<th>Material</th>
<th>Se mg kg$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Earth’s Crust</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Igneous rocks</strong></td>
<td></td>
</tr>
<tr>
<td>Ultramafic rocks</td>
<td>0.05</td>
</tr>
<tr>
<td>Mafic rocks</td>
<td>0.05</td>
</tr>
<tr>
<td>Granite</td>
<td>0.01–0.05</td>
</tr>
<tr>
<td>Volcanic rocks</td>
<td>0.35</td>
</tr>
<tr>
<td>Volcanic rocks, USA</td>
<td>0.10</td>
</tr>
<tr>
<td>Volcanic rocks, Hawaii</td>
<td>2.00</td>
</tr>
<tr>
<td>Volcanic tuffs</td>
<td>9.15</td>
</tr>
<tr>
<td><strong>Sedimentary rocks</strong></td>
<td></td>
</tr>
<tr>
<td>Marine carbonates</td>
<td>0.17</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.03–0.08</td>
</tr>
<tr>
<td>Sandstone</td>
<td>0.05</td>
</tr>
<tr>
<td>W. USA shale</td>
<td>1–675</td>
</tr>
<tr>
<td>Wyoming shale</td>
<td>2.3–52</td>
</tr>
<tr>
<td>S. Korean shale</td>
<td>0.1–41</td>
</tr>
<tr>
<td>Carbon-shale China</td>
<td>206–280</td>
</tr>
<tr>
<td>Mudstone</td>
<td>0.1–1500</td>
</tr>
<tr>
<td>Phosphate</td>
<td>1–300</td>
</tr>
<tr>
<td>USA Coal</td>
<td>0.46–10.7</td>
</tr>
<tr>
<td>Australian coal</td>
<td>0.21–2.5</td>
</tr>
<tr>
<td>Chinese stone-coal</td>
<td>6500</td>
</tr>
<tr>
<td>Oil</td>
<td>0.01–1.4</td>
</tr>
</tbody>
</table>

Sources: (Fordyce, 2013; Jacobs, 1989; Malisa, 2001; Plant et al., 2003).

A study by Tamari et al. (1990) compared selenium concentrations in 46 sedimentary rocks and 115 igneous rocks from a geological survey in Japan. The average concentration in igneous rocks was 8.6 µg kg$^{-1}$ compared to 88.1 µg kg$^{-1}$ in sedimentary rocks. Of the sedimentary rocks, selenium was highest in shales ranging from 41.7 - 811 µg kg$^{-1}$ and in mudstones ranging from 172 – 1280 µg kg$^{-1}$ with values lower than the typical values in table 2.1. The high concentrations of selenium observed in these sedimentary rocks is perhaps due to the high abundance of organic matter and detritus which is strongly associated with selenium in the environment (Malisa, 2001). Selenium is particularly enriched in organic rich, black shales as they are formed in deep water oceans where reducing conditions cause toxic trace elements to accumulate (Alloway, 2013). However, in
the study by Tamari et al. (1990), more igneous rocks were sampled compared to sedimentary and therefore the average values could shift with an equal sample size.

2.2.2. Selenium in Sulfide Minerals

Selenium in shale is also strongly associated with sulfide minerals such as pyrite (FeS$_2$) as well as with organic matter (Alloway, 2013). High selenium concentrations occur in rocks containing sulfide mineral deposits because sulfur and selenium have similar chemical properties and crystallography allowing the isomorphic substitution of sulfur with selenium. Selenium is also found in the sulfide minerals chalcopyrite (CuFeS$_2$), pyrrhotite (FeS), and sphalerite (ZnS) (Fordyce, 2013; Neal, 1995). According to Shamberger (1983), the average crustal abundance of selenium is difficult to determine due to the separation of sulphur and selenium in magma during rock formation.

A study by Bujdoš et al. (2005) found a correlation of selenium with sulfur in rocks from the Sˇ obov surface mine in Slovakia. Selenium concentrations reached up to 18.1 mg kg$^{-1}$ in the rocks sampled and X-ray diffraction (XRD) analysis revealed these selenium-rich source rocks consisted predominantly of pyrophillite, pyrite and illite. The data suggests the source of selenium in the environment is the weathering and leaching of these sulfide minerals with rainwater which immobilised selenium. This is also supported by a study by Yang et al. (1983) which observed selenium in the lattice of pyrite minerals in coal shales underlying the seleniferous soils in the Enshi district of China. A study by Matamoros-Veloza et al. (2014) also identified a correlation with selenium and pyrite in shale using microfocus spectroscopy and microprobe analysis, however sequential analysis showed selenium was also associated in small amounts with organic matter.

2.2.3. Selenium Speciation in Rocks

The speciation of selenium determines the mobility and bioavailability in the environment (Jacobs, 1989; Neal, 1995), however very little literature exists on the speciation of selenium in source rocks. A study by Wen et al. (2006) analysed the speciation of selenium in the solid organic matter (kerogen) extracts from the
Laerma Se-Au deposit in carbonaceous cherts and slates, and the Yutangba Se deposit between carbonaceous chert and shale in China. Transmission electron microscopy (TEM) revealed 75% of selenium in the Laerma deposit was organically bound selenium and therefore more resistant to weathering and leaching into soils (Fernández-Martínez and Charlét, 2009; Li et al., 2008). However, selenium bearing minerals such as pyrite, arsenopyrite and tiemannite were also present. In the Yutangba deposit 66% of selenium existed as nanograins of pure elemental selenium (Se⁰) embedded in the kerogen. Elemental selenium is easily weathered and oxidised to bioavailable selenate and selenite oxyanions (Girling, 1984), which could explain the cause of selenium poisoning in the area (Wen et al., 2006).

In a study by Mamoros-Veloza et al. (2014), the distribution and speciation of selenium was analysed in five typical shale samples from West Yorkshire using microfocus spectroscopy. X-ray absorption near edge structure (XANES) analysis of the framboidal and euhedral pyrite containing selenium showed the predominant species resembled the adsorption edge energy of selenium sulfide (Se⁻II) which is immobile, seleno-L-cysteine (Se⁻II) which is an organic selenium compound and elemental selenium (Se⁰). Extended X-ray absorption fine structure (EXAFS) data showed that both pyrite grains have different selenium bonding sites and in the euhedral grains of pyrite selenium was substituted for sulfur which supports Fordyce (2013) and Neal (1995).

2.3. Selenium in Soils

2.3.1. Selenium Speciation in Soils

Understanding the speciation of selenium in soils and thus the mobility and bioavailability is important in determining the fate of selenium in the natural environment (Tolu et al., 2013). The speciation of selenium in soil is largely influenced by the pH, redox potential and ionic strength. However, the abundance of organic matter, mineralogy and the activity of soil microorganisms also play an important role (Fordyce, 2013). In aerobic soils which are neutral to slightly alkaline, selenium exists mainly as the soluble oxyanions selenite (Se⁴⁺) and selenate (Se⁶⁺) (figure 2.1). Selenate is more soluble than selenite and is readily leached from the
surface layer of soils and easily taken up by plants (Girling, 1984). In the presence of organic matter and iron oxides, selenite is more readily adsorbed onto soil particles than selenate (Jacobs, 1989; Neal, 1995). In organic-rich soils with acidic, reducing conditions selenium exists mainly as insoluble selenide (Se$^{-II}$) and elemental selenium (Se$^0$) (Fernández-Martínez and Charlet, 2009; Fordyce, 2007; Neal, 1995).

Figure 2.1. The cycling of selenium in soil showing the changes in species adapted from Dungan and Frankenberger (1999).

Selenium also exists in soils as organic selenium compounds which can be immobilised. However, the amino acids selenomethionine, selenocystine and selenocysteine are bioavailable organic species which can be incorporated into proteins. Methylation of selenium shown in figure 2.1 can produce more volatile methyl species such as dimethylselenide (DMSe, [CH$_3$]2Se), dimethyl diselenide (DMDSe, [CH$_3$]2Se$_2$), dimethyl selenone ([CH$_3$]2SeO$_2$), methane selenol (CH$_3$SeH), and dimethyl selenenyl sulfide (DMSeS, [CH$_3$]2SeS) which are released from the soil (Dungan and Frankenberger, 1999). However, a large proportion of selenium species in soils still remain unidentified (Fordyce, 2007).

2.3.2. Soil Microbial Activity

The transformations of selenium in soils from both inorganic and organic species involve the activity of microorganisms in oxidation-reduction reactions (Chasteen
and Bentley, 2002). According to the literature reviewed, approximately 16 species of microorganisms have been identified which grow anaerobically in organic substrates which transform bioavailable selenate (Se\textsuperscript{VI}) and selenite (Se\textsuperscript{IV}) oxyanions to immobile elemental selenium (Se\textsuperscript{0}) (Oremland et al., 2004; Stolz and Oremland, 1999). The most studied microorganism involved in selenium respiration is \textit{Thauera selenatis}. A study by Debieux et al. (2011) analysed selenium transformations by \textit{T. selenatis} growing anaerobically in cultures using selenate as the sole electron acceptor and an acetate medium. TEM analysis revealed selenium nanospheres approximately 150 nm in diameter in the growth medium which was secreted by \textit{T. selenatis} within the cytoplasm of the cells. Once the culture was centrifuged to remove the \textit{T. selenatis} cells the solution turned red in colour. This occurred as the reduction of selenate by microorganisms forms red amorphous elemental selenium (Se\textsuperscript{0}) (Chasteen and Bentley, 2002; Dungan and Frankenberger, 1999).

Microbial populations are therefore useful detoxification mechanisms in highly toxic soils as they can reduce selenium into less bioavailable species (Dungan and Frankenberger, 1999). A study by Pearce et al. (2009) looked at the microbial reduction of bioavailable selenite (Se\textsuperscript{IV}) in anaerobic environments as a potential remediation strategy. Analysis of cultures containing the microorganisms \textit{Geobacter sulfurreducens} and \textit{Shewanella oneidensis} by Inductively coupled plasma atomic emission spectroscopy (ICP-AES) showed a reduction in selenite (Se\textsuperscript{IV}) which corresponded to an increase in elemental selenium (Se\textsuperscript{0}) followed by an increase in selenide (Se\textsuperscript{II}) after 500 hours. TEM images show Se\textsuperscript{0} nanospheres similar to Debieux et al. (2011) on the surface of the rod shaped microorganisms after 24 hours.

Microorganisms can tolerate a wide range of selenium concentrations from trace amounts to highly toxic (5 µg L\textsuperscript{-1} - 2000 µg L\textsuperscript{-1}) (Chasteen and Bentley, 2002). A study by Fellowes et al. (2013) analysed the microbial activity in the selenium toxic soils of County Meath, Ireland where 156.2 µg g\textsuperscript{-1} selenium was present predominantly as a reduced organic phase. Microcosm experiments incubated at 20°C over 4 weeks showed selenium was resistant to different aerobic and anaerobic conditions and remained in a reduced organic phase despite high microbial activity. The XANES
energy spectra of the anoxic microcosms amended with selenate (Se\textsuperscript{VI}) showed rapid reduction to immobile elemental selenium (Se\textsuperscript{0}) represented by a red colour change similar to Debieux et al. (2011). The autoclaved control microcosm showed no selenate reduction indicating selenate is reduced by the activity of resistant microbial species (Fellowes et al., 2013).

2.3.3. Selenium Associated with Organic Matter

Soil fractions high in organic matter can prevent selenite and selenate oxyanions leaching from the surface by forming a strong organo-selenium complex (Fernández-Martínez and Charlet, 2009; Li et al., 2008). In a study by Coppin et al. (2009) on a silty clay loam soil, the organic matter fractions of the soil contained the greatest concentrations of selenium. Selenium sorption experiments showed that 92% of selenite was sorbed in the organic fraction compared to only 44% in the mineral fraction. However, it is possible that the sorption onto organic matter is indirect, due to the interaction of selenium with clays and iron oxides on the surface of organic matter (Jacobs, 1989).

A positive trend between selenium and organic matter was also observed by Fellowes et al. (2013) in the selenium toxic soils of County Meath, Ireland originally identified by Fleming (1962). The selenium concentration peaked at 156.2 µg g\textsuperscript{-1} in an organic-rich horizon with a TOC of 33.8% at 30-40 cm depth (figure 2.2) which suggests the source of selenium is drainage and not the underlying geology as suggested by Jacobs (1989). XANES analysis showed selenium was present as an organic selenium phase which resembled the adsorption edge energy of selenomethionine (SeMet) which can be incorporated into plant proteins (Brown and Shrift, 1982; Sors et al., 2005). However, dissimilarities in the post-edge structure were observed and therefore SeMet could not be concluded. The presence of lipid biomarkers of medium molecular weight suggested selenium accumulated by the burial of organic selenium species within Sphagnum plant material (Fellowes et al., 2013).
Figure 2.2. The positive trend of total organic carbon and negative trend of SiO$_2$ with selenium concentration in a soil core collected in County Meath, Ireland after Fellowes et al. (2013).

According to Gustafsson and Johnsson (1992), large accumulations of selenium in the organic fractions of soil can immobilise selenium and decrease the availability to plants. In a study of Swedish podzol soils, selenium was enriched in organic matter particularly in the horizon where there is a large anion adsorption capacity. The distribution of organic sulfur was different to selenium indicating different biochemical cycling in soil despite both elements sharing similar chemical properties (Sors et al., 2005; White et al., 2007). Addition of selenite to the soil in solution showed rapid fixation onto organic matter as no selenium oxyanions were present in the leachate. It is likely selenium is present in the podzols as organic selenium compounds although none were identified as part of the study (Gustafsson and Johnsson, 1992).

It is evident from the literature more research is required to understand the mechanisms for incorporating selenium onto organic matter and to identify the organic selenium compounds formed. It is estimated that 50% of selenium exists in
soils as organic selenium compounds, however very few have been identified in the literature (Fordyce, 2007). Unidentified organoselenium compounds are generally of high molecular weight and may differ in mobility and bioavailability compared to selenium oxyanions (Tolu et al., 2014).

2.3.4. Selenium Associated with Soil Minerals

According to Goldberg and Glaubig (1988), selenium sorption in soils occurs on aluminium, iron oxides, clay minerals and calcite. As mentioned previously in a study by Coppin et al. (2009), selenite (Se\textsuperscript{IV}) sorption is greatest in the organic fraction (>400 mg Se kg\textsuperscript{-1}) of the soil compared to the mineral fraction (<200 mg Se kg\textsuperscript{-1}). However, transmission electron microscopy (TEM) and scanning electron microscopy (SEM) analysis of the organic fraction showed selenium was associated with clay minerals such as mica and illite. In the mineral fraction, SEM-EDS mapping showed selenium hotspots correlated with Al and Fe. Fe corresponded to iron oxides, however no aluminium oxides were observed by SEM and therefore Al is likely to be present in clay minerals. Selenium is easily adsorbed onto the surface of clay minerals and oxides by ligand exchange (Neal, 2005).

According to Plant et al. (2003) the sorption of selenite (Se\textsuperscript{IV}) onto iron oxides and clay minerals depends on the pH of the soil. A study by Hamdy and Gissel-Nielsen (1977) investigated the effect of pH on the fixation of selenite onto clay minerals and iron oxides over a 10 day equilibrium period. Of the clay minerals, kaolinite fixed larger amounts of selenite compared to montmorillonite and vermiculite. However, iron oxide was more effective and fixed more than 50% of the selenite. The fixation of selenite onto clay minerals was greatest between pH 3 and 5 and rapidly decreased as the pH increased demonstrating the importance of pH (Plant et al., 2003). Fixation by iron oxides remained constant between pH 3 and 9 which illustrates how the sorption capacity of iron is greater than clay minerals (Coppin et al., 2009). In acidic environments selenite is adsorbed onto iron oxides to form minerals such as ferric selenite (Fe\textsubscript{2}(OH)\textsubscript{4}SeO\textsubscript{3}) (Plant et al., 2003; Presser and Swain, 1990). However, most selenite minerals are soluble and will not persist in the natural environment (Masscheleyn et al., 1991).
In calcareous soils, calcite adsorbs selenite at greater amounts than kaolinite and montmorillonite due to the high reactivity of the mineral. In clay minerals only the edges are sites for selenite sorption, however in calcite the whole surface area of the mineral is involved. In Imperial soils, the sorption of selenite onto calcite rises from pH 6 to 8 and is optimal between pH 8 and 9 (Goldberg and Glaubig, 1988). In an experimental study by Reeder et al. (1994) at low temperatures, selenium concentrations greater than 1000 mg kg$^{-1}$ caused selenite to incorporate into calcite in aqueous solution. This may be due to the substitution of carbonate (CO$_3$) by selenite (Se$^{IV}$) and selenate (Se$^{VI}$) oxyanions in calcite (CaCO$_3$) however they both have differences in point symmetry.

Calcite may be important in determining the distribution and concentration of selenium oxyanions in the natural environment. However, the selenium cycle in soil is a much more complex system than the experimental studies on synthesised minerals in the laboratory. The fixation of selenium in soils is influenced by the microbial activity and the organic matter content of the soil (Hamdy and Gissel-Nielsen, 1977) and therefore the data from experiments in a laboratory are not representative of the natural environment (Girling, 1984). It is clear from the literature the microbial transformations of selenium in soils is an area of growing interest although the associations of selenium with the mineral and organic fractions of soil is still poorly understood.

2.4. Selenium in Plants

2.4.1. Selenium Uptake in Different Plant Species

Species of plants vary in their ability to accumulate and tolerate high concentrations of selenium in soils and are categorised into groups of ‘hyperaccumulators’, ‘secondary accumulators’ and ‘non-accumulators’ (Brown and Shrift, 1982; Terry et al., 2000; White et al., 2004). Selenium hyperaccumulator plants can accumulate selenium concentrations greater than 1000 mg kg$^{-1}$ DW (dry weight) (Beath et al., 1941; Brown and Shrift, 1982; El Mehdawi and Pilon-Smits, 2011). Many native shrubs and flowering plants grown in seleniferous areas of America are hyperaccumulators and include species from the genus Astragalus, Xylorhiza,
Oonopsis and Stanleya (Brown and Shrift, 1982; Terry et al., 2000). Many of these species can accumulate concentrations of selenium up to 15,000 mg kg\(^{-1}\) without growth problems (Beath et al., 1941; El Mehdawi and Pilon-Smits, 2011). A study by Feist and Parker (2001), looked at the concentration of selenium in species of the hyperaccumulator princes plume (Stanleya pinnata) growing in 25 seleniferous sites. The selenium concentrations in the leaves of Stanleya pinnata ranged from 1-1200 mg kg\(^{-1}\) showing large variation in selenium uptake. However, selenium concentrations in hyperaccumulator plants also fluctuate seasonally according to a study by Galeas et al. (2007). The highest selenium concentration in Stanleya pinnata was 12,700 mg kg\(^{-1}\) observed in spring and in Astragalus bisulcatus it was 4250 mg kg\(^{-1}\) observed in winter. During the year, Stanleya pinnata decreased by approximately 6000 mg kg\(^{-1}\) and in Astragalus bisulcatus by 2000 mg kg\(^{-1}\). Therefore, the time of year the plants are collected and analysed could affect the concentrations observed in the literature.

Secondary selenium accumulators typically accumulate selenium concentrations ranging from 100-1000 mg kg\(^{-1}\) DW. A number of shrubs and herbaceous plants are defined as secondary accumulators and include species from the genus Aster, Astragalus, Atriplex, Castilleja, Commandra, Grayia, Grindelia, Gutierrezia, Machaeranthera and Sideranthus (Brown and Shrift, 1982; Terry et al., 2000). Secondary accumulators are not constrained to seleniferous soil and can also colonise soils low in selenium (Brown and Shrift, 1982; White et al., 2007). A study by Yuan et al. (2012) classified Adenocaulon himalaicum, a species of trail plants grown in the Enshi district of China as a secondary selenium accumulator species as concentrations reached 563.60 mg kg\(^{-1}\) DW in the roots. Some cruciferous vegetables (Brassica spp) can accumulate selenium overlying seleniferous soils as shown by a soil culture study by Bañuelos et al (1997) on Indian Mustard (Brassica juncea) which accumulated concentrations from 407 mg kg\(^{-1}\) to 769 mg kg\(^{-1}\) without showing signs of selenium toxicity. This is supported by a study by Slekovcev and Goessier (2005) on Brassicas which accumulated selenium in the leaves of onion with 37.74 mg kg\(^{-1}\), followed by radish with 36.54 mg kg\(^{-1}\), cabbage with 11.9 mg kg\(^{-1}\) and garlic with 19.33 mg kg\(^{-1}\) which were grown in outdoor beds.
Selenium non-accumulators typically contain concentrations less than 25 mg kg\(^{-1}\) DW, although some species may accumulate enough selenium to cause toxicity in livestock (Brown and Shrift, 1982; Terry et al., 2000; White et al., 2004). Most species of grasses, forage and crop plants are considered non-accumulators as concentrations rarely exceed 100 mg kg\(^{-1}\) DW without experiencing toxic effects (Brown and Shrift, 1982; El Mehdawi and Pilon-Smits, 2011; White et al., 2007). Symptoms of plant selenosis include chlorosis of the leaves and necrosis at the tips of old leaves (Cartes et al., 2005). A study by Slekovoc and Goessier (2005) revealed perennial ryegrass (*Lolium perenne* L.), common ryegrass (*Lolium multiflorum* Lam.) and Orchard grass (*Dactylis glomerata* L.) do not accumulate selenium when growing in high selenium soils. The ryegrass concentration ranged from 0.08- 0.081 mg kg\(^{-1}\) and the Orchard grass 0.069-0.082 mg kg\(^{-1}\), both in the deficient range. However, selenium concentrations fluctuate seasonally in non-accumulators and are highest in the summer months which may have influenced results (Galeas et al., 2007). According to White et al. (2004), non-accumulator species are unable to colonise seleniferous soils. However, a study by Yuan et al. (2012) observed the uptake of 79.39 mg kg\(^{-1}\) DW selenium in the leaves of the non-accumulator white clover (*Trifolium repens* L.) growing in the seleniferous soils of the Enshi district in China.

### 2.4.2. Selenium Speciation in Plants

The ability of plants to accumulate selenium is also determined by the selenium species supplied to the plant. According to a study by Zayed et al. (1998) on four different plant species, accumulation of selenium in leaves is greatest when selenate (Se\(^{VI}\)) is applied to cultures followed by selenomethionine (SeMet) and selenite (Se\(^{IV}\)). However, accumulation in the roots is greatest when SeMet is applied followed by selenite and selenate. The overall uptake and accumulation of selenium was the least in plant tissues when selenite was applied. It is likely the variation in uptake is caused by the different transport mechanisms for each chemical species of selenium (Sors et al., 2005).
Hyperaccumulators can accumulate high concentrations of selenium without experiencing toxic affects due to their ability to exclude the amino acid selenocysteine (SeCys) which is a precursor of the organic selenium compound selenomethionine (SeMet) into proteins. Hyperaccumulators convert SeCys using enzymes into se-methylselenocysteine (MeSeCys), γ-glutamyl-se-methylselenocysteine (GGMeSeCys) and selenocystathionine (SeCyth) which are non-protein amino acids (Brown and Shrift, 1982; Sors et al., 2005). This is supported by a study by Freeman et al. (2006) as XANES spectra showed the predominant species of selenium in two accumulator plants was the non-protein amino acid MeSeCys. In the young leaves of Astragalus bisulcatus selenium was present as 86-98% MeSeCys and in Stanleya pinnata selenium was present as 71-98% MeSeCys.

Figure 2.3. The Se K-edge XANES energy spectra for the hyperaccumulator plants A. bisulcatus and S. pinnata after Freeman et al. (2006).
Similarly, in a study by Quinn et al. (2011), the XANES spectra revealed the predominant species of selenium accumulating in the flowers of *Stanleya pinnata* was MeSeCys. However, according to Freeman et al. (2006), MeSeCys has similar Se-K XANES spectra to SeMet, γ-GMeSeCys and SeCysth making it difficult to distinguish if MeSeCys is the predominant organic species as shown in figure 2.2. In the trichomes of the leaves of *Astragalus bisulcatus* where selenium is highest, LC-MS extracts showed 53% MeSeCys and 47% γ-GMeSeCys which differ significantly from the XANES spectra. However, in the globular structures of the leaf edges and tips of *Stanleya pinnata* where selenium is highest, liquid chromatography–mass spectrometry (LC-MS) extracts showed 88% MeSeCys and 12% SeCysth which supports the XANES spectra results. Therefore, a combination of techniques is advised to confirm selenium species in samples.

The secondary selenium accumulator Indian mustard (*Brassica juncea*) can accumulate selenium in the tissues of the plant regardless of the chemical species supplied to the plant (Zayed et al., 1998). Quinn et al. (2011) studied the selenium uptake in the flowers of *Brassica juncea* treated with 80 µM of sodium selenate and the XANES spectra revealed the flowers of the plant contained primarily MeSeCys (58%), followed by selenate, selenite and selenocysteine. However, studies on *Brassica juncea* leaves where selenium was highest showed the predominant species was selenate (de Souza et al., 1998; Freeman et al., 2006). A study by Slekovec and Goessier (2005) also showed the dominant peak was selenate in the vegetables radish (*Raphanus sativus*), onion (*Allium sepa L.*) and cabbage (*Brassica oleracea*) by high performance liquid chromatography (HPLC) in combination with inductively coupled plasma mass spectrometry (ICP-MS). However, the chromatogram for garlic revealed a more dominant peak than selenate which was an unidentified selenium compound which may be organic.

The uptake of selenium in the non-accumulator species perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) can occur when sodium selenate or sodium selenite is applied to soils (Smith and and Watkinson, 1984). This is because application of selenate (Se\textsuperscript{VI}) or selenite (Se\textsuperscript{IV}) increases the amount of bioavailable selenium to non-accumulators (Cartes et al., 2005). Selenate application causes
selenium to accumulate highest in the shoots, whereas selenite application causes selenium to accumulate highest in the roots (Smith and and Watkinson, 1984). Laboratory trials on the non-accumulator species wheat (*Triticum*) revealed that application of selenite was more toxic than selenate by inhibiting the roots more than the shoots (Lyons et al., 2005). It is suggested selenate remains as inorganic selenium in the shoots, whereas selenite is incorporated into organic selenium compounds (Cartes et al., 2005; Zayed et al., 1998) and therefore into amino acids and proteins causing enzyme dysfunction (de Souza et al., 1998; Zayed et al., 1998). This is supported by Wu and Huang (1992) who observed inhibition of plant growth with increased selenoprotein concentration in non-accumulators. However, the short timescale of pot experiments in these studies could influence the selenium concentrations measured as selenate uptake in the roots is faster than selenite (El Mehdawi and Pilon-Smets, 2011; Zhao et al., 2005). Therefore, over longer timescales the difference may be less significant in relation to toxicity (Smith and and Watkinson, 1984) and more research needs to focus on *in situ* growing experiments.

2.5. Sampling Site - Selenium Toxicity in West Limerick

Chronic selenium toxicity in livestock has been a historical issue in areas of West Limerick, Ireland, with farmers documenting symptoms in livestock including hair loss and cracks in hooves. The cause of chronic selenium toxicity is grazing pastures overlying seleniferous soils however the exact concentration mechanism is unknown. Although grasses are regarded as non-accumulator plants, a study by Fleming and Walsh (1956) found concentrations in pastures collected from County Limerick as high as 450 mg kg\(^{-1}\). Cases of selenium toxicity in the area usually occur in autumn, however outbreaks are unpredictable as they can occur one year and not in others (Rogers et al., 1990).

The main control on selenium distribution in soils is the underlying geology (Jacobs, 1989) which consists of Carboniferous limestone, shale and mudstone in Limerick. The source of selenium in the soils and pastures of West Limerick is believed to be the Clare Shale Formation which consists of black, pyritic shales (Finch and Ryan,
1966; Mcgrath and Fleming, 2008). Atkinson (1967) suggests selenium in the Limerick area is controlled by the effects of glaciation, drainage and the distribution of iron oxides. However, Fleming and Walsh (1956) observed the highest selenium concentrations in the horizons of soil highest in organic matter suggesting selenium in the Limerick area is controlled by the soil organic matter content. In Limerick, selenium toxicity typically occurs in neutral to alkaline soils which are poorly drained due to the low-lying topography (Fleming, 1962; Mcgrath and Fleming, 2008).

Figure 2.4. Spatial distribution map of selenium which indicates selenium concentrations are in the low to deficient range in Limerick (black square), adapted from Fay et al. (2007).
The distribution of selenium toxic soils is irregular and they often occur in close proximity to deficient soils. Geochemical mapping is used to determine the spatial distribution of chemical elements and compounds in soils (Johnson, 2008). This information can be used to understand the geochemical processes occurring in the study area which is important for environmental monitoring and management of land (Lancianese and Dinelli, 2014). In the literature, maps of selenium distribution in Ireland are on scales >10 km² and therefore selenium toxic soils have often been overlooked as shown in figure 2.4 produced by the Environment Protection Agency and TEAGASC. According to McGrath and Fleming (2008), maps would need to be on a 10 m² scale to determine the regional distribution of selenium in Limerick soils. A study by (Atkinson, 1967) analysed the distribution of selenium in rocks with concentrations ranging from 0 - >10 mg kg⁻¹, however it is not clear if this reflects the distribution in soils. Each year, new unexplained cases of livestock selenosis occur in farms which have not experienced problems previously (Rogers et al., 1990) and therefore it is likely most of the data reported is out-dated due to the constant biogeochemical cycling of selenium in the environment.

2.6. References


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Chapter 3 – Research Methods

This chapter discusses the analytical techniques used to characterise the inorganic and organic chemistry, microbiology and mineralogy in environmental samples collected as part of the thesis project.

3.1. X-ray Techniques

3.1.1. X-ray Fluorescence

X-ray fluorescence (XRF) is an atomic emission method used to quantitatively determine the concentration of trace elements and major compounds in environmental samples. XRF is a non-destructive method (Brouwer, 2003) widely used in environmental science due to the high precision accuracy, short preparation time and the ability to run multi-element analysis on a number of samples at once (PANalytical, 2015). The XRF technique uses an X-ray beam to radiate atoms within the sample with enough energy to expel an electron from the inner atomic shell (k-shell) as shown in figure 3.1. This produces a gap in the shell which causes the atom to become unstable. To restore stability, the gap in the shell is filled by an outer shell electron (L-shell). The energy of the L-shell is greater than the k-shell which causes excess energy to be emitted as a secondary X-ray photon. The energy of the emitted fluorescence X-ray is characteristic for each individual element. Therefore, by measuring the intensity of the emitted energies the concentration of individual elements can be determined. XRF systems are separated into two groups which are energy dispersive (EDXRF), with an elemental range from sodium (Na) to uranium (U) and wavelength dispersive (WDXRF), with an elemental range from beryllium (Be) to uranium (U) (Brouwer, 2003).

In order to analyse samples by XRF, environmental samples such as soil and rock must firstly be dried at 70°C and powdered to homogenise the material. The particle size must be less than 50 µm and large organic components such as stems and roots removed. The samples are made into 40 mm diameter wax pellets for inserting into the XRF machine by compressing 3 g of wax with 12 g of sample under a 10 tonne load. Sample pellets are analysed using a PANanalytical Axios sequential
X-ray fluorescence spectrometer at The University of Manchester. The major elements are expressed in wt% and trace elements in ppm, with detection limits of 0-6 ppm depending on the element. Element concentrations are determined from identification of individual peak intensities in the spectra compared to standard spectra using Omnian and Pro-Trace software.

![Diagram of X-ray interactions](image)

Figure 3.1. Simplified diagram showing the three main interactions of X-rays with matter (left) and characteristic radiation showing the emission of an electron (right), after Brouwer (2003).

The main limitation of EDXRF spectrometers is the resolution and sensitivity is less optimal for light elements compared to WDXRF. Whereas, WDXRF spectrometers have a lower efficiency than EDXRF and require more power (200-4000) which is expensive (Brouwer, 2003). XRF techniques only determine element concentrations and are not able to analyse the concentration of individual element species or distinguish between isotopes.

3.1.2. X-ray Diffraction

X-ray diffraction (XRD) is an analytical technique used to analyse the crystallinity of a material. Soil samples are first dried at 70°C, powdered to homogenise the material and mixed with amyl acetate. The sample is fixed to the sample holder on a glass slight and bombarded with X-rays. At the University of Manchester samples are analysed by a Bruker D8Advance equipped with a Lynxeye detector and a Cu Kα X-ray source. Samples are scanned with a step size of 0.02° and a counting time of 0.2
s per step from 5 to 70°2θ. The X-ray beam is focused using a Göbel mirror which is comparable to synchrotron techniques. X-rays of fixed wavelength are diffracted from the crystalline material at an angle which depends on the spacing between the lattice planes (figure 3.2).

Figure 3.2. An illustration showing the Bragg equation after Borchert (2014).

The sample is rotated to ensure the correct angle is achieved and to increase the X-rays interaction with the lattice planes. The relationship between the wavelength of the incident X-rays (λ), the angle of incidence (θ) and the spacing between the lattice planes (d) can be explained using bragg’s law: \( n\lambda = 2d\sin\theta \), where n is the order of reflection (integer). The spacing of lattice planes (d) is characteristic for each mineral (Borchert, 2014) and the resultant peaks can be compared to known standards using the International Centre for Diffraction Data (ICDD) to identify the mineral. Identifying minerals in soil samples by XRD is often difficult due to the amorphous nature of sample.

3.1.3. Synchrotron Radiation

Synchrotron radiation produces high energy X-rays which can be applied to conventional X-ray techniques to analyse the organic and inorganic species of particular elements. Synchrotron radiation can be applied to both crystalline and amorphous samples including soil, rock and plant material (Winkel et al., 2012). The high energy X-rays are produced by generating electrons which are accelerated around a storage ring at high speeds by 48 bending magnets. During the process, a
linear accelerator and booster synchrotron accelerate the speed of the electrons. Electrons lose energy as the path of the beam is curved by the magnets resulting in the emission of electromagnetic radiation which is channelled through beamline stations. I18 is a microfocus spectroscopy beamline at Diamond Light Source used in Environmental Sciences to analyse complex heterogeneous samples using techniques such as µ-XRF imaging, µ-XRD and µ-XAS (X-ray absorption spectroscopy). I18 uses a micron-sized beam with a high brightness and energies of 2.05-20.5 keV to collect high spatial resolution information in samples (Mosselmans et al., 2009). A schematic of the optical elements on the I18 beamline is shown in figure 3.3 which consists of a 25 m toroid mirror, a double crystal monochromator and two bimorph Kirkpatrick-Baez mirrors.

![Figure 3.3. Simple schematic of the I18 beamline at the Diamond Light Source facility after Mosselmans et al. (2009).](image)

**X-ray Absorption Spectroscopy**

X-ray absorption spectroscopy (XAS) is a technique which uses synchrotron radiation to analyse the atomic and electronic structure of a sample. XAS is used in this project to analyse selenium species in soil and plant material. XAS is divided into two regions, the near-edge X-ray absorption (XANES) region and the extended X-ray fine structure (EXAFS) region shown in figure 3.4 (Penner-Hahn, 2004). Generally, the XANES region reveals information about the electronic structure and
geometry (Penner-Hahn, 2004) and can be used to identify various inorganic selenium species and selenium-containing amino acids such as selenocysteine, and selenomethionine by comparing to standard spectra (Chasteen and Bentley, 2003). Linear combination fitting (LCF) can be performed on the XANES spectra using Athena software to fit the data against standard spectra (Ravel et al., 2005). The EXAFS region extracts quantitative information on structural parameters (Penner-Hahn, 2004) and can be used to identify the spatial distribution of particular selenium species and analyse sorption sites (Winkel et al., 2012).

XAS analysis of selenium is often difficult due to beam induced reduction and oxidation of the sample which results in unusable EXAFS data and poor quality XANES data even at 77K temperatures. However, this can be overcome by running the sample at liquid helium temperatures (10K). Another limitation of the technique is soil and plant samples which are below ppm are too low to obtain good quality EXAFS data. Therefore, only highly seleniferous environmental samples can analysed (Winkel et al., 2012).

Figure 3.4. Example of an XAS spectra showing the XANES and EXAFS regions for a protein containing [2Fe-2S] after Penner-Hahn (2004).
3.2. Chemical Analysis

3.2.1. Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Inductively coupled plasma mass spectrometry is a quantitative method for determining the concentrations of both metal and non-metal elements in aqueous samples within the ppt range (Winkel et al., 2012). ICP-MS is a widely used technique for analysing selenium in aqueous environmental samples due to the sensitivity, rapid timescale and high-quality control of interferences. An ICP-MS can be coupled to a High pressure liquid chromatography (HPLC) system to analyse the concentrations of individual species in elements. Solid samples such as soil and plant material need to be digested prior to analysis using a mixture of acids such as nitric acid (HNO$_3$), hydrogen peroxide (H$_2$O$_2$) and hydrofluoric acid (HF). 200 mg of freeze dried samples is added to PFA vessels and digested at 170°C using a MARS microwave digestion. Digested samples need to be heated to evaporate the HF before acidifying the sample in 2% ultra-pure HNO$_3$. Aqueous samples only require acidification in 2% nitric acid except for speciation analysis using HPLC which only requires a dilution in ultra-pure deionised water.

The initial step is to vaporise the aqueous sample through a pneumatic nebuliser to produce an aerosol which can be introduced to the ICP. A spray chamber shown in figure 3.5 removes aerosol droplets which are larger in size to ensure only the smaller droplets continue into the argon plasma in the quartz tube. The tube is surrounded by a magnetic field created by a current passing through a copper coil which causes free electrons to collide with argon atoms. Collisions continue until the high temperature is stable and the heat causes the aerosol to ionise by expelling an electron. Ions move through the vacuum system to the mass spectrometer which separates the ions according to the m/z ratio. This produces a spectrum with individual peak intensities that are representative of the element concentration (Agilent Technologies, 2006).
Quantifying selenium concentration is difficult as selenium has 5 stable isotopes and therefore the signal for measuring a single isotope is decreased. Compared to other trace elements, selenium has a high ionization potential and therefore has less efficient ionisation in the plasma. Argon-based polyatomic interference in $^{80}$Se and $^{78}$Se isotopes are also common (Uchida and Ito, 1997) which makes precise quantification of selenium at low concentrations difficult (Winkel et al., 2012).

3.2.2. Ion Chromatography

Ion chromatography (IC) is a reliable and sensitive method of analysis primarily used for the determination of inorganic ions in aqueous samples. However the technique can be used to separate organic acids, carbohydrates and amino acids. Samples require no acidification prior to analysis as the instrument can be operated using water as the only reagent (Haddad, 2004). At the University of Manchester, anion analysis is performed on a Dionex ICS5000 which is a dual channel system used to analyse soil sequential extracts and soil microcosm solutions in this thesis. To analyse a sample, a pump is used to push the mobile phase (liquid) from a reservoir through the guard column and then the analytical column where they interact with the ion exchange sites in the resin. When analysing anions, the mobile phase competes with the resin which contains positively charged sites. The resin causes the particular anion to move from the stationary phase to the mobile phase. The ions pass into a suppressor which
reduces the background conductivity of the mobile phase and increases the conductivity of the ions compared to the background. A peak is produced as the ions pass through a conductivity detector which can be compared to calibration standards to identify the ion and the concentration determined from the peak area (Haddad, 2004). The main limitation when analysing anions is the interference with any coinciding substances that are higher in concentration such as organic acids.

3.2.3. Spectrophotometry

Spectrophotometry is a technique used to analyse the concentration of solutes by measuring the absorption of light by the solution at a specific wavelength. The technique involves a colorimetric assay and a set of standards for the solute to determine the concentration from a calibration curve (Hofmann, 2010). The technique can be used to analyse iron concentration in solution using the ferrozine assay (Stookey, 1970) which forms a purple coloured solution in the presence of Fe(II) and is used in this thesis to analyse soil microcosm solutions. The UV/Vis spectrophotometer is set at a wavelength of 562.0 nm to measure iron. A blank ferrozine solution is first analysed in the spectrophotometer in a disposable cuvette to standardise before analysing the sample.

![Figure 3.6. Optical arrangement in a dual-beam spectrophotometer with optical paths shown in green after Hofmann (2010).](image)

The light source is both a tungsten filament bulb and a deuterium bulb so that the spectrophotometer emits visible and UV light. A prism called a monochromator is placed between the sample and the source of light which separates the incoming light by refraction (figure 3.6). The light source reaches a half mirror which reflects part of the light to a photodiode which calculates the original intensity of light ($I_0$).
The remaining light travels through the sample in the ferrozine solution and the intensity recorded by the photodiode. The intensity can be used to calculate the concentration from a calibration curve according to Beer–Lambert law (Hofmann, 2010).

3.3. Organic Analysis

3.3.1. Loss on Ignition

The release of CO₂ from solid environmental samples is measured by loss on ignition (LOI) which determines the carbon originating from organic matter and carbonates. 1 g of dried, powdered sample is weighed into a clean pre-ignited crucible and heated to 110°C for 1 hour, then transferred to a desiccator to cool and reweighed. This process is repeated at 1000°C and both results corrected for water content. The LOI is calculated as the difference between the initial and final sample weights divided by the initial sample weight multiplied by 100 (Schumacher, 2002). To determine the total organic carbon (TOC) in the samples and not CO₂ originating from carbonate, the LOI method is repeated, and the samples are heated to 400°C instead of 1000°C. A conversion factor of 1.724 is used to convert the results into total organic carbon, based on the assumption that organic matter contains 58% organic C (Nelson and Sommers, 1996).

3.3.2. Total Dissolved Organic Carbon

The release of CO₂ from aqueous environmental samples is measured using a Shimadzu TOC-C CPN analyser to calculate the total dissolved organic carbon (TDOC). Firstly, the total carbon (TC) is calculated using the combustion catalytic oxidation method by injecting 15 ml of filtered sample into the sample port and heating to 680°C in a furnace. The total inorganic carbon (TIC) is calculated by acidifying the sample in a quartz medium. The release of CO₂ from both the combustion (TC) and acidification (TIC) is measured using an infrared detector and the TDOC is calculated by subtracting the TIC concentration from the TC concentration in mg L⁻¹.
3.3.3. *Pyrolysis Gas Chromatography Mass Spectrometry (Py-GC/MS)*

Pyrolysis GC/MS is a fast and efficient analytical technique used to characterise the complex mixture of organic compounds in environmental samples such as sub-milligram sized soil and plant material (Ralph and Hatfield, 1991). The initial step is pyrolysis which involves injecting 2-4 mg of powdered sample without the need for pre-treatment using solvents to extract the compounds (Bull, 2008; Kusch, 2012). The sample is placed into a pre-cleaned quartz tube with a known concentration and volume of androstane standard. Quartz wool is inserted into the ends of the tube to prevent sample loss during insertion into the pyrolysis unit (Christy et al., 1999). The tube is inserted into a chemical data systems (CDS) 5200 series pyroprobe filament and heated to high temperatures (>600°C) in the absence of oxygen which causes the thermal degradation of complex molecules. The breakage of chemical bonds is caused by reactions with free radicals which produces volatile compounds that have been separated by the relative strength of the molecular bonds (Kusch, 2012).

![Pyrolysis Gas Chromatography Mass Spectrometry Unit Diagram](image)

Figure 3.7. Diagram of a pyrolysis gas chromatography mass spectrometer unit, adapted from UCLA (2015).

The thermally stable compounds can now be passed through a gas chromatography column by an inert carrier gas (helium) as shown in figure 3.7. Organic rich samples are introduced into the GC column in split mode with a ratio of 20:1 rather than the
entire material at once. The GC column used at the University of Manchester is an Agilent 7890A, which is a 30 m coiled tube coated with 55 diphenyl-dimethylpolyolsiloxane with a thickness of 0.25 µm. The pyrolysis moieties interact with the GC column and are passed through a heated transfer line which separates the volatile compounds into ions (Bull, 2008). For soil samples the oven is set to a maximum temperature of 320°C programmed at 40°C for 3 minutes then 6°C/min to 320°C and held for 10 min.

The GC column is coupled to a mass spectrometer (MS) where the positively charged ions are separated by an electric and magnetic field into chemical moieties relating to mass (Bull, 2008). An example of a MS is an Agilent 5975C MSD single quadrupole MS which operates in ionization (EI) mode. Ionisation occurs with a kinetic energy of the impacting electrons of 70 eV. The EI source is set to a temperature of 230°C (max 250°C) and the MS quadrupole temperature is programmed at 150°C (max 200°C). The organic moieties are analysed by the GC/MS scanning a mass range of m/z 50-700.

Py-GC/MS Analysis

The chromatograms obtained by Py-GC/MS can be analysed to identify the organic moieties using MSD Chem Station software and comparison with data in the literature. The software has a NIST (National Institute of Standards Technology) library which is a database containing 107,866 known compounds (AgilentTechnologies, 2000). The organic moieties are identified by the relative mass measured by the peak in the total ion chromatogram at particular GC retention times. The corresponding spectra and chemical formula for each identified peak in the chromatogram can be viewed in the Chem Station software. The identified organic compounds can be reduced into a dataset arranged by the average retention time, mass, compound name, and peak area for interpretation. The concentration of the compound is calculated by manual integration of the peak areas. The different organic moieties can be divided into groups of aromatics, polysaccharides, lignin markers, phenols, nitrogen containing compounds and lipids according to the Vancampenhout method (Vancampenhout et al., 2009).
The limitations of Py-GC/MS are samples need to be high in organic matter for the compounds to be detected. Unlike normal gas chromatography which retains a clean insert, pyrolysis often introduces considerable amounts of low molecular weight compounds and the insert does not stay inactive for long. At a pyrolysis temperature of 700°C the breakdown of some complex molecules into volatile compounds is low, for example the breakdown of carbohydrate polymers is less efficient than lignin. Other limitations include the identification of peaks as some isomers are non-distinguishable from the mass spectra. For example, eugenol, cis-isoeugenol and trans-isoeugenol are difficult to interpret from the total ion chromatogram alone (Ralph and Hatfield, 1991). Furthermore, not all organic compounds have been identified in the NIST database and some peaks often overlap on the ion chromatograms making identification difficult.

3.4. Microbiological Techniques

3.4.1. DNA Extraction

16S pyrosequencing techniques are used for microbial community analysis of environmental samples. The first step is to extract the genetic information from the sample which involves separating the deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) followed by purification. For this project a PowerSoil DNA isolation kit (MO BIO Laboratories INC, Carlsbad, CA, USA) is used to extract the DNA from soil microcosm samples. To release the DNA and RNA, cell lysis needs to occur using the bead beating method which involves a combination of mechanical homogenisation using glass beads and chemical breakdown using anionic surfactants. A buffer solution is also used to prevent the alteration of the genetic material so only the cell membrane is broken down. The total genomic DNA is captured on a silica membrane and is washed and removed from the membrane (Miller et al., 1999).

The 16S rDNA gene region of the DNA extract is then amplified by polymerase chain reaction (PCR). PCR involves denaturing the DNA double helix, attachment of oligonucleotide primers to the 16S rDNA gene and finally DNA polymerase synthesis. In this project the fluorescently labelled primers (5′-AGAGTTTGATCCTGGCTCAG-3′ and 1492R 5′-TACGGYTACCTTGTAGACTT-3′) are
used according to the methods of (Rizoulis et al., 2016). The DNA is stained, placed in an agarose gel and separated using electrophoresis. The DNA is observed under UV light to identify 1500 base pair products.

Figure 3.8. An example protocol for PCR amplification of the 16S rRNA gene with primers targeting the V4 and V5 variable region after Lakshmanan et al. (2017)

3.4.2. DNA Sequencing

To sequence the PCR amplicons of the 16S rRNA, the V4 hyper variable region is targeted (forward primer, 515F, 5’-GTGYCAGCMGCCGCGGTAA-3’; reverse primer, 806R, 5’-GGACTACHVGGGTWTCTAAT-3’) for 2× 250-bp paired-end sequencing using a Illumina® MiSeq platform (illumine, San Diego, CA, USA) (Caporaso et al., 2011). An example protocol for PCR amplification is shown in figure 3.8. After PCR amplification, the products are cleaned and normalised using a SequelPrep Normalisation Kit (Fisher Scientific, Loughborough, UK) to get the most sensitive and reliable results. The normalised pools of PCR amplicons are run according to the method of (Kozich et al., 2013) and the acquired sequences organised by barcodes. A MiSeq error correction is applied and chimeras are removed which are
not genuine 16S rRNA gene sequences. Operational taxonomic units (OTUs) are classified at the 97% similarity level and singletons removed. Rarefaction analysis is performed using the detected OTUs in Qiime which is an open source bioinformatics pipeline. The taxonomic assignment to the species level is conducted using the ribosomal database project (RDP) classifier (Wang et al., 2007). The OTUs which could not be classified to the species level are classified to the identified phylum, class or family.

3.5. References

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Chapter 4 – Research Paper 1

Selenium Uptake from Soil to Plants: An XAS Study

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Abstract

Selenium (Se) uptake and accumulation in plants is important as it is a vital micronutrient in humans and animals. However, to avoid deficiency or toxicity the recommended dietary intake is limited to a narrow range between 40 μg d\textsuperscript{−1} and 400 μg d\textsuperscript{−1}. Selenium toxicity in livestock has been a historical problem in County Limerick as a result of grazing pastures overlying seleniferous soils. In order to understand the uptake of selenium from soil to plants, soil samples collected at 10-20 cm from 5 different agricultural sites were analysed. Concentrations of selenium of up to 1265.8 mg kg\textsuperscript{−1} were identified in the soil which exceeds the world’s seleniferous soil value. Soil cores were extracted from two sites and analysis shows selenium was concentrated at 30-40 cm depth associated with the total organic carbon. Pastures grazed by livestock consisted of Lolium perenne (perennial ryegrass), interspersed with Festuca arundinacea (tall fescue), Dactylis glomerata (cocksfoot), Phleum pretense (timothy grass) and Trifolium repens (white clover). These plant species are considered Se non-accumulators but contained concentrations toxic to livestock and uptake and translocation was highest in the white clover species. An \textit{in situ} growing experiment was conducted using the Se accumulator species Brassica oleracea. The leaves of the cabbage and kale accumulated up to 971.7 mg kg\textsuperscript{−1} Se and 1000.4 mg kg\textsuperscript{−1} molybdenum (Mo). X-ray absorption near edge spectroscopy (XANES) analysis indicated selenium was
present in the seleniferous soil predominantly in a reduced immobile phase as elemental Se (Se\(^0\)) and also the organic species selenomethionine (SeMet) which is bioavailable. SeMet was also the main species identified in both the Se non-accumulator and Se accumulator plants. This study provides important information for selenium bioavailability in soil, uptake and translocation in pasture species and the potential for phytoextraction using *Brassica oleracea* species.

**Keywords:** XANES, speciation, selenium uptake, translocation, *Brassicas*, bioaccumulation

### 4.1. Introduction

Selenium uptake and accumulation in plants is important to human and animal health as plants are the main dietary source of vital micronutrients in many parts of the world. The recommended dietary intake in humans is limited to a narrow range between 40 μg d\(^{-1}\) and 400 μg d\(^{-1}\) which is essential to avoid deficiency or toxicity related health problems occurring (Fordyce, 2007; WHO, 1996). The uptake of selenium in plants is determined by the concentration and bioavailability of selenium in the soil. Selenium accumulates in soils as immobile selenides (Se\(^{\text{II}}\)) and elemental selenium (Se\(^0\)) but uptake in plants is greater when selenium is present as the bioavailable organic species selenomethionine (SeMet) and selenocysteine (SeCys), and the inorganic species, selenate (Se\(^{\text{VI}}\)) and selenite (Se\(^{\text{IV}}\)) (Fordyce, 2013; Guignardi and Schiavon, 2017; Neal, 1995; Zayed et al., 1998). Selenium is not essential to plants but is assimilated into a number of organic selenium compounds in a similar manner to sulfur uptake which increases plant growth and antioxidant activity (Pilon-Smits et al., 2017). The translocation and distribution of selenium in different parts of the plant is influenced by the species of selenium supplied in the soil (Terry et al., 2000). According to Zayed et al. (1998), accumulation in the roots of plants is largest when SeMet is supplied from the soil whereas accumulation in the leaves is largest when selenate is supplied. However, the distribution is also affected by the phase of growth and physical functioning of the plant (Terry et al., 2000).
Different plant species vary in their ability to accumulate and tolerate high concentrations of selenium due to their genetic properties (Pilon-Smits et al., 2017). It is suggested selenium hyperaccumulator plants convert selenium species into less harmful volatile compounds and prevent incorporation into proteins and thus avoid toxicity (Guignardi and Schiavon, 2017). Secondary selenium accumulator species can tolerate concentrations from 100-1000 mg kg$^{-1}$ (DW) dry weight (Brown and Shrift, 1982; Terry et al., 2000) and include species of *Brassicas* such as Indian mustard, onion, cabbage and garlic. There is an environmental interest in accumulator plants as a sustainable method of removing toxic metals from contaminated land and water, termed phytoremediation (Salt et al., 1995). The accumulator species concentrates selenium in plant tissues thus removing high concentrations from the soils. The Se accumulator plant is beneficial to human health as it can be used in selenium deficient areas as a dietary supplement (Pilon-Smits et al., 2017). Selenium non-accumulator species can only tolerate selenium concentrations below 100 mg kg$^{-1}$ DW and rarely exceed 25 mg kg$^{-1}$ DW and include species of grasses and crops plants (Brown and Shrift, 1982; Terry et al., 2000; White et al., 2004).

Prolonged exposure to selenium concentrations of only 2-5 mg kg$^{-1}$ DW in pastures has shown to cause toxicity in livestock which is a particular problem for small farm holdings (de Souza et al., 1998; Terry et al., 2000; Wilber, 1980; Wu et al., 1996b). Selenium toxicity in livestock which is characterized by the loss of hair and hooves has been a historical problem in County Limerick, Ireland, identified in 1951 (Fleming, 1961; Fleming and Walsh, 1956; McGrath and Fleming, 2008). Molybdenum toxicity also often occurs in these areas and is characterised by a fading, rough coat and scouring in livestock. One Limerick study showed soil selenium concentrations of 150 mg kg$^{-1}$ in the same areas as molybdenum concentrations of 53 mg kg$^{-1}$ (Fleming and Walsh, 1956) although they recognized that uptake and toxicity will depend on soil factors such as pH, drainage and the organic matter content (McGrath and Fleming, 2008). The bioavailability of selenium to plants is found mainly in soils with an alkaline pH value and high redox potentials (Winkel et al., 2012) and therefore most of the research on plant
bioavailable selenium has focused on selenium oxyanions. However, more research is needed on the uptake and bioavailability of different selenium organic compounds present in anoxic soils as many have not been identified (Fordyce, 2007). Correlations between soil selenium and uptake in plants have been observed in controlled laboratory growing experiments more successfully than in-situ field studies (Zhao et al., 2005). This highlights the need for selenium research focused on realistic field studies, examining the behaviour of selenium in the natural environment where complex bio-physiochemical conditions prevail.

Understanding the mobility of selenium species in soils and the uptake and assimilation in plants is essential to avoid health implications in livestock and humans (Fordyce, 2013). Therefore, the aim of this work was to use field studies to examine the bioavailability of selenium in different seleniferous soils and examine the uptake in the overlying vegetation to understand the processes underlying the terrestrial cycling of selenium. The objectives were to examine the bulk chemistry and selenium species in (i) soils to determine the bioavailability and toxicity to overlying vegetation, (ii) different parts of non-accumulator plants (grass species and white clover) to measure the uptake and the risk to livestock health and (iii) different parts of Se accumulator plants (Brassicas) to understand the detoxification mechanisms and the potential for phytoremediation of soils.

4.2 Methodology

4.2.1 Field Site and Sampling Techniques

Soils underlying pastures were sampled from 5 seleniferous sites (TL, TJ, FF, MO and PK) in West Limerick and were collected at 10-20 cm depth to determine the distribution of selenium and other toxic trace elements. Sites were selected using data provided by TEAGASC, The Irish Agricultural and Food Development Authority and published chemical data on selenium in soils and vegetation (Atkinson, 1967; Fleming, 1961; McGrath and Fleming, 2008). A full dataset of sites studied is provided in the supplementary information (table 8.1-8.5). The field study revealed that this area of West Limerick contained natural soils with extraordinarily high Se values (up to 1265.8 mg kg$^{-1}$). The sites are all relatively low lying grassland in
gentle topography (30 m variation) with organic rich soils overlying Carboniferous limestones, mudstones and shales. Soil cores were collected from sampling locations TL and FF to determine the changes in selenium concentration with soil profile depth. The soil cores were sampled using a Van Walt corer with stainless steel cylinders measuring 22 cm in length and 4.4 cm in diameter. The first complete core was stored aerobic ally for bulk chemical analysis, and the second replicate core was stored under oxygen free nitrogen at the site to maintain an anoxic environment for speciation analysis. The cores were tightly capped and stored at 4°C to reduce microbial activity.

Figure 4.1. Map showing soil and plant sampling locations in agricultural sites FF, MO, PK, TL and TJ in County Limerick, Ireland.

Pastures grazed by livestock were sampled from the 5 seleniferous sites (TL, TJ, FF, MO and PK). The vegetation consisted of several species of perennial grasses: *Lolium perenne* (perennial ryegrass), interspersed with *Festuca arundinacea* (tall fescue), *Dactylis glomerata* (cocksfoot), *Phleum pretense* (timothy grass) and *Trifolium repens* (white clover). An in-situ growing experiment was conducted at a site with an established Se soil concentration (site FF) using *Brassica oleracea* (red flare cabbage, hispi cabbage and premier kale) which are known to accumulate selenium (Fleming, 1961; Fleming and Walsh, 1956; Slekovc and Goessier, 2005).
The *Brassicas* were grown in an area from which the grass was removed, over a period of 11 weeks (4\textsuperscript{th} May – 22\textsuperscript{nd} July 2016) under natural conditions. The fully grown plants were harvested by placing them undisturbed in 20x20x20 cm pots including the underlying soil and watered using rainwater collected in a local reservoir at the site to avoid plant stress during transportation.

4.2.2. Plant Chemical Analysis

As the grass pasture species were interspersed they were analysed in bulk, separated into shoot and root material. White clover and the *Brassica* species red flare cabbage, hispi cabbage and premier kale were separated into leaf, stem and root material. Once separated the plant material was rinsed in deionised water to remove any soil particles prior to chemical analysis. To determine the bulk concentrations of key elements, the plant material was frozen at -80°C and freeze-dried to remove water. The dried plant material was powdered using a pestle and mortar to ensure thorough mixing and 0.1-0.2 g was added to PFA microwave vessels in triplicate. In a fume cupboard 6 ml nitric acid (HNO\textsubscript{3}) and 3 ml hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) was added to digest the material overnight. The next day 1 ml of hydrofluoric acid (HF) was added to the vessels and after 1 hour they were sealed and digested at 170°C in a MARS microwave digestion unit for a total of 55 minutes at 100% of 800W (max of 12 samples per batch). Once the vessels depressurized, the acidified sample was poured into 120 ml PTFE and heated on a hot plate until the HF evaporated. The residue was dissolved in 2% ultra-pure HNO\textsubscript{3}, reheated to incipient dryness and the residue washed and diluted to 50ml with 2% ultra-pure HNO\textsubscript{3}. In each batch, samples were digested in triplicate with a certified reference material CRM 402 – white clover (Se 6.7 +/- 0.25 mg kg\textsuperscript{-1}) with an average recovery of 6.73 mg kg\textsuperscript{-1} and a blank (all <0.1 mg kg\textsuperscript{-1} Se) to validate the chemical extraction. For chemical analysis, 10 ml was filtered (> 0.45µm) and analysed for selenium (\textsuperscript{82}Se), molybdenum (\textsuperscript{95}Mo), arsenic (\textsuperscript{75}As), uranium (\textsuperscript{238}U) and vanadium (\textsuperscript{51}V) using an inductively coupled plasma mass spectrometer (ICP-MS) Agilent 7500cx equipped with a pressurized octopole collision/reaction cell. Standard reference materials for trace elements in natural water were used to measure the accuracy of the analytical run (NIST-1643, NIST-1640 and TM25.5). The mean and standard
error was calculated for each triplicate sample to measure the precision of the data. The translocation factor (TF) was calculated for all the plant samples analysed which defines the plants ability to translocate the trace elements from roots through to the shoots or leaves of the plant \((TF = C_{\text{shoot}}/C_{\text{root}})\), which determines the phytoextraction capability (Nirola et al., 2015). \(C_{\text{shoot}}\) in the white clover, kale and cabbages was subdivided into leaf and stem material and the TF calculated separately; in the grass species the bulk shoot concentration was used. A TF>1 indicates the plant can translocate efficiently from root to shoot. The bioaccumulation factor was calculated for the grass species which is the ratio of the trace element concentration in the plant material to the soil \((BAF = C_{\text{plant}}/C_{\text{soil}})\), which is used to estimate the plants ability to accumulate the trace elements (Zhou et al., 2016). \(C_{\text{plant}}\) was subdivided into shoot and root material and the BAF calculated separately.

4.2.3. Bulk Soil Characterisation

To prepare the soil cores for chemical analysis they were first separated into 10 cm sections. All soil samples collected from sites TL, TJ, FF, MO and PK and the cores were dried at 50°C and powdered (<50 μm) to homogenise the sample and remove any root material. Wax mounted pellets were prepared using 12 g of sample and 3 g of wax pressed under a 10 tonne load. The pellets were analysed by X-ray fluorescence (XRF) using a PANanalytical Axios sequential X-ray Fluorescence Spectrometer. The concentrations of elements (mg kg\(^{-1}\)) were determined by comparing the spectra peak intensities with known calibration standards and blank specimens using the pro-trace software. Omnian software was used to accurately calculate the major concentrations (wt%) which adjusts to sample and matrix effects. A certified reference material GBW 0731 - Chinese sediment (Se 0.20 ± 0.05 mg kg\(^{-1}\)) was used to validate the method. Another 1 g of soil was separated for loss on ignition (LOI) to calculate the moisture loss (H\(_2\)O wt%) and carbon dioxide (CO\(_2\) wt%) released by heating to 110°C and 1000°C. The weight loss of the soil was used to quantify the XRF major concentrations. 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 (Nelson and Sommers, 1996). The Se, Mo, U and As concentrations measured at 10-20 cm depth were plotted using Surfer® software to determine suitable core sampling locations in sites TL and FF.

4.2.4. Synchrotron Analysis

X-ray absorption spectroscopy (XAS) analysis of the Se K-edge (12.654 keV) was undertaken at Diamond Light Source (DLS), 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 which was cooled in a container of liquid nitrogen to achieve a temperature of -150°C which avoids ice crystal formation and subsequent sample damage. Sub-samples of soil were collected from the highly seleniferous horizons of two soil cores in an anaerobic cabinet and sealed in 1 ml epipendorfs to avoid oxidation. The sub-samples were centrifuged at 6000 rpm for 15 min to remove the supernatant and flash frozen in liquid nitrogen to remove the pellet. Samples were set in an optimum cutting compound (OCT) matrix to avoid element redistribution (Vavpetič et al., 2015) and cryosectioned in a microtome at -20°C to a thickness of 30-50 microns for plant material and 100 microns for soils. 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.

The samples were cryo-fixed onto the sample holder in liquid nitrogen and mounted on an x-y-z stage inclined at 45° to the incident beam. The I18 beamline covers an energy range between 2.0 and 20.7 keV using Si(III) monochromating crystals (Mosselmans et al., 2009) which allows the analysis the Se K-edge. The beamline experiment was conducted at a temperature of 10K using a liquid helium cryostat to prevent in situ beam reduction or oxidation. The beamline energy was calibrated using Au foil (8.072 keV) and a range of powdered selenium and molybdenum standards measured in transmission mode to allow comparison with the spectra of individual species. Selenium standards included sodium selenide (Na₂Se, Se⁻II), selenocystine (C₆H₁₂N₂O₄Se₂, Se⁻II), selenomethionine (C₅H₁₁NO₂Se, Se⁻II).
 elemental selenium (Se$^0$), sodium selenite (NaSeO$_3$, Se$^{IV}$) and sodium selenate (NaSeO$_4$, Se$^{VI}$).

Synchrotron micro X-ray fluorescence maps (μ-XRF) of the samples were collected in fluorescence mode using a germanium solid state detector at 13.0 keV for selenium fluorescence with a fixed beam size of 5 μm. Points of interest (POI) displaying high selenium counts, as well as a range of counts, were selected for X-ray absorption near-edge structure (XANES) analysis for species information. The μ-XRF spectra were processed into elemental maps using PyMCA 5.2.2 software (Solé et al., 2007). The selected XANES spectra were normalised using Athena software and linear combination fitting (LCF) was performed to fit the data against the standard spectra (Ravel et al., 2005).

4.3. Results

4.3.1. Characterisation of the Seleniferous Soils

The trace and major elemental composition of soil was analysed in 500 g samples collected at 10–20 cm depth from 5 different dairy cattle pasture sites (labelled TL, FF, MO, PK and TJ) in West Limerick (Figure 4.1) to determine their concentration of selenium, and the concentration of a range of major and trace elements. All sites have elevated Se soil contents, and historical issues with cattle selenosis. The XRF data for Se, Mo, As, U and V are summarised in table 4.1 and show the average selenium concentration for all sites ranged from 13 mg kg$^{-1}$ at site TL to 426 mg kg$^{-1}$ at site MO, which are indicative of toxic levels of Se in the soils. The highest concentration of selenium was observed in a sample at site MO with 1265.8 mg kg$^{-1}$, this site also had the largest range with a lowest concentration of only 1 mg kg$^{-1}$ sampled in other areas of the site (table 4.1). The remaining 4 sites all had minimum selenium concentrations <1 mg kg$^{-1}$, however the highest concentrations were highly variable with 28.4 mg kg$^{-1}$ at site TJ, 88.2 mg kg$^{-1}$ at site TL, 489.1 mg kg$^{-1}$ at site FF and 682 mg kg$^{-1}$ at site PK.

Site FF and MO which are closest in proximity also contained the most elevated concentrations of molybdenum, arsenic and uranium. The molybdenum
concentrations were highest at site FF but ranged from a relatively low value of 2.8, to 1627.5 mg kg\(^{-1}\), followed by site MO which ranged from only 3.2 to 898.1 mg kg\(^{-1}\). Uranium concentrations were also enriched but ranged from a low value of 8.3 to 599.5 mg kg\(^{-1}\) at site FF and 17 to 407 mg kg\(^{-1}\) at site MO. The arsenic concentrations were lower and 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 concentration for all sites had a smaller range and was highest at site PK with 175.7-195.2 mg kg\(^{-1}\). The spatial variation of the Se, Mo, As and U in soils at 10-20 cm depth at sites FF and TL is shown in figures 4.2 and 4.3, displaying the highly heterogeneous nature of these sites; the reasons for the concentration and distribution is presented elsewhere (supplementary report 8.1).

Table 4.1. The minimum, maximum and mean chemical data for bulk soil samples collected at 10-20 cm depth from 5 agricultural sites determined by X-ray Fluorescence (values do not include soil core data).

<table>
<thead>
<tr>
<th>Site</th>
<th>n</th>
<th>Se (mg kg(^{-1}))</th>
<th>Mo (mg kg(^{-1}))</th>
<th>As (mg kg(^{-1}))</th>
<th>U (mg kg(^{-1}))</th>
<th>V (mg kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>TL</td>
<td>Min</td>
<td>0.0</td>
<td>0.2</td>
<td>7.5</td>
<td>5.2</td>
<td>54.3</td>
</tr>
<tr>
<td></td>
<td>Max</td>
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<td>39.8</td>
<td>13.0</td>
<td>85.9</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
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<td>0.7</td>
<td>16.1</td>
<td>7.0</td>
<td>72.4</td>
</tr>
<tr>
<td>TJ</td>
<td>Min</td>
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<td>1.0</td>
<td>6.2</td>
<td>6.8</td>
<td>20.7</td>
</tr>
<tr>
<td></td>
<td>Max</td>
<td>28.4</td>
<td>19.0</td>
<td>17.1</td>
<td>26.6</td>
<td>80.9</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>18.0</td>
<td>6.6</td>
<td>13.5</td>
<td>14.9</td>
<td>47.6</td>
</tr>
<tr>
<td>FF</td>
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<td>0.0</td>
<td>2.8</td>
<td>0.0</td>
<td>8.3</td>
<td>48.4</td>
</tr>
<tr>
<td></td>
<td>Max</td>
<td>489.1</td>
<td>1627.5</td>
<td>99.8</td>
<td>599.5</td>
<td>189.7</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>109.3</td>
<td>104.6</td>
<td>21.5</td>
<td>67.7</td>
<td>103.3</td>
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<tr>
<td>MO</td>
<td>Min</td>
<td>1.0</td>
<td>3.2</td>
<td>0.0</td>
<td>17.0</td>
<td>77.8</td>
</tr>
<tr>
<td></td>
<td>Max</td>
<td>1265.8</td>
<td>898.1</td>
<td>115.3</td>
<td>407.0</td>
<td>195.2</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>426.0</td>
<td>180.1</td>
<td>42.5</td>
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<td>133.1</td>
</tr>
<tr>
<td>PK</td>
<td>Min</td>
<td>0.9</td>
<td>1.9</td>
<td>0.4</td>
<td>10.0</td>
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</tr>
<tr>
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<td>682.0</td>
<td>34.9</td>
<td>63.1</td>
<td>12.4</td>
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</tr>
<tr>
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<td>Mean</td>
<td>172.8</td>
<td>17.0</td>
<td>21.7</td>
<td>11.1</td>
<td>195.2</td>
</tr>
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</table>
Figure 4.2. Map showing the spatial distribution of Se, Mo, As and U concentrations (mg kg$^{-1}$) in site TL, produced using Surfer® software. Contours based on sample sites chosen to represent the vegetation and slope variations in the field.
Figure 4.3. Map showing the spatial distribution of Se, Mo, As and U concentrations (mg kg$^{-1}$) in site FF, produced using Surfer® software. Contours based on sample sites chosen to represent the vegetation and slope variations in the field.
4.3.2. Soil Core Characterisation

A more detailed analysis of the trace and major elemental composition of soil was undertaken by collecting and analysing a complete soil core from two of the sites, FF and TL, to understand the distribution of selenium with depth in the soil profile and to compare the plant bioaccumulation of Se from soil via the rhizosphere. Soil core locations were selected using data on the selenium distribution in soil samples collected at 10-20 cm depth (figures 4.2 and 4.3) and the translocation of Se concentrations in grass samples (see table 4.3 and 4.4). The results shown in table 4.2 show the top horizon of soil contained the lowest selenium concentration in core FF-2 with 99.1 mg kg\(^{-1}\). The concentration in the top horizon of core TL-1 was 55.2 mg kg\(^{-1}\) although selenium was even lower at 10-20 cm depth. The highest selenium concentrations in both soil cores were at a depth horizon of 30–40 cm with 171 mg kg\(^{-1}\) in core TL-1 and 926 mg kg\(^{-1}\) in core FF-2.

In core FF-2, the most seleniferous horizon also contained 191.2 mg kg\(^{-1}\) molybdenum, 94.2 mg kg\(^{-1}\) arsenic and 354.9 mg kg\(^{-1}\) uranium. Although these elements were highly concentrated in the soil they did not show a strong positive correlation with selenium through the soil profile except for molybdenum with a R\(^2\) value of 0.52. The highest uranium concentration of 658.8 mg kg\(^{-1}\) was also observed directly above the seleniferous horizon at a depth of 20-30 cm. In core TL-1 the most seleniferous horizon contained elevated concentrations of arsenic with 54.6 mg kg\(^{-1}\) and uranium with 13 mg kg\(^{-1}\) but only 2.4 mg kg\(^{-1}\) molybdenum. These elements had strong 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.
Figure 4.4. Selenium and total organic carbon (TOC) concentration with depth of the soil profile in core TL-1.

Figure 4.5. Selenium and total organic carbon (TOC) concentration with depth of the soil profile in core FF-2.
Table 4.2. XRF data showing the concentration of selected trace and major elements for two soil cores TL-1 and FF-2 every 10 cm interval. R^2 = the correlation of selenium with other elements within the soil profile where a value closer to 1 is the better fit to the regression line. TOC = total organic carbon and is calculated by the loss on ignition method.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Depth cm</th>
<th>Se mg kg(^{-1})</th>
<th>Mo mg kg(^{-1})</th>
<th>As mg kg(^{-1})</th>
<th>U mg kg(^{-1})</th>
<th>V mg kg(^{-1})</th>
<th>Mn mg kg(^{-1})</th>
<th>Cu mg kg(^{-1})</th>
<th>CaO wt%</th>
<th>Fe(_2)O(_3) wt%</th>
<th>SiO(_2) wt%</th>
<th>Al(_2)O(_3) wt%</th>
<th>TOC wt%</th>
</tr>
</thead>
<tbody>
<tr>
<td>TL-Core 1</td>
<td>0-10</td>
<td>55.2</td>
<td>0.8</td>
<td>25.2</td>
<td>7.2</td>
<td>82.0</td>
<td>260.9</td>
<td>67.5</td>
<td>3.1</td>
<td>3.7</td>
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<td></td>
<td>10-20</td>
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<td>6.9</td>
<td>81.1</td>
<td>312.8</td>
<td>64.9</td>
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<td>3.8</td>
<td>57.1</td>
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<td>7.1</td>
</tr>
<tr>
<td></td>
<td>20-30</td>
<td>58.2</td>
<td>1.0</td>
<td>26.0</td>
<td>7.5</td>
<td>76.8</td>
<td>280.2</td>
<td>62.1</td>
<td>4.2</td>
<td>3.8</td>
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<tr>
<td></td>
<td>30-40</td>
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<td>54.6</td>
<td>13.0</td>
<td>75.8</td>
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<td>4.3</td>
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<td>R(^2)</td>
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<td>0.90</td>
<td>0.93</td>
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<td>0.01</td>
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<td>12.9</td>
<td>88.7</td>
<td>119.9</td>
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<td>107.4</td>
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</tr>
<tr>
<td></td>
<td>30-40</td>
<td>926.8</td>
<td>191.9</td>
<td>132.3</td>
<td>354.9</td>
<td>138.0</td>
<td>221.8</td>
<td>120.0</td>
<td>8.0</td>
<td>4.3</td>
<td>4.8</td>
<td>1.3</td>
<td>35.9</td>
</tr>
<tr>
<td></td>
<td>40-50</td>
<td>268.6</td>
<td>163.3</td>
<td>94.2</td>
<td>78.7</td>
<td>86.9</td>
<td>214.4</td>
<td>17.4</td>
<td>32.7</td>
<td>2.3</td>
<td>8.3</td>
<td>1.4</td>
<td>11.2</td>
</tr>
<tr>
<td></td>
<td>R(^2)</td>
<td>-</td>
<td>0.52</td>
<td>0.41</td>
<td>0.01</td>
<td>0.27</td>
<td>0.12</td>
<td>0.14</td>
<td>0.00</td>
<td>0.00</td>
<td>0.19</td>
<td>0.17</td>
<td>0.09</td>
</tr>
</tbody>
</table>
It should be noted that major element analysis shows the SiO$_2$ concentration was higher in the top 0-10 cm of soil compared to the bottom 40-50 cm at both sites. SiO$_2$ was 56.5 wt% at the top compared to the 40.5 wt% in the bottom in core TL-1 and 28.6 wt% at the top compared to the 8.3 wt% in the bottom in core FF-2. This could indicate an influx of sediment at the surface at both sites. Indeed at both sites the soil profiles were developed on a distinctive grey clay, possibly lacustrine in origin. The CaO concentration was lower in the top 0-10 cm of soil compared to the bottom 40-50 cm which could be an influence of the underlying lithology, in both cases a dark muddy limestone. CaO was 3.1 wt% at the top compared to 18.8 wt% at the bottom at site TL-1 and 3.2 wt% at the top compared to 32.7 wt% at the bottom in site FF-2. In site TL-1, the total organic carbon (TOC) correlated with the selenium with an $R^2$ value of 0.96 and a concentration of 15.1 wt% in the seleniferous horizon (figure 4.4). In core FF-2 the TOC was much higher overall and reached a maximum of 37.3 wt% at 30-40 cm although this did correlate directly with selenium (figure 4.5). X-ray diffraction analysis shows the dominant crystalline mineral in both cores was quartz. However, TL-1 revealed minor amounts of muscovite and clinochlore whereas FF-2 had minor amounts of dolomite, microcline, muscovite and calcite. Sub-samples from the most seleniferous horizon of both soil cores were selected for further analysis by XAS to determine the speciation.

4.3.3. Bulk Chemistry of Se in Grass Species and White Clover (Non-accumulators)

The concentration of Se, along with that of Mo, As, U and V, was determined in grass shoot and root material collected from the five different dairy cattle pasture sites TL, FF, MO, PK and TJ. In sample TL-1, white clover was mixed within the grass species and was separated into leaf, stem and root material and also analysed. The ICP-MS data in tables 4.3 and 4.4 show that the highest selenium concentrations in the non-accumulator plants analysed were in the white clover leaves of TL-1 with 296.15 mg·kg$^{-1}$ followed by the stem with 249.48 mg·kg$^{-1}$ and the roots with 150.44 mg·kg$^{-1}$. This was significantly higher than the concentration in the grass material from the same location with 48.89 mg·kg$^{-1}$ in the shoots and 67.86 mg·kg$^{-1}$ in the roots which indicates white clover is more efficient in the uptake of selenium.
However, As, U and V concentrations were <1 mg·kg\(^{-1}\) in the leaf and stem material and <5 mg·kg\(^{-1}\) in the root material despite elevated concentrations in the underlying soil.

In general, selenium was most concentrated in the root material of the grass samples compared to the shoot material with the exception of sample FF-2. The highest selenium concentration was 112.2 mg·kg\(^{-1}\) in the roots of site TL-2 which also contained 78.05 mg·kg\(^{-1}\) in the shoots. Interestingly, the concentrations in the soils collected from site TL were lower than FF, MO, and PK but the highest in the plant material. The lowest selenium concentration was observed in the grass shoots of sample PK-2 with 1.32 mg·kg\(^{-1}\) which also contained only 4.28 mg·kg\(^{-1}\) in the roots. In all grass samples, molybdenum was most concentrated in the shoot material compared to the root material, contrasting with Se. The highest molybdenum concentration was in the grass shoots of sample FF-2 with 79.63 mg·kg\(^{-1}\) which also contained 15.02 mg·kg\(^{-1}\) in the roots. In general, uranium was most concentrated in the grass root material compared to the shoot material with the exception of FF-2, similar distribution to selenium. The highest uranium concentration was in sample TJ-1 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 shoot material. The highest arsenic concentration was in sample FF-1 with 179.67 mg·kg\(^{-1}\) in the roots but contained only 0.30 mg·kg\(^{-1}\) in the shoots. The highest vanadium concentration was in sample PK-1 with 132.91 mg·kg\(^{-1}\) in the roots but contained only 1.84 mg·kg\(^{-1}\) in the shoots.

As the high concentrations of Se, Mo, As, U and V varied between the roots and shoots, the translocation factor (TF) was calculated (table 4.3 and 4.4) to determine the ability of the non-accumulator species to translocate the elements from the root to the shoot. In all 5 sites, the translocation factor in the grass samples was greatest for molybdenum with the lowest value of 1.24 at site PK-1 to the highest of 5.30 in site FF-2. With all values greater than 1, the grass species are more efficient in translocation of molybdenum from root to shoot compared to other elements.
Table 4.3. ICP-MS data showing the mean concentration of Se, Mo, As, U and V for triplicate selenium non-accumulator samples ± the standard error for site TL and TJ. TF = the translocation factor, a value >1 indicates the plant can translocate the element effectively from the root to the shoots or leaf and stem of the plant.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Material</th>
<th>82 Se mg kg⁻¹</th>
<th>95 Mo mg kg⁻¹</th>
<th>75 As mg kg⁻¹</th>
<th>238 U mg kg⁻¹</th>
<th>51 V mg kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>TL-1</td>
<td>Clover Leaf</td>
<td>296.15 ± 4.50</td>
<td>15.98 ± 0.25</td>
<td>0.27 ± 0.01</td>
<td>0.05 ± 0.00</td>
<td>0.70 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>Clover Stem</td>
<td>249.58 ± 7.20</td>
<td>17.27 ± 0.29</td>
<td>0.23 ± 0.00</td>
<td>0.03 ± 0.00</td>
<td>0.38 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>Clover Root</td>
<td>150.44 ± 16.19</td>
<td>4.89 ± 0.25</td>
<td>1.54 ± 0.05</td>
<td>0.34 ± 0.01</td>
<td>4.63 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>TF = Leaf/Root</td>
<td>1.97</td>
<td>3.27</td>
<td>0.18</td>
<td>0.14</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>TF = Stem/Root</td>
<td>1.66</td>
<td>3.53</td>
<td>0.15</td>
<td>0.09</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Grass Shoot</td>
<td>44.89 ± 7.23</td>
<td>6.20 ± 0.05</td>
<td>0.39 ± 0.02</td>
<td>0.05 ± 0.00</td>
<td>0.92 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Grass Root</td>
<td>67.86 ± 2.57</td>
<td>4.59 ± 0.49</td>
<td>30.10 ± 0.77</td>
<td>1.40 ± 0.04</td>
<td>37.69 ± 1.40</td>
</tr>
<tr>
<td></td>
<td>TF = Shoot/Root</td>
<td>0.66</td>
<td>1.35</td>
<td>0.01</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>TL-2</td>
<td>Grass Shoot</td>
<td>78.05 ± 7.81</td>
<td>3.19 ± 0.49</td>
<td>1.24 ± 0.08</td>
<td>0.18 ± 0.00</td>
<td>4.45 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>Grass Root</td>
<td>112.10 ± 1.72</td>
<td>1.80 ± 0.03</td>
<td>6.64 ± 0.28</td>
<td>0.79 ± 0.03</td>
<td>18.58 ± 2.24</td>
</tr>
<tr>
<td></td>
<td>TF = Shoot/Root</td>
<td>0.70</td>
<td>1.77</td>
<td>0.19</td>
<td>0.23</td>
<td>0.24</td>
</tr>
<tr>
<td>TJ-1</td>
<td>Grass Shoot</td>
<td>5.77 ± 0.67</td>
<td>11.59 ± 0.95</td>
<td>0.66 ± 0.06</td>
<td>0.47 ± 0.04</td>
<td>2.29 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>Grass Root</td>
<td>17.27 ± 0.16</td>
<td>6.94 ± 0.05</td>
<td>10.46 ± 0.11</td>
<td>6.18 ± 0.04</td>
<td>30.89 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>TF = Shoot/Root</td>
<td>0.33</td>
<td>1.67</td>
<td>0.06</td>
<td>0.08</td>
<td>0.07</td>
</tr>
<tr>
<td>TJ-2</td>
<td>Grass Shoot</td>
<td>1.19 ± 0.07</td>
<td>13.68 ± 0.07</td>
<td>0.21 ± 0.00</td>
<td>0.12 ± 0.00</td>
<td>0.64 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Grass Root</td>
<td>5.09 ± 0.06</td>
<td>4.14 ± 0.02</td>
<td>19.72 ± 0.15</td>
<td>3.97 ± 0.02</td>
<td>11.17 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>TF = Shoot/Root</td>
<td>0.23</td>
<td>3.30</td>
<td>0.01</td>
<td>0.03</td>
<td>0.06</td>
</tr>
</tbody>
</table>
Table 4.4. ICP-MS data showing the mean concentration of Se, Mo, As, U and V for triplicate selenium non-accumulator samples ± the standard error for site FF, PK and MO. TF= the translocation factor, a value >1 indicates the plant can translocate the element effectively from the root to the shoots of the plant.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Material</th>
<th>Se</th>
<th>Mo</th>
<th>As</th>
<th>U</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg kg(^{-1})</td>
<td>mg kg(^{-1})</td>
<td>mg kg(^{-1})</td>
<td>mg kg(^{-1})</td>
<td>mg kg(^{-1})</td>
</tr>
<tr>
<td>FF-1</td>
<td>Grass Shoot</td>
<td>4.13 ± 0.24</td>
<td>35.83 ± 0.47</td>
<td>0.30 ± 0.03</td>
<td>0.11 ± 0.00</td>
<td>1.40 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>Grass Root</td>
<td>22.31 ± 2.51</td>
<td>14.62 ± 1.17</td>
<td>179.67 ± 17.18</td>
<td>0.76 ± 0.07</td>
<td>50.19 ± 4.89</td>
</tr>
<tr>
<td></td>
<td>TF = Shoot/Root</td>
<td>0.19</td>
<td>2.45</td>
<td>0.00</td>
<td>0.15</td>
<td>0.03</td>
</tr>
<tr>
<td>FF-2</td>
<td>Grass Shoot</td>
<td>33.80 ± 2.15</td>
<td>79.63 ± 1.13</td>
<td>0.83 ± 0.01</td>
<td>4.41 ± 0.05</td>
<td>2.55 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Grass Root</td>
<td>26.29 ± 0.13</td>
<td>15.02 ± 0.21</td>
<td>4.15 ± 0.06</td>
<td>4.10 ± 0.04</td>
<td>27.29 ± 0.28</td>
</tr>
<tr>
<td></td>
<td>TF = Shoot/Root</td>
<td>1.29</td>
<td>5.30</td>
<td>0.20</td>
<td>1.07</td>
<td>0.09</td>
</tr>
<tr>
<td>PK-1</td>
<td>Grass Shoot</td>
<td>3.93 ± 0.21</td>
<td>23.24 ± 0.13</td>
<td>0.63 ± 0.02</td>
<td>0.41 ± 0.00</td>
<td>1.84 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>Grass Root</td>
<td>31.10 ± 0.17</td>
<td>18.79 ± 0.11</td>
<td>26.98 ± 0.12</td>
<td>4.02 ± 0.02</td>
<td>132.91 ± 0.76</td>
</tr>
<tr>
<td></td>
<td>TF = Shoot/Root</td>
<td>0.13</td>
<td>1.24</td>
<td>0.02</td>
<td>0.10</td>
<td>0.01</td>
</tr>
<tr>
<td>PK-2</td>
<td>Grass Shoot</td>
<td>1.32 ± 0.12</td>
<td>14.76 ± 0.14</td>
<td>0.78 ± 0.02</td>
<td>0.42 ± 0.01</td>
<td>7.34 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>Grass Root</td>
<td>4.28 ± 0.22</td>
<td>5.52 ± 0.17</td>
<td>3.05 ± 0.12</td>
<td>0.95 ± 0.04</td>
<td>30.46 ± 1.03</td>
</tr>
<tr>
<td></td>
<td>TF = Shoot/Root</td>
<td>0.31</td>
<td>2.67</td>
<td>0.26</td>
<td>0.45</td>
<td>0.24</td>
</tr>
<tr>
<td>MO-1</td>
<td>Grass Shoot</td>
<td>48.25 ± 0.63</td>
<td>55.69 ± 0.47</td>
<td>0.30 ± 0.02</td>
<td>1.02 ± 0.04</td>
<td>0.84 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>Grass Root</td>
<td>84.27 ± 1.21</td>
<td>13.16 ± 0.55</td>
<td>1.17 ± 0.11</td>
<td>1.11 ± 0.02</td>
<td>1.50 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>TF = Shoot/Root</td>
<td>0.57</td>
<td>4.23</td>
<td>0.26</td>
<td>0.92</td>
<td>0.56</td>
</tr>
</tbody>
</table>
Table 4.5. ICP-MS data showing the mean concentration of Se, Mo, As, U and V for triplicate Brassica samples ± the standard error from a growing experiment at site FF-2. TF = the translocation factor, a value >1 indicates the plant can translocate the element effectively from the root to the leaf and stem parts of the plant.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Material</th>
<th>82 Se mg kg(^{-1})</th>
<th>95 Mo mg kg(^{-1})</th>
<th>75 As mg kg(^{-1})</th>
<th>238 U mg kg(^{-1})</th>
<th>51 V mg kg(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>FF-3</td>
<td>Kale Leaf</td>
<td>971.72 ± 18.08</td>
<td>1000.35 ± 14.91</td>
<td>0.20 ± 0.01</td>
<td>0.63 ± 0.04</td>
<td>0.37 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>Kale Stem</td>
<td>394.28 ± 2.04</td>
<td>181.23 ± 2.78</td>
<td>0.16 ± 0.02</td>
<td>0.29 ± 0.06</td>
<td>0.15 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>Kale Root</td>
<td>570.59 ± 6.38</td>
<td>309.18 ± 3.09</td>
<td>0.47 ± 0.01</td>
<td>4.74 ± 0.19</td>
<td>1.11 ± 0.05</td>
</tr>
<tr>
<td>TF = Leaf/Root</td>
<td>1.70</td>
<td>3.24</td>
<td>0.43</td>
<td>0.13</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>TF = Stem/Root</td>
<td>0.69</td>
<td>0.59</td>
<td>0.34</td>
<td>0.06</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hispi Cabbage Leaf</td>
<td>826.47 ± 10.72</td>
<td>443.24 ± 3.41</td>
<td>0.22 ± 0.01</td>
<td>0.93 ± 0.05</td>
<td>0.39 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>Hispi Cabbage Stem</td>
<td>539.87 ± 2.63</td>
<td>260.91 ± 2.53</td>
<td>0.36 ± 0.01</td>
<td>0.38 ± 0.02</td>
<td>0.13 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>Hispi Cabbage Root</td>
<td>643.00 ± 8.47</td>
<td>288.63 ± 1.78</td>
<td>0.76 ± 0.01</td>
<td>9.38 ± 0.29</td>
<td>0.83 ± 0.03</td>
</tr>
<tr>
<td>TF = Leaf/Root</td>
<td>1.29</td>
<td>1.54</td>
<td>0.29</td>
<td>0.10</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>TF = Stem/Root</td>
<td>0.84</td>
<td>0.90</td>
<td>0.48</td>
<td>0.04</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Red Cabbage Leaf</td>
<td>787.75 ± 42.31</td>
<td>271.61 ± 13.45</td>
<td>0.03 ± 0.03</td>
<td>0.20 ± 0.02</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Red Cabbage Stem</td>
<td>336.15 ± 8.38</td>
<td>162.78 ± 0.39</td>
<td>0.26 ± 0.01</td>
<td>0.32 ± 0.00</td>
<td>0.13 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>Red Cabbage Root</td>
<td>389.97 ± 6.45</td>
<td>187.55 ± 1.98</td>
<td>0.53 ± 0.01</td>
<td>4.60 ± 0.22</td>
<td>0.57 ± 0.04</td>
</tr>
<tr>
<td>TF = Leaf/Root</td>
<td>2.02</td>
<td>1.45</td>
<td>0.06</td>
<td>0.04</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>TF = Stem/Root</td>
<td>0.86</td>
<td>0.87</td>
<td>0.50</td>
<td>0.07</td>
<td>0.23</td>
<td></td>
</tr>
</tbody>
</table>
The translocation factor for selenium was below 1 in all grass samples apart from location FF-2 which had a value of 1.29. However, the highest translocation factor for selenium was found in the white clover sample from site TL-1, shown in table 4.3. The translocation from root to leaf was 2.01 and the translocation from root to stem was 1.71 which suggests white clover may be useful for phytoextraction as translocation is efficient. The translocation factor for arsenic did not exceed 0.26 and vanadium did not exceed 0.56 indicating poor translocation. The translocation factor for uranium was below 1 with the exception of location FF-2. As the translocation factor was highest in the non-accumulators sampled from TL-1 and FF-2, these samples were selected for further analysis by X-ray Absorption Spectroscopy (XAS).

4.3.4. Bulk concentration of Se in Brassicas (Se accumulator)

The concentration of Se, Mo, As, U and V were determined by ICP-MS in three Brassica oleracea varieties (hispi cabbage, red flare cabbage and premier kale) grown on a 4x3 m experimental plot at site FF is shown in table 4.5. In all 3 Brassica plants the selenium was most concentrated in the leaves followed by the root material and stem material which differs from the non-accumulator white clover which was lowest in the root. The selenium concentration in the leaves ranged from 971.72 mg-kg\(^{-1}\) in the kale to 787.75 mg-kg\(^{-1}\) in the red flare cabbage. The concentration in the roots ranged from 643 mg-kg\(^{-1}\) in the hispi cabbage to 389.97 mg-kg\(^{-1}\) in the red flare cabbage. The concentration in the stem ranges from 539.87 mg-kg\(^{-1}\) in the hispi cabbage to 336.15 mg-kg\(^{-1}\) in the red flare cabbage. In all 3 Brassica plants, molybdenum was most concentrated in the leaves followed by the root material and then stem material, similar to selenium. The concentration in the leaves ranged from 1000.35 mg-kg\(^{-1}\) in the kale to 271.61 mg-kg\(^{-1}\) in the red flare cabbage. Similar to the non-accumulator plants, the uptake of arsenic, vanadium and uranium was lower than selenium and molybdenum. The highest concentration of arsenic was 0.76 mg-kg\(^{-1}\) in the roots of the hispi cabbage, uranium was 9.38 mg-kg\(^{-1}\) in the roots of hispi cabbage and vanadium was 1.11 mg-kg\(^{-1}\) in the roots of kale. The translocation factor from the root to leaf ranged from 1.29 – 2.02 for selenium which indicates the plants are effective in phytoextraction. Similar to the
non-accumulator species, the translocation of molybdenum was more efficient than Se, with a translocation factor from root to leaf of 1.45-3.24. The translocation factors for arsenic, uranium and vanadium were all below 1. Samples were selected from the premier kale and hispi cabbage for further analysis by X-ray absorption spectroscopy to compare the selenium species to the non-accumulator plants.

4.3.5. *Micro-focus X-Ray Fluorescence (μ-XRF) and X-ray Absorption Spectroscopy*

Cryosectioned samples of Se non-accumulator plants (tall fescue grass and white clover), Se accumulator plants (premier kale and hispi cabbage) and seleniferous soil from site TL-1 and site FF-2 at 30-40 cm depth were selected for μ-XRF and speciation analysis by μ-XAS. The XANES from selected points of interest from μ-XRF maps were compared to a range of selenium standards (figure 4.6). Standards chosen represent species likely to be present in both plant and soil material and representing potential molecular Se-coordination environments.

Figure 4.6. Normalised XANES spectra for both inorganic and organic selenium standards (Se K-edge) in the oxidation states -II, 0, IV and VI (see text).
The Se K-edge energies for reference standards, based on the maximum of 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 (C$_5$H$_{11}$NO$_2$Se, Se$^{II}$), 12657 eV for red elemental selenium (Se$^0$), 12657 eV for sodium selenide (Na$_2$Se, Se$^{II}$) and 12656 eV for selenocystine (C$_6$H$_{12}$N$_2$O$_4$Se$_2$, Se$^{II}$). Tables 4.6-4.8 show the results of the linear combination fitting for selenium species in plant and soil samples completed on merged duplicates of the normalised spectra, using ATHENA software.

**Soil Selenium Species**

The linear combination fits shown in table 4.6 for the seleniferous soil horizon (30-40 cm) sampled from site TL-1 showed elemental selenium was the most abundant selenium species with 63-89% identified in the 3 POI. The µ-XRF map (figure 4.7) shows a cluster of selenium with high counts, represented by POI-3 which contained 81% elemental selenium. The fit was improved with addition of 19% selenide to achieve a low R-factor of 0.003. The µ-XRF map shows areas of lower Se concentration which are more dispersed within the soil material and represented by POI-2 and POI-4. The fits were improved with the addition of the organic species SeCys$_2$ in POI-4 and both SeCys$_2$ and SeMet in POI-2, in combination with elemental selenium. In site FF-2, elemental selenium was the most abundant in POI-1 with 92% followed by POI-2 with 76%. The µ-XRF map shows POI-1 is also in a cluster of high selenium counts and the fit was improved with 8% selenide to achieve an R-factor of 0.008, similar to POI-3 in site TL-1. However, SeMet was the most abundant in POI-4 with 71% and POI-3 with 76%. The fit for POI-3 was improved with 24% SeCys$_2$ which suggests it is a purely organic species however the R-factor was slightly higher than the other POI with 0.02. In both soil samples, there was no correlation of selenium with the other elements identified within the sample. The soil samples were abundant in iron however the µ-XRF maps show the areas of high iron counts do not correlate with selenium (supplementary figure 8.1).
Figure 4.7. $\mu$-XRF maps showing selenium in a section through a soil pellet with the selected points of interest (POI) for Se $K$-edge XANES analysis labelled 1-4 in soil FF-1 (left) and labelled 1-3 in soil MO-1 (top). The $\mu$-XRF is on a ‘pseudo-temperature’ scale with high Se counts in red and areas with no counts in dark blue. Bottom: Normalised Se $K$-edge XANES spectra showing the selenium species in selected POI, the red elemental Se and SeMet Standards.
Table 4.6. Linear combination fits (%) of the Se K-edge XANES spectra for selected points of interest (POI) in the μ-XRF maps of the seleniferous horizon (30-40 cm) in soil TL-1 and FF-1 measured using ATHENA software. The goodness of fit is determined by the R-Factor and Reduced Chi squared measured using ATHENA software and the lower the value the better the fit.

<table>
<thead>
<tr>
<th>Sample</th>
<th>POI</th>
<th>SeCys$_2$</th>
<th>SeMet</th>
<th>Selenide</th>
<th>Elemental Se</th>
<th>Selenite</th>
<th>Selenate</th>
<th>R-Factor</th>
<th>Reduced chi-square</th>
</tr>
</thead>
<tbody>
<tr>
<td>TL-1 Soil</td>
<td>1</td>
<td>11</td>
<td>-</td>
<td>-</td>
<td>89</td>
<td>-</td>
<td>-</td>
<td>0.008</td>
<td>0.002</td>
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<td></td>
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<td>32</td>
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<td>63</td>
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<td>-</td>
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<td>0.001</td>
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<td>-</td>
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<td>92</td>
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<td>-</td>
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<td>0.003</td>
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<td>-</td>
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<td>76</td>
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<td>0.001</td>
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<td>-</td>
<td>71</td>
<td>-</td>
<td>18</td>
<td>11</td>
<td>-</td>
<td>0.007</td>
<td>0.002</td>
</tr>
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</table>
**Se Non-Accumulator Species**

The linear combination fits for the selenium non-accumulator plants showed selenomethionine (SeMet) was the dominant selenium species in the leaf, stem and root material analysed (table 4.7). In the tall fescue grass blade analysed from site TL-1 SeMet ranged from 88-92%, whereas in the grass blades collected from site FF-2 SeMet abundance was lower and ranged from 75%-79%. The µ-XRF maps in figure 4.8 show the highest selenium counts were observed in the parallel veins of the grass blades from both sites. In site TL-1, the SeMet value was 92% on the parallel vein of the grass blade (POI-1). The fit was improved with the addition of minor amounts of selenite and selenate species to achieve a low R-factor of 0.005. In grass sample FF-2, the highest SeMet value was 79% also on the parallel veins (POI-2). The fit was improved with the addition of 8% of the organic species selenocystine (SeCys₂) and the inorganic species selenite (9%) and selenate (4%) to achieve a low R-factor of 0.005. In the clover leaf collected from site TL-1 the abundance of SeMet was slightly lower than in the grass blades from the same location ranging from 76%-84%. The µ-XRF map in figure 4.9 shows the highest selenium counts were observed at POI-4 in the midrib of the white clover leaf sample with 82% SeMet. The fit was also improved with the addition of 8% SeCys₂ followed by selenite (7%) and selenate (3%) to achieve a low R-factor of 0.005. However, the stem of the white clover had a slightly higher SeMet abundance ranging from 85-88%. High selenium counts in the µ-XRF map were observed at POI-1 located in the phloem of the stem which had 87% SeMet. The fit was also improved with the addition of 9% selenite and 4% selenate to achieve an R-factor of 0.009.
Figure 4.8. Top: µ-XRF maps showing selenium in grass blades from sites TL-1 and FF-2 and the selected points of interest (POI) 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 with no counts in dark blue. Bottom: Normalised Se K-edge XANES spectra showing the selenium species in selected POI and the SeMet Standard.
Figure 4.9. μ-XRF maps showing selenium in a white clover leaf (right) with selected points of interest (POI) for Se K-edge XANES analysis labelled 1-4 and a cross section through a white clover stem (bottom) with selected POI labelled 1-3. The μ-XRF is on a ‘pseudo-temperature’ scale with high Se counts in red and areas with no counts in dark blue. Left: Normalised Se K-edge XANES spectra showing the selenium species in selected POI and the SeMet Standard.
Table 4.7. Linear combination fits (%) of Se K-edge XANES spectra for selected points of interest (POI) in the µ-XRF maps of the Se non-accumulator plants measured using ATHENA software. The goodness of fit is determined by the R-Factor and Reduced Chi squared and the lower the value the better the fit.

<table>
<thead>
<tr>
<th>Sample</th>
<th>POI</th>
<th>SeCys₂</th>
<th>SeMet</th>
<th>Selenide</th>
<th>Elemental Se</th>
<th>Selenite</th>
<th>Selenate</th>
<th>R-Factor</th>
<th>Reduced chi-square</th>
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<td>0.005</td>
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<td>8</td>
<td>79</td>
<td>-</td>
<td>-</td>
<td>9</td>
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<td>0.005</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>7</td>
<td>79</td>
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<td>-</td>
<td>10</td>
<td>4</td>
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<td>0.002</td>
</tr>
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<td>92</td>
<td>-</td>
<td>-</td>
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<td>0.005</td>
<td>0.001</td>
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<td>4</td>
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<td>0.002</td>
</tr>
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<td>-</td>
<td>84</td>
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<td>82</td>
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<td>-</td>
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<td>0.002</td>
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</tbody>
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87
*Se Accumulator Species*

Similar to the Se non-accumulator plants, SeMet was the most abundant species in the leaf, stem and root material in both *Brassica* plants analysed. In the premier kale samples, SeMet ranged from 76-80% in the leaf material 67-72% in the stem material and 83-81% in the root material (table 4.8). The µ-XRF map in figure 4.10 showing a cross section through a kale stem has a Se-hotspot within the cortex (POI-3) with a SeMet value of 67%. The fit was improved with 18% selenite, 11% SeCys₂ and 4% selenite to achieve an r-factor of 0.005. The slight oxidation of the selenium species in POI-3 could be caused by the close proximity to an air pocket in the stem. In contrast, the stem of the hispi cabbage sample contained no SeCys₂ in the linear combination fit, similar to the white clover stem. In the cabbage samples, SeMet ranged from 70-84% in the leaf material, 87-95% in the stem material and 76-81% in the root material. The µ-XRF maps for both the hispi cabbage and kale showing a cross-section through a root have high selenium counts towards the epidermis (figure 4.10 and 4.11). POI-1 in the kale root contained 83% SeMet with an r-factor of 0.003 and POI-3 in the cabbage contained 81% SeMet with an r-factor of 0.005 and both fits included SeCys₂, selenite and selenite. Comparison of the XANES spectra for all the Se accumulator and non-accumulator samples with the SeMet standard showed that the white line absorbance peak is much lower and the maximum of the first derivative ranged from 12657.5 eV to 12658 eV which could indicate a lower SeMet concentration or breakage of C-Se bonds.
Figure 4.10. Left: μ-XRF maps showing selenium in a cross section of a premier kale leaf vein, stem and root with selected points of interest (POI) 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 with no counts in dark blue. Right: Normalised Se K-edge XANES spectra showing the selenium species in selected POI and the SeMet Standard.
Figure 4.11. Top: µ-XRF maps showing selenium in a hispi cabbage leaf, cross section through a stem and root with selected points of interest (POI) 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 with no counts in dark blue. Bottom: Normalised Se K-edge XANES spectra showing selenium species in the selected POI and the SeMet Standard.
Table 4.8. Linear combination fits (%) of the Se K-edge XANES spectra for selected points of interest (POI) in the μ-XRF maps of *Brassica oleracea* (Se accumulator plants) from site FF measured using ATHENA. The goodness of fit is determined by the R-Factor and Reduced Chi squared and the lower the value the better the fit.

<table>
<thead>
<tr>
<th>Sample</th>
<th>POI</th>
<th>SeCys$_2$</th>
<th>SeMet</th>
<th>Selenide</th>
<th>Elemental Se</th>
<th>Selenite</th>
<th>Selenate</th>
<th>R-Factor</th>
<th>Reduced chi-square</th>
</tr>
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<td>12</td>
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<td>-</td>
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<td>76</td>
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<td>11</td>
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<td>81</td>
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<td>0.005</td>
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4.4. Discussion

4.4.1. Selenium Distribution and Concentration in Soils

The average selenium concentrations observed in the soils collected from 5 cattle pasture sites (TL, TJ, FF, MO, PK) in County Limerick were all far above the global soil average concentration of 0.44 mg kg\(^{-1}\) (Fordyce, 2007) and the trigger action value (TAV) for agricultural land of 3-10 mg kg\(^{-1}\) (table 4.1) (Kabata-Pendias, 2011). All sites can be defined as ‘seleniferous’ as the maximum soil values were well within the world’s seleniferous range of 1-1200 mg kg\(^{-1}\) with the exception of a sample in site MO which had a concentration of 1265.8 mg kg\(^{-1}\) which even exceeded the world’s seleniferous value (Fernández-Martínez and Charlet, 2009; Jacobs, 1989; Neal, 1995). However, Site TL, TJ and FF also contained areas of soil with concentrations <0.1 mg kg\(^{-1}\) which are considered selenium deficient (Dhillon and Dhillon, 2003). Therefore, the data collected shows the distribution of selenium in these agricultural soils is extremely heterogeneous, with small seleniferous hotspots in close proximity to low and deficient areas (figure 4.2 and 4.3). This supports the conclusions of McGrath and Fleming (2008) who stated that selenium would need to be mapped on a 0.01 km\(^{2}\) scale to determine the regional distribution. At present, selenium distribution in Limerick is mapped on a 10 km\(^{2}\) scale and is identified as in the deficient to low range (0.0501-0.75 mg kg\(^{-1}\)) which indicates seleniferous hotspots have been overlooked (Fay et al., 2007).

The average molybdenum concentrations were also well above the world soil average of 1.1 mg kg\(^{-1}\) with the exception of site TL. Average values for site TJ and PK were within the TAV range of 5-20 mg kg\(^{-1}\) and site FF and MO were more than 5 times the upper TAV value (Kabata-Pendias, 2011) and all can be categorised as molybdeniferous (>0.3 mg kg\(^{-1}\)) (McGrath and Fleming, 2008). A soil sample collected from site FF had a molybdenum concentration of 1627.5 mg kg\(^{-1}\), and with the highest selenium value represent some of the highest natural concentrations recorded worldwide. Previously the seleniferous soils in County Limerick have been shown to contain high molybdenum concentrations (Fleming and Walsh, 1956) and a similar general spatial distribution to selenium. These were on limited data sets
and the data presented here show the true nature of the Se and Mo distributions. In all sites studied, the average arsenic concentrations exceeded the world soil average of 6.83 mg kg\(^{-1}\) and were within the TAV of 10-65 mg kg\(^{-1}\). The average uranium concentrations also exceeded the world soil average of 3 mg kg\(^{-1}\) and concentrations were as high as 599.5 mg kg\(^{-1}\) in site FF. Site MO and PK also exceeded the world average value for vanadium of 129 mg kg\(^{-1}\) (Kabata-Pendias, 2011). What is evident is that these soils pose a serious risk to livestock health as these trace elements are bioavailable via the overlying perennial grass and white clover.

### 4.4.2. Selenium Distribution and Concentration in Pastures (Non-Accumulators)

The results of this study indicate that pastures containing a mixture of *Lolium perenne* (perennial ryegrass), *Festuca arundinacea* (tall fescue), *Dactylis glomerata* (cocksfoot) and *Phleum pretense* (timothy grass) can uptake both selenium and molybdenum while growing in seleniferous and molybdeniferous soils (table 4.4-4.5). In the grass shoots, 7 out of the 9 samples analysed exceeded the recommended limit in pastures of 2-5 mg kg\(^{-1}\) put in place to protect grazing livestock from toxicity (Terry et al., 2000; Wu, 2004; Wu et al., 1996a). Concentrations are as high as 78.05 mg kg\(^{-1}\) in the shoots of grass sample TL-1 which poses a risk of livestock developing chronic selenium toxicity related diseases such as alkali disease (Rogers et al., 1990). The risk is the chance that the environmental hazard (selenium) will cause harm to the animal. Selenium uptake and translocation was highest in the grass samples collected from site TL compared to any other site despite the higher concentrations of selenium observed in the soils collected from site FF, MO and PK (table 4.1). The white clover (*Trifolium repens L.*) collected from site TL-1 was even more efficient in selenium uptake and translocation (296.15 mg kg\(^{-1}\) in leaf) compared to the grass forage from the same location. The uptake of selenium in white clover was also observed in a study by Yuan et al. (2012) with 79.39 mg kg\(^{-1}\) in the leaves grown in the seleniferous soils of the Enshi district, China. Concentrations observed in this study suggest white clover could be categorised as a secondary selenium accumulator as concentrations are above 100 mg kg\(^{-1}\) (Brown and Carter, 1969; Terry et al., 2000) and selenium was
more concentrated in the leaf material compared to the roots. Therefore, white clover seeding should be avoided in pastures overlying seleniferous soils.

The translocation of molybdenum from grass root to shoot was higher than selenium in all sites analysed. Molybdenum concentrations were elevated in all sites apart from TL-1 which reflected the low concentrations in the soil (table 4.1). Grass shoots sampled from site FF, MO, TJ and PK all contained Mo concentrations which exceeded the maximum tolerable level for livestock of 5-10 mg kg\(^{-1}\) (NRC, 2005). In particular, sample FF-2 which contained almost 8 times the upper tolerable level with 79.63 mg kg\(^{-1}\) and therefore poses a very high risk to livestock health from molybdenosis. Arsenic, uranium and vanadium pose less of a risk to livestock health as these elements have a poor translocation from roots to shoots.

4.4.3. Selenium Bioaccumulation in Soil and Pastures

Selenium concentrations in plants largely reflect the concentrations in the soil environment (Fordyce, 2007) however, comparison of the selenium concentrations in grass samples TL-1 and FF-2 (table 4.3 and 4.4) with the underlying rhizosphere concentrations in the soil cores (table 4.2) shows that bioaccumulation in the grass was greatest at site TL-1 despite the lower soil concentration. Therefore, the uptake may be influenced by other bio-physio-chemical factors such as the soil pH, redox conditions, competitive ions, mineralogy or organic matter content (Fordyce, 2007). A bioaccumulation factor (BAF) >1 was determined for the accumulation of selenium in the roots from the soil in site TL-1 representing efficiency, whereas at site FF-2 the factor was <1. Although the concentration of selenium at 0-10 cm depth was lower in site TL-1, both sites exceed the TAV of 3–10 mg kg\(^{-1}\). In site FF-2, the molybdenum concentrations at 0-10 cm depth also exceed the TAV of 5-20 mg kg\(^{-1}\) (Kabata-Pendias, 2011) however, the concentration was below the TAV in core TL-1. The BAF for molybdenum in TL-1 was >5 indicating efficient plant uptake and therefore the very low rhizosphere concentrations may be a result of rapid removal of bioavailable molybdenum species from the soil. The BAF for molybdenum was also higher than selenium in site FF-2, although not as efficient as site TL-1.
Table 4.9. Bioaccumulation factor (BAF) calculated for site TL-1 and FF-2. BAF >1 indicates the grass is efficient in accumulating the trace element from the soil from 0-10 cm depth.

<table>
<thead>
<tr>
<th>Site</th>
<th>BAF</th>
<th>Se</th>
<th>Mo</th>
<th>As</th>
<th>U</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>TL-1</td>
<td>Grass Shoot/Soil</td>
<td>0.81</td>
<td>7.74</td>
<td>0.02</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Grass Root/Soil</td>
<td>1.23</td>
<td>5.74</td>
<td>1.19</td>
<td>0.19</td>
<td>0.46</td>
</tr>
<tr>
<td>FF-2</td>
<td>Grass Shoot/Soil</td>
<td>0.34</td>
<td>3.48</td>
<td>0.06</td>
<td>0.05</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Grass Root/Soil</td>
<td>0.27</td>
<td>0.66</td>
<td>0.32</td>
<td>0.05</td>
<td>0.23</td>
</tr>
</tbody>
</table>

As the BAF for site FF-2 was lower, this suggests the selenium is immobilised causing it to accumulate within the soil. This could be due to the high concentration of total organic carbon present in the first 40 cm of core FF-2 compared to site TL-1 (figure 4.5) as selenium is strongly associated with organic matter (OM) (Zhang and Moore, 1996). The most seleniferous horizon of soil TL-1 also contained a high TOC concentration (15 wt%) which could explain the accumulation at this depth. This is supported by a study by Johnsson (1991) which showed a decrease in selenium uptake when soil contained >11% OM, however soils which contained 5-11% OM showed an increase in selenium uptake. The presence of high levels of organic matter can stimulate the microbial reduction of selenium to less bioavailable species due to the increase in available electron donors (Zhang and Moore, 1996). However, high selenium accumulation in soil FF-2 may also be influenced by the abundance of calcite which was not present in the site TL-1. Selenium adsorption onto calcite is greater than to soil clays such as kaolonite and montmorillonite (Goldberg and Glaubig, 1988) and therefore calcite may be the host for selenium in soil profile FF-2.

4.4.4. Selenium Speciation and Bioavailability

The uptake of selenium in plants is also influenced by the presence of bioavailable species and XANES analysis showed that the most seleniferous horizon in site TL-1 and FF-1 were dominated by elemental selenium (Se⁰) which is unavailable for plant uptake and suggests microbial reduction processes are occurring in the soil causing selenium to become immobile at this depth horizon (Fernández-Martínez and Charlet, 2009). In both sites the LCF fits were improved with the reduced inorganic species selenide (Se⁻²), which is a volatile compound also formed in microbial
reduction processes, however the reduced organic selenium species SeCys$_2$ and SeMet which are bioavailable and associated with plant material (Fernández-Martínez and Charlet, 2009) were also needed to improve the fits. The presence of both mobile organic Se species and immobile inorganic Se species makes this a complex soil system. The µ-XRF maps show high areas of reduced inorganic selenium (Se$^0$ and Se$^{II}$) in both sites (POI-1 in FF-2 and POI-3 in TL-1) which could be selenium nanoparticles (50-100 nm size) as they are significantly more concentrated than the sorbed organic selenium which is more dispersed within the soil material. Se$^0$ nanoparticles can be produced in soils by microorganisms such as *Geobacter sulfurreducens*, *Shewanella oneidensis* and *Veillonella atypica* which reduce the highly bioavailable and toxic selenium oxyanions (Pearce et al., 2008; Pearce et al., 2009). Although Se$^0$ nanoparticles are associated with biological activity, the solubility and fate in natural soil systems are still poorly defined (Winkel et al., 2015).

The organic species SeMet was also dominant in the seleniferous soil horizon of site FF-1 and present to a lesser extent in TL-1, perhaps due to the lower total organic carbon content of the soil in TL-1. The XANES species analysis in both soil profiles is supported by the sequential extraction data for site TL and FF discussed elsewhere (chapter 6) which shows the elemental selenium fraction was highest, followed by the organic selenium fraction. Fellowes et al. (2013) also observed an organic selenium species within an organic-rich horizon of soil which resembled SeMet but the species could not be defined due to differences in the XANES post edge structure. The predominant Se species identified in the root and shoot material of the tall fescue grass and the leaf, stem and root material from the white clover was also SeMet (table 4.7) and therefore, the seleniferous soil horizons are high in decaying plant matter. SeMet is known to accumulate in the roots of plants which was observed in most of the grass samples (Zayed et al., 1998). SeMet can cause toxicity in plant tissues in high concentrations due to the incorporation into plant proteins in place of the sulfur occurring amino acids methionine (Met) (Brown and Shrift, 1982; Sors et al., 2005; Terry et al., 2000). Slight chlorosis of the tall fescue grass blades were observed (supplementary figure 8.3) which suggests
phytotoxicity from selenium. However, the LCF for the non-accumulator plants were not 100% SeMet and were improved with the addition of minor amounts of the oxyanions selenite and selenate, and with or without the organic species SeCys$_2$. The presence of only small amounts of selenium oxyanions may indicate limited oxidation in the rhizosphere.

4.4.5. Phytoextraction using Se Accumulators

As expected the accumulation of selenium was highest in the Brassica oleracea plants grown at site FF-2 (table 4.5) compared to the grass with similar concentrations in the kale, hispi cabbage and red flare cabbage as a study by (Bañuelos et al., 1997) on the Brassica juncea species, Indian mustard. The high Se concentrations of up to 971.72 mg kg$^{-1}$ in the leaves poses a huge risk to human health as this is the edible part of the plant and the intake limit to avoid toxicity is recommended as 400 µg$^{-1}$ per day (Fordyce, 2007; WHO, 1996). However, the ability of the Brassica species to accumulate both selenium and molybdenum in concentrations of up to nearly 1000 mg kg$^{-1}$ demonstrates how they can be effective for phytoremediation of seleniferous soils (Salt et al., 1995). The kale and cabbage accumulated selenium highest in the leaves which is likely caused by more translocation from root to shoot via xylem transport which differs from other non-accumulator plants (Brown and Shrift, 1982; Winkel et al., 2015).

The predominant phase identified in the selenium accumulator plants was also the organic selenium species SeMet (table 4.6). This suggests the ability of different plant species to accumulate and translocate selenium to different parts of the plant is not affected by the selenium species. Other factors such as the rate of transpiration or rate of xylem transport in different plant species may affect the accumulation (Renkema et al., 2012). However, SeMet has similar XANES spectra to the organic selenium species γ—glutamyl-se-methylselenocysteine (γ-GMeSeCys), selenocystathionine (SeCysth) and Se-methylselenocysteine (MeSeCys) and therefore it may be challenging to differentiate between these species. MeSeCys is often present in selenium hyperaccumulators plants as it is a selenium occurring amino acid that does not incorporate into proteins which prevents selenium toxicity.
in plant tissue (El Mehdawi and Pilon-Smits, 2011; Freeman et al., 2006). However, comparison of the standard XANES spectra for SeMet and MeSeCys in Ryser et al. (2005), show MeSeCys has K Edge energy 0.5 eV higher than SeMet and therefore it is unlikely to be present in the Brassica samples analysed. Chlorosis of the leaves was also observed in the Brassica plants (supplementary figure 8.4) which suggests mild selenium phytotoxicity and supports the presence of SeMet. The hispi cabbage was most affected by chlorosis which had the highest selenium concentration in the roots compared to the premier kale and red flare cabbage. As the Brassica plants in this study can accumulate high concentrations of organic selenium they could be used for phytoextraction to increase dietary intake in selenium deficient areas.

4.5. Conclusions

This research shows the distribution of selenium and molybdenum in soils collected from 5 pasture sites from County Limerick is very inhomogeneous and spatially focused with concentrations ranging from deficient to highly toxic. The highest selenium concentration was 1265.8 mg kg\(^{-1}\) which even exceeds the world’s seleniferous concentration range and the highest molybdenum concentration was 1627.5 mg kg\(^{-1}\) which is highly molybdeniferous. Soil cores collected from site TL and FF show selenium is most concentrated at a depth horizon of 30-40 cm and is strongly associated with the total organic carbon. The high organic matter content and activity of microorganisms has caused selenium to accumulate in the soil in an immobile phase and XANES analysis showed elemental selenium was the predominant species. However, the presence of organic selenium species in the soil such as the amino acid SeMet poses a risk to livestock health as it is bioavailable for uptake in the overlying grass. As a result, grass species accumulated selenium in concentrations toxic to livestock health predominantly as SeMet. However, Se concentrations were highest mainly in the roots (up to 112.10 mg kg\(^{-1}\)). The presence of relatively minor selenium oxyanions in the plant material indicates limited oxidation in the rhizosphere.

The translocation of selenium from root to shoot was higher in the white clover plant compared to the grass from the same location. White clover accumulated
selenium highest in the leaves with concentrations of up to 296.15 mg kg\(^{-1}\) and could therefore be described as a Se accumulator species and should be avoided in seeding pastures overlying seleniferous soils. The translocation of molybdenum was more efficient than selenium in the non-accumulator species and therefore poses a risk of molybdenum poisoning in livestock. The translocation of selenium and molybdenum from root to shoot was also high in the Se accumulator species cabbage and kale, with concentrations of selenium up to 971.72 mg kg\(^{-1}\) and molybdenum of up to 1000.35 mg kg\(^{-1}\) in the leaves. The selenium species identified in the XANES was predominantly SeMet which may explain the chlorosis of the leaf tissue. The ability of the *Brassicas oleracea* plants to remove high concentrations of selenium from such a complex soil system is useful for phytoextraction which can be used to supplement deficient areas of the world.

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Chapter 5 – Research Paper 2

Microbial Transformations of Selenium Species in Toxic Irish Soils

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Abstract

Selenium (Se) is an essential element required by both humans and animals with the narrowest range between deficiency and toxicity than any other element. The biogeochemical cycling of selenium in the natural environment involves redox reactions which are controlled by the activity of microorganisms. A field area in West Limerick, Ireland, with historical selenium toxicity in livestock was identified with high concentrations of selenium (Se), molybdenum (Mo) and uranium (U) associated with the organic carbon in soils. Soil cores were collected from the site for chemical analysis and anaerobic samples collected at 10-20 cm depth for Se speciation, microcosm experiments and microbial community analysis. Selenium was present in soil MO-1 predominantly in a reduced phase as selenide (Se\textsuperscript{II}), elemental se (Se0) and organic species (Se\textsuperscript{II}). No selenite (Se\textsuperscript{IV}) and selenate (Se\textsuperscript{VI}) oxyanions were detected in the soil solutions and therefore the soluble phase is a bioavailable organic species such as SeMet which decreased in anaerobic conditions. Molybdenum was present in the soil predominantly as Mo\textsuperscript{IV} species associated with iron (Fe). The bioavailable species molybdate was identified in the soil solution which increased in anaerobic conditions with uranium which has implications for selenium remediation. Nitrate and microaerophillic conditions hindered selenium removal from solution and Fe\textsuperscript{III} reduction to Fe\textsuperscript{II}. However the microbial community remained relatively stable to changing physiochemical
conditions. This study provides information on selenium and molybdenum speciation and cycling in the natural environment which is important for agricultural remediation and prevention of selenium related diseases in livestock.

**Keywords:** Soil chemistry, selenium, molybdenum, speciation, microbial transformations.

### 5.1. Introduction

Selenium is an essential element required by both humans and animals for enzymatic and thus cellular processes, however, it has the narrowest range of any element between deficiency and toxicity (Fordyce, 2007; Stolz et al., 2006). The biogeochemical cycling of selenium in the natural environment involves redox reactions which are often controlled by the activity of microorganisms (Dowdle and Oremland, 1998; Herbel et al., 2003) and understanding these processes is important for the prevention of toxicity and deficiency related diseases. Microorganisms are known to tolerate high concentrations (and trace concentrations) of selenium in the environment (Chasteen and Bentley, 2002) and elevated selenium concentrations occur naturally in soils which are often also enriched with molybdenum (McGrath and Fleming, 2008). Microbial activity influences the chemical speciation and oxidation state of selenium in the soil and thus changes the bioavailability and toxicity to plants and herbivorous animals.

Microbial transformations include oxidation, reduction (assimilatory and dissimilatory), methylation and demethylation and these transformations may be concurrent (Dungan and Frankenberger, 1999). The oxidation of reduced selenium species Se$^0$ and Se$^{-II}$ to bioavailable selenium oxyanions Se$^{IV}$ and Se$^{VI}$ occurs at a much slower rate than reduction (Dowdle and Oremland, 1998) as reduced selenium species have shown to be more resistant to microbial activity regardless of the soil redox conditions (Fellowes et al., 2013). Assimilatory reduction occurs when selenium oxyanions are incorporated into organic compounds such as the amino acids selenomethionine (SeMet) and selenocysteine (SeCys) which are, however, still bioavailable to plants and, thus, animals. An important part of the
selenium cycle is the dissimilatory reduction and the methylation of bioavailable selenium species as these microbial reactions can be employed as remediation strategies in seleniferous environments (Dungan and Frankenberger, 1999).

In dissimilatory reduction, the selenate ($\text{Se}^{\text{VI}}$) and selenite ($\text{Se}^{\text{IV}}$) oxyanions can be used as an electron acceptor in microbial metabolism and reduced to less bioavailable forms such as elemental selenium ($\text{Se}^0$) (Dungan and Frankenberger, 1999). In anaerobic conditions, selenium can be further reduced to metal selenides ($\text{Se}^{\text{II}}$) which are immobile and therefore reduce Se toxicity (Pearce et al., 2009). Dissimilatory selenium reduction results in the production of elemental selenium ‘nanospheres’ which have found to have useful electro-optical properties and could be harvested for nanotechnological applications (Oremland et al., 2004; Pearce et al., 2009; Stolz and Oremland, 1999). The reduction of both selenium oxyanions has been observed on organic substrates using 16 different microorganisms (Dungan and Frankenberger, 1999; Oremland et al., 2004; Oremland et al., 1990). Selenium reduction can occur via sulfate or nitrate reducers and archaea (Lenz et al., 2008). Similarly, the molybdate ($\text{Mo}^{\text{VI}}$) anion which is the most oxidised and bioavailable form of molybdenum can be reduced by sulfate reducing bacteria such as $D$. desulfuricans (Lloyd, 2003; Tucker et al., 1996).

Methylation is thought to be a microbial mechanism which can detoxify and protect the surrounding environment (Dungan and Frankenberger, 1999) and could be implemented as a bioremediation strategy in seleniferous soils. Microbial methylation is the formation of volatile organic species such as dimethylselenide (Lenz and Lens, 2009) from both selenium oxyanions and organic selenium compounds present in Se-contaminated sediments, soil and water (Doran, 1982). There are 8 identified selenium volatising bacteria including $F$avobacterium sp, $P$seudomonas sp, $P$seudomonas fluorescens K27, Corynebacterium sp, Aeromonas sp, $R$hodocyclus tenuis, $R$hodospirillum rubrum and Aeromonas veronii (Dungan and Frankenberger, 1999). However, in the presence of high concentrations of heavy metals, the oxyanions nitrate ($\text{NO}_3^-$) and nitrite ($\text{NO}_2^-$) in concentrations of $>0.1\text{M}$ have been shown to inhibit selenium methylations by microorganisms (Dungan and Frankenberger, 1999; Thompson-Eagle and Frankenberger, 1990).
The remediation of selenium contaminated soils is difficult due to the inherently heterogeneous nature of soils in terms of physio-chemical conditions, and the presence of selenium in several oxidation states which are controlled by both biotic and abiotic reactions (Lenz and Lens, 2009). Similarly, the soil biogeochemistry of molybdenum is complex as the oxidation state can vary from Mo\textsuperscript{II} to Mo\textsuperscript{VI} in the environment (Xu et al., 2013) and thus Mo speciation depends on the same conditions as Se speciation. Natural, highly seleniferous and molybdeniferous soils occur in West Limerick, Ireland which have formed in low lying, poorly drained, mildly acidic to alkaline conditions and rich in organic matter (Fleming, 1961; Mcgrath and Fleming, 2008). The source of selenium and molybdenum is believed to be drainage from the local Carboniferous black shales which has led to a serious toxicity issue for livestock (Mcgrath and Fleming, 2008) in this major dairy producing part of Ireland.

The aim of this study was to characterise the behaviour of selenium and molybdenum in a horizon of natural soil from two well characterised sites in West Limerick where the levels of these elements are very high, up to 1265.8 mg kg\textsuperscript{-1} Se (see table 4.1, chapter 4), focusing on the relationship between soil geochemical conditions, Se speciation and the microbial community. To achieve these aims, the study employs four main experimental methodologies: (i) determine the bulk inorganic and organic chemistry of two soil cores, (ii) X-ray absorption spectroscopy (\(\mu\)-XAS) of the most seleniferous soil horizons to determine the distribution of Se and Mo species, (iii) microcosm experiments to observe Se and Mo mobility with changing bio-physiochemical conditions, (iv) DNA sequencing techniques to understand the microbial activity involved in selenium cycling in the natural environment.
5.2. Methodology

5.2.1. Field Site and Sampling Techniques

Prior to this study, soil samples were collected from agricultural sites across West Limerick Ireland at 10-20 cm depth to identify seleniferous and molybdeniferous areas (supplementary table 8.1-8.3) following information provided by Teagasc and in the literature (Fleming, 1961; Mcgrath and Fleming, 2008). Suitable soils for microbial studies with ‘toxic’ levels of Se and Mo were identified at the bottom of two gentle sloping grassed fields MO-1 and FF-1 as shown in figure 5.1. The sites are underlain by dark Carboniferous limestones; 500 m to the west relatively higher (15 m) ground is underlain by Carboniferous mudstones and shales (see chapter 6, figure 6.1), forming the catchment area for these two sites. Soil cores were sampled in each field for chemical analysis to determine the changes in selenium concentration with soil profile depth. The soil cores were collected using a Van Walt corer and transferred into tightly sealed polypropylene tubes prior to analysis.

Figure 5.1. Topographical map showing the location of soil samples taken in the field sites FF-1 and MO-1 which contained elevated Se and Mo concentrations. B-1 and B-2 were sampled upslope and contained <5 mg kg\(^{-1}\) Se. Green area indicates field boundaries.
For speciation analysis and microcosm experiments, the soil was collected at 10-20 cm depth by digging a small trench and the soil kept anaerobic by flushing the sampling container with oxygen-free nitrogen. The pH and Eh of the soil was taken in the field using 10 ml of deionised water, mixed with an equal volume of soil. For microbial ecology analysis, a further two soil samples B-1 and B-2 were taken in adjacent fields for comparison. The two samples were collected from a higher elevation where the soil was well drained and contained low concentrations (<5 mg kg\(^{-1}\)) of selenium and molybdenum. All soils were stored in the dark at 4°C to reduce microbial activity which affects selenium speciation, prior to analysis.

5.2.2. Soil Chemical Analysis

Soil samples were dried at 50°C, root material removed and the soil powdered (<50 μm) to homogenise the sample. Wax mounted pellets were prepared using 12 g of sample and 3 g of wax pressed under a 10 tonne load. The pellets were analysed by X-ray fluorescence (XRF) using a PANanalytical Axios sequential X-ray Fluorescence Spectrometer. The concentrations of elements (mg kg\(^{-1}\)) were determined by comparing the spectra peak intensities with known calibration standards and blank specimens using the pro-trace software. Omnian software was used to accurately calculate the major concentrations (wt%) which adjusts to sample and matrix effects. A certified reference material GBW 0731 - Chinese sediment (Se 0.20 ± 0.05 mg kg\(^{-1}\)) was used to validate the method. Another 1 g of soil was separated for loss on ignition analysis (LOI) by measuring the weight loss of the soil at 110°C to calculate the moisture loss (H\(_2\)Owt%) and then the carbon dioxide (CO\(_2\) wt%) released by heating to 1000°C, the latter defined the organic matter and carbonate present which is could be used in quantifying the major element concentrations. Another 1 g was used to calculate the total organic carbon (TOC) content of the soils by measuring the weight loss of the soil between 110°C and 400°C and applying a correction factor assuming 58% of organic matter contains organic carbon (Nelson and Sommers, 1996).
5.2.3. Synchrotron Techniques

Approximately 0.5 g of soil sample MO-1 at 10-20 cm depth was analysed by X-ray absorption spectroscopy (XAS) at Diamond Light Source, Didcot, UK to determine the distribution of the selenium and molybdenum species present. The sample was kept anaerobic and centrifuged at 12,000 rpm for 10 minutes. The pellet was frozen in liquid nitrogen and cut in a cross-section. The pellet was cryosectioned to 100 µm in an optimum cutting compound (OCT) matrix at -20°C and placed within a Kapton® tape parcel. The sample was sectioned in triplicate and transported in dry ice (-78.5°C) to the I18 microfocus spectroscopy beamline at Diamond Light Source. The frozen soil section was mounted onto the sample holder, loaded on an x-y-z stage and measured with a liquid helium cryostat at 10K, to avoid beam oxidation. Micro X-ray fluorescence maps (µ-XRF) of the soil were collected at 13.0 keV for selenium fluorescence and 20.4 keV for molybdenum fluorescence with a fixed beam size of 5 µm. Points of interest (POI) were chosen from the images displaying high and moderate molybdenum and selenium concentrations for X-ray absorption near-edge structure (XANES) analysis. Selenium and molybdenum standards were measured in transmission mode and included sodium selenide (Na₂Se, Se²⁻), selenocystine (C₆H₁₂N₂O₂Se₂; Se²⁻), selenomethionine (C₅H₁₁NO₂Se; Se²⁻), elemental selenium (Se₀), sodium selenite (Na₂SeO₃, Se⁴⁺), sodium selenate (Na₂SeO₄; Se⁶⁺), molybdite (MoO₃; Mo⁶⁺), ammoniumtetrathiomolybdate (MoS₄(NH₄)₂; Mo⁴⁺) molybdenumdisulfide (MoS₂; Mo⁴⁺) and molybdenumdithiocarbamate (Mo[S₂CN(C₂H₅)₂]₄; Mo⁴⁺). The µ-XRF data was processed into elemental images using PyMCA 5.2.2 software (Solé et al., 2007). Linear combination fitting was performed on the XANES spectra using Athena software to fit the data against standard spectra (Ravel et al., 2005).

5.2.4. Microcosm Experiment

A microcosm experiment was set up to assess the fate of selenium in soils MO-1 and FF-1 with changing physiochemical conditions. Approximately 20 g of soil from the 10-20 cm depth interval was placed into a 100 ml serum bottle with 40 ml of synthetic groundwater. The synthetic groundwater was produced by weighing salts
in mg L\(^{-1}\) of deionised water according to Wilkins et al. (2007): 0.006 KCl, 0.0976 MgSO\(_4\).7H\(_2\)O, 0.081 MgCl\(_2\).6H\(_2\)O, 0.1672 CaCO\(_3\), 0.0829 Na\(_2\)SiO\(_2\), 0.0275 NaNO\(_3\), 0.0094 NaCl, 0.2424 NaHCO\(_3\). The synthetic groundwater is based on the Drigg site in West Cumbria which differs from Irish groundwater concentrations. The microcosm experiment was set up with 4 different conditions: 1) anaerobic, 2) anaerobic with addition of 10 mM sodium nitrate, 3) anaerobic with addition of 10 mM sodium sulfate, 4) micro-aerophilic created by flushing 3 needles with 10 ml of sterilised air every 2 days. The microcosm experiment was carried out in triplicate with anaerobic autoclaved controls. Prior to sampling, the bottles were shaken to mix the sediment with the groundwater and 3.5 ml of soil suspension was removed with a sterilised 0.45 µm needle on days 0, 3, 7, 14, 21, 28 and 42.

5.2.5. Chemical Analysis of Microcosms

The pH and Eh of the soil suspension was measured, before taking a 100 µl sub-sample for ferrozine analysis to determine the concentration of ferrous iron (Fe\(^{II}\)) and total iron. For Fe\(^{II}\) the sub-sample was digested in 4.9 ml of 0.5 M HCl for 1 hour and 50 µl was mixed with 2.45 ml ferrozine in a cuvette and the absorbance of the solution analysed on a spectrophotometer at 562 nm. For total Fe, 200 µl of 6.25 M hydroxylamine (HAH) was added and left overnight before mixing with the ferrozine and measuring the absorbance again (Lovley and Phillips, 1988). Another sub-sample (2 ml) of the soil suspension was centrifuged for 5 minutes at 12,000 rpm, filtered with a 0.45 µm filter and 1.5 ml of the supernatant analysed for chemical elements using an inductively coupled plasma mass spectrometer (ICP-MS) Agilent 7500cx equipped with a pressurized octopole collision/reaction cell. The remaining 0.5 ml of supernatant was analysed using Ion chromatography (IC) using a Dionex ICS5000 for inorganic anions selenate, selenite, molybdate, sulfate, nitrate, nitrite, chloride, bromide and oxalate. To measure the precision of the chemical data, the mean and standard error was calculated for the triplicate microcosm samples. The remaining soil suspension was frozen at -80 °C at the end of the experiment for DNA analysis.
5.2.6. Microbial Ecology Analysis

Samples were selected on day 0 and 42 of the microcosm experiment for microbial ecology analysis, along with the two samples (B-1 and B-2) taken from an area upslope from the site, with low selenium concentrations. A PowerSoil DNA isolation kit (MO BIO Laboratories INC, Carlsbad, CA, USA) was used to extract DNA from 200 µl of soil slurry. The 16 rDNA gene region of the sample was amplified by polymerase chain reaction (PCR) using fluorescently labelled primers (5’-AGAGTTTGATCCTGCTAGCC-3’ and 1492R 5’-TACGGYTACCTTGTTACGACTT-3’) and methods according to (Rizoulis et al., 2016). The DNA was stained and observed in an agarose gel under UV light to identify base pair products. An Illumina® MiSeq platform (illumine, San Diego, CA, USA) was used to sequence PCR amplicons of 16S rRNA by targeting the V4 hyper variable region (forward primer, 515F, 5’-GTGYCAGCMGCCGCGGTAA-3’; reverse primer, 806R, 5’-GGACTACHVGGGTWTCTAAT-3’) for 2 × 250-bp paired-end sequencing (Illumina) (Caporaso et al., 2011). A Roche FastStart High Fidelity PCR system (Roche Diagnostics Ltd, Burges Hill, UK) was used to perform PCR amplification in 50 µl reactions. PCR products were purified and normalised using a SequelPrep Normalisation Kit (Fisher Scientific, Loughborough, UK) and the amplicons pooled in equimolar ratios and the run according to the method of (Kozich et al., 2013). The obtained sequences were analysed using Qiime (Caporaso et al., 2011) and trimming was performed using Cutadapt (Martin, 2011), FastQC1 and Sickle software (Joshi and Fass, 2011). Operational taxonomic units (OTUs) were classified at the 97% similarity level and taxonomic assignment conducted by the Ribosomal Database Project (RDP) classifier (Wang et al., 2007).

5.3. Results

5.3.1. Chemical Characterisation of the Soil Cores

Two soil cores were selected for analysis with the highest selenium (FF-1) and molybdenum (MO-1) concentrations from a study of agricultural pastures in County Limerick. Both soil cores were collected from the lowest lying land between gentle slopes in two adjacent fields separated by a small stream (figure 5.1). There is no
evidence of tillage in either field and the site was poorly drained. The soils were both dark brown and organic-rich and formed above a distinctive grey calcareous clay layer, possibly lacustrine in origin (see supplementary report 8.1). The soils were slightly acidic and reducing as MO-1 had a pH of 6.0 and an Eh of 126 mV, and FF-1 had a pH of 6.51 and an Eh of 100 mV measured at a depth horizon of 10-20 cm.

The XRF results in table 5.1 show that selenium is enriched in soil core MO-1 from 0-30 cm compared to the trigger action value (TAV) for agricultural soil of 3-10 mg kg\(^{-1}\) (Kabata-Pendias, 2011) and was highest at a depth horizon of 20-30 cm with 670.1 mg kg\(^{-1}\) selenium. The seleniferous soil horizon also contained 1081.6 mg kg\(^{-1}\) molybdenum, 79.4 mg kg\(^{-1}\) arsenic and 185.2 mg kg\(^{-1}\) uranium. Molybdenum was highly concentrated in the soil and showed a positive correlation with selenium (\(R^2\) value of 0.58). However, uranium had an \(R^2\) value of 0.99 and arsenic had an \(R^2\) of 0.91 indicating a very strong positive correlation with selenium throughout the soil profile. Soil profile FF-1 was 20 cm deeper than MO-1 and was enriched in selenium from 0-60 cm compared to the TAV. The selenium concentration was highest at a depth horizon of 10-20 cm with 945.2 mg kg\(^{-1}\) and this horizon also contained 252.7 mg kg\(^{-1}\) molybdenum, 221.6 mg kg\(^{-1}\) uranium and 45.3 mg kg\(^{-1}\) arsenic. Similar to site MO-1, molybdenum correlated positively with selenium in the soil profile of FF-1 with an \(R^2\) value of 0.52. However, uranium had an \(R^2\) value of 0.93 and arsenic had an \(R^2\) of 0.99 again indicating a very strong positive correlation with selenium throughout the soil profile.

It should be noted that major element analysis shows the concentration of SiO\(_2\) decreased with the depth of the soil profile from 40.6 wt% at 0-10 cm to 1.2 wt% at 50-60 cm in soil FF-1 which indicates an influx of clay or quartz-rich sediment, possible from the clastic sediments to the west. However the concentration of SiO\(_2\) in MO-1 increased from 34.3 wt% at 0-10 cm to 57.1 wt% at 30-40 cm. Both soil samples showed an increase in CaO with the depth of the soil profile from 3.7 wt% at 0-10 cm to 50 wt% at 50-60 cm in FF-1 and from 4.1 wt% at 0-10 cm to 10.7wt% at 30-40 cm in MO-1 which is an influence of the underlying dark muddy limestone bedrock.
Table 5.1. X-ray fluorescence data showing the concentration of major and trace elements in soil core MO-1 and FF-1. $R^2$ = the correlation of selenium with other elements in the soil profile where a value closer to 1 is the better fit to the regression line. TOC was calculated by the loss on ignition method.

<table>
<thead>
<tr>
<th>Site</th>
<th>Depth (cm)</th>
<th>Se (mg kg$^{-1}$)</th>
<th>Mo (mg kg$^{-1}$)</th>
<th>As (mg kg$^{-1}$)</th>
<th>U (mg kg$^{-1}$)</th>
<th>V (mg kg$^{-1}$)</th>
<th>Mn (mg kg$^{-1}$)</th>
<th>Cu (mg kg$^{-1}$)</th>
<th>CaO (wt%)</th>
<th>Fe$_2$O$_3$ (wt%)</th>
<th>SiO$_2$ (wt%)</th>
<th>Al$_2$O$_3$ (wt%)</th>
<th>TOC (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MO-1</td>
<td>0-10</td>
<td>381.2</td>
<td>152.5</td>
<td>22.8</td>
<td>129.3</td>
<td>57.2</td>
<td>163.0</td>
<td>83.6</td>
<td>4.1</td>
<td>6.0</td>
<td>34.3</td>
<td>5.0</td>
<td>23.1</td>
</tr>
<tr>
<td></td>
<td>10-20</td>
<td>504.5</td>
<td>400.9</td>
<td>53.0</td>
<td>159.0</td>
<td>102.6</td>
<td>111.8</td>
<td>125.0</td>
<td>5.5</td>
<td>8.9</td>
<td>34.6</td>
<td>4.9</td>
<td>19.9</td>
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<tr>
<td></td>
<td>20-30</td>
<td>670.1</td>
<td>1081.6</td>
<td>79.4</td>
<td>185.2</td>
<td>122.9</td>
<td>54.1</td>
<td>127.3</td>
<td>4.1</td>
<td>10.8</td>
<td>44.1</td>
<td>7.8</td>
<td>13.1</td>
</tr>
<tr>
<td></td>
<td>30-40</td>
<td>7.0</td>
<td>178.6</td>
<td>0.0</td>
<td>19.7</td>
<td>90.4</td>
<td>100.8</td>
<td>57.0</td>
<td>10.7</td>
<td>3.9</td>
<td>57.1</td>
<td>10.8</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>$R^2$</td>
<td>-</td>
<td>0.58</td>
<td>0.91</td>
<td>0.99</td>
<td>0.22</td>
<td>0.11</td>
<td>0.89</td>
<td>0.80</td>
<td>0.92</td>
<td>0.43</td>
<td>0.39</td>
<td>0.42</td>
</tr>
<tr>
<td>FF-1</td>
<td>0-10</td>
<td>186.2</td>
<td>9.6</td>
<td>11.9</td>
<td>16.2</td>
<td>145.8</td>
<td>405.5</td>
<td>57.9</td>
<td>3.7</td>
<td>3.2</td>
<td>40.6</td>
<td>5.8</td>
<td>20.9</td>
</tr>
<tr>
<td></td>
<td>10-20</td>
<td>945.2</td>
<td>252.7</td>
<td>45.3</td>
<td>221.6</td>
<td>387.7</td>
<td>620.2</td>
<td>44.6</td>
<td>6.4</td>
<td>7.0</td>
<td>13.8</td>
<td>2.0</td>
<td>29.3</td>
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<tr>
<td></td>
<td>20-30</td>
<td>480.3</td>
<td>171.9</td>
<td>23.9</td>
<td>147.9</td>
<td>186.3</td>
<td>540.1</td>
<td>35.2</td>
<td>22.1</td>
<td>4.4</td>
<td>14.7</td>
<td>2.2</td>
<td>18.9</td>
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<td></td>
<td>30-40</td>
<td>177.1</td>
<td>157.7</td>
<td>10.3</td>
<td>50.5</td>
<td>75.7</td>
<td>600.2</td>
<td>16.8</td>
<td>40.3</td>
<td>3.0</td>
<td>8.4</td>
<td>1.2</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>40-50</td>
<td>209.5</td>
<td>110.4</td>
<td>8.8</td>
<td>66.1</td>
<td>72.4</td>
<td>567.5</td>
<td>15.8</td>
<td>40.7</td>
<td>2.3</td>
<td>7.1</td>
<td>1.0</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>50-60</td>
<td>121.3</td>
<td>124.4</td>
<td>6.7</td>
<td>40.2</td>
<td>19.7</td>
<td>481.9</td>
<td>4.1</td>
<td>50.0</td>
<td>1.9</td>
<td>1.2</td>
<td>0.2</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>$R^2$</td>
<td>-</td>
<td>0.59</td>
<td>0.99</td>
<td>0.93</td>
<td>0.30</td>
<td>0.22</td>
<td>0.37</td>
<td>0.96</td>
<td>0.00</td>
<td>0.00</td>
<td>0.69</td>
<td></td>
</tr>
</tbody>
</table>
Total organic carbon (TOC) was highest close to the surface in both soil profiles and was highest at a depth horizon of 0-10 cm in MO-1 with 23.1 wt% and at a depth horizon of 10-20 cm in FF-1 with 29.3 wt%. The TOC concentrations show a positive correlation with selenium in the soil profile with an $R^2$ value of 0.42 in MO-1 and 0.69 in FF-1 (table 5.1). As only 4 samples were analysed in soil profile MO, the data from both soil profiles were combined in figure 5.2 and shows the positive correlation of TOC and Se with an $R^2$ value of 0.58. The Fe$_2$O$_3$ concentration was low in both soil profiles ranging from 1.9 wt% to 4.4 wt% in MO and 3.9 wt% to 10.8 wt% in FF-1 but both showed a strong positive correlation with selenium with an $R^2$ of 0.96 and 0.92.

5.3.2. X-Ray Absorption Spectroscopy

Soil MO-1 was selected for detailed analysis by microfocus XAS ($\mu$-XAS) to identify selenium and molybdenum species at 10-20 cm depth. The $\mu$-XRF maps showing the distribution of selenium and molybdenum in the soil are shown in figure 5.3. The XANES from selected points of interest (POI) were compared to a range of selenium and molybdenum standards (figure 5.4 and figure 5.5) using the linear combination fitting tool in ATHENA. Standards chosen represent species likely to be present in soil material. The Se K-edge energies for reference standards, based on the

![Graph showing the positive correlation between selenium (mg kg$^{-1}$) and TOC (wt%) concentration in soil cores MO-1 and FF-1 (Combined $R^2 = 0.58$).](image-url)

Figure 5.2. Graph showing the positive correlation between selenium (mg kg$^{-1}$) and TOC (wt%) concentration in soil cores MO-1 and FF-1 (Combined $R^2 = 0.58$).
maximum of first derivative of XANES spectra (e0) were 12656 eV for selenocystine 
(C$_6$H$_{12}$N$_2$O$_4$Se$_2$; Se$^{II}$), 12657 eV for sodium selenide (Na$_2$Se; Se$^{II}$), 12657 eV for red 
elemental selenium (Se$^{0}$), 12658 eV for selenomethionine (C$_5$H$_{11}$NO$_2$Se; Se$^{II}$), 12662 
eV for sodium selenite (NaSeO$_3$; Se$^{IV}$) and 12665 eV for sodium selenate (Na$_2$SeO$_4$; 
Se$^{VI}$). (The spectra for selenate and selenite are not shown on figure 5.4 due to the 
presence of only reduced species but can be found in figure 4.2, Chapter 4). The 
molybdenum $K$-edge energies for the reference standards were 20016 eV for 
molybdenum trioxide (MoO$_3$; Mo$^{VI}$), 20008 eV for the molybdenumdisulfide (MoS$_2$; 
Mo$^{IV}$), 20009 eV for the molybdenumdithiocarbamate (Mo[S$_2$CN(C$_2$H$_5$)$_2$]$_4$; Mo$^{IV}$) and 
20001 eV for the ammoniumtetrathiomolybdate (MoS$_4$(NH$_4$)$_2$; Mo$^{IV}$).

Figure 5.3. µ- XRF maps showing selenium dispersed throughout soil MO-1 and selected 
points of interest (POI) S1-S3 for Se $K$-edge XANES analysis. The µ-XRF is on a ‘pseudo 
temperature scale with high Se counts in red and areas with no counts (spaces due to soil 
drying) in dark blue. The black rectangle shows the area selected for µ-XRF maps of iron 
(Fe) and molybdenum (Mo) where high counts (red) appear to correlate. Selected POI for 
Mo $K$-edge XANES analysis are labelled M1-M3.

The Se µ-XRF map in figure 5.3 shows the highest selenium counts (in red) were at 
POI-S3 and linear combination fitting in table 5.2 showed that selenium was in a 
reduced inorganic phase comprising of 64% elemental selenium and 36% selenide.

114
Table 5.2. Linear combination fits (%) of the Se K-edge XANES spectra for selected points of interest (POI) in the μ-XRF map of soil sample MO-1 measured in ATHENA. The goodness of fit is determined by the R-Factor and Reduced Chi-squared and the lower the value the better the fit.

<table>
<thead>
<tr>
<th>POI</th>
<th>SeCys$_2$</th>
<th>SeMet</th>
<th>Selenide</th>
<th>Elemental Se</th>
<th>Selenite</th>
<th>Selenate</th>
<th>R-Factor</th>
<th>Reduced Chi-squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>-</td>
<td>0.294</td>
<td>0.362</td>
<td>0.344</td>
<td>-</td>
<td>-</td>
<td>0.006</td>
<td>0.002</td>
</tr>
<tr>
<td>S2</td>
<td>-</td>
<td>0.232</td>
<td>0.666</td>
<td>0.102</td>
<td>-</td>
<td>-</td>
<td>0.007</td>
<td>0.002</td>
</tr>
<tr>
<td>S3</td>
<td>-</td>
<td>-</td>
<td>0.362</td>
<td>0.638</td>
<td>-</td>
<td>-</td>
<td>0.005</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 5.3. Linear combination fits (%) of the Mo K-edge XANES spectra for selected points of interest (POI) in the μ-XRF map of soil sample MO-1 measured in ATHENA. The goodness of fit is determined by the R-Factor and Reduced Chi-squared and the lower the value the better the fit.

<table>
<thead>
<tr>
<th>POI</th>
<th>MoDT</th>
<th>MoDTC</th>
<th>Molybdenum disulfide</th>
<th>Tetrathiomolybdate</th>
<th>Molybdenum trioxide</th>
<th>R-Factor</th>
<th>Reduced Chi-squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>0.086</td>
<td>0.013</td>
</tr>
<tr>
<td>M2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>0.040</td>
<td>0.007</td>
</tr>
<tr>
<td>M3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>0.061</td>
<td>0.009</td>
</tr>
</tbody>
</table>
Figure 5.4. Normalised Se K-edge XANES spectra for reduced selenium species and the 3 points of interest (POI) selected from the Se μ-XRF map of soil MO-1.

Figure 5.5. Normalised Mo K-edge XANES spectra for reduced molybdenum species and the 3 points of interest (POI) selected from the Mo μ-XRF map of soil MO-1.
The selenium did not directly correlate with any other elements analysed, although the selenium was surrounded by low amounts of iron (green in Fe µ-XRF map). The selenium counts were lower at POI-S1 and POI-S2 where the selenium appears to be more dispersed within the soil material and both LCF fits were improved with the addition of the organic species SeMet. POI-S1 contained 36% selenide followed by 34% elemental Se and 30% SeMet, whereas POI-S2 contained 66% selenide, 23% SeMet and 10% elemental Se. The R-factor for the 3 POI ranged from 0.005-0.007 indicating a good fit. All linear combination fits showed that selenium is predominantly in a reduced phase within the soil which is consistent with the soil Eh and pH.

A section of the Se µ-XRF map was selected for Mo µ-XRF analysis to determine the correlation between selenium and molybdenum in the soil. The Mo µ-XRF map shows that the high molybdenum counts (red) at POI-M2 and POI-M3 correlate with areas of high iron counts (red in Fe µ-XRF map) but lower selenium counts (green in Se µ-XRF map). POI-S3 with the highest selenium counts (red) also shows no molybdenum present despite a correlation observed in the soil core horizons ($R^2 = 0.58$). The linear combination fits show that predominant molybdenum species in all points of interest was molybdenum trioxide ($\text{Mo}^{VI}$) with a 100% linear combination fit. The r-factor for the fit was higher compared to the selenium fits and ranged from 0.040-0.086. There was also a slight difference in the edge structure (figure 5.5) which suggests the molybdenum is present as a $\text{Mo}^{VI}$ molybdenum species similar, but not identical, to $\text{MoO}_3$.

5.3.3. Microcosm Experiment

Soil collected from near the surface at a depth horizon of 10-20 cm from site FF-1 and MO-1 was selected for a series of microcosm experiments under different physiochemical conditions over 42 days to help understand the fate and microbial cycling of selenium and other toxic trace elements in these soils. Although selenium was present in the ICP-MS data, no selenate or selenite oxyanions were detected in the IC analysis. This suggests selenium is present in solution as a mobile organic
species such as SeMet which was identified in the linear combination fits by XAS in the original soil material of MO-1 (figure 5.4).

The ICP-MS data in figure 5.6 shows that, in the anaerobic microcosm for site MO-1, the selenium concentration in the soil solution was much less than the soil (504.5 mg kg$^{-1}$) which is due to the dominance of reduced Se species ($\text{Se}^{\text{III}}$ and $\text{Se}^{0}$) observed in the XAS analyses which are immobile. Selenium in the soil solution decreased from 951.8 µg L$^{-1}$ on day 0 to 654.8 µg L$^{-1}$ on day 3 and then decreased rapidly between day 3 and day 7 to 52.6 µg L$^{-1}$. The rapid decrease of selenium in the microcosm solution indicates anaerobic conditions favour more immobile selenium species or methylation to volatile species. The rate of selenium removal from solution was faster at site FF-1 and the initial concentration in solution was lower which may be the influence of more reduced conditions (Eh = 100 mV). In FF-1 the selenium concentration in the soil solution was also much lower than the soil material (945.2 mg kg$^{-1}$) and decreased rapidly from 558.1 µg L$^{-1}$ on day 0 to 26.3 µg L$^{-1}$ on day 3.

The concentration of selenium also decreased in the anaerobic microcosms amended with 10 mM sodium sulfate in both soils showing sulfate does not change the anaerobic conditions or selenium species. In site MO-1, selenium decreased from 873.3 µg L$^{-1}$ on day 0 to 808.9 µg L$^{-1}$ on day 3 and then rapidly decreased to 63.2 µg L$^{-1}$ on day 7. Similar to the anaerobic microcosm, the selenium concentration in the soil solution from site FF-1 was initially lower than MO-1 and decreased from 620.1 µg L$^{-1}$ to 26.8 µg L$^{-1}$ on day 3. However, in the anaerobic microcosm amended with 10mM sodium nitrate the selenium concentration decreased at a slower rate. In soil MO-1, the selenium concentration was 890.7 µg L$^{-1}$ on day 0 and decreased slowly to 439.8 µg L$^{-1}$ by day 42. In soil FF-1, the selenium concentration was 615.1 µg L$^{-1}$ on day 0 and decreased to 39.7 µg L$^{-1}$ on day 14 of the experiment. Nitrate likely acted as an electron acceptor as the nitrate concentration also decreased to 0 µg L$^{-1}$ in all the microcosm conditions except from the nitrate amended which decreased to 160 mg L$^{-1}$ in site MO-1 and 12.6 mg L$^{-1}$ in FF-1.
Figure 5.6. The changes in Se, Mo and U concentration in the soil solution (µg L⁻¹) with different physiochemical conditions over a 42 day microcosm experiment. Concentrations are determined by ICP-MS and the data plotted are average values of triplicate samples with the standard error.
The rate of selenium removal in the soil solution of the microaerophilic microcosms was also slower than the anaerobic microcosms; however it was faster than the anaerobic microcosm amended with sodium nitrate. In sample MO-1, selenium decreased from 1007.3 µg L\(^{-1}\) on day 0 to 148.8 µg L\(^{-1}\) on day 21. This was also observed in sample FF-1 and selenium decreased from 616 µg L\(^{-1}\) on day 0 to 32 µg L\(^{-1}\) on day 7. This may suggest the samples have become slightly anaerobic during the experiment which favoured selenium removal.

The concentrations of uranium and molybdenum were also elevated in the soil profiles (table 5.1) and microcosm experiments show these toxic trace elements behave similarly with changing physiochemical conditions, but different in behaviour to selenium. In the anaerobic microcosms, the molybdenum concentration steadily increased from day 0 to day 42 from 517.1 µg L\(^{-1}\) to 2158.7 µg L\(^{-1}\) in soil MO-1 and from 29.8 µg L\(^{-1}\) to 260.8 µg L\(^{-1}\) in FF-1 (figure 5.6). Uranium also increased from day 0 to day 42 from 226 µg L\(^{-1}\) to 3187.3 µg L\(^{-1}\) in soil MO-1 and from 16.3 µg L\(^{-1}\) to 192.7 µg L\(^{-1}\) in soil FF-1. Therefore, anaerobic conditions increase the solubility of molybdenum and uranium in solution (while decreasing that of selenium). However, the concentrations of molybdenum and uranium in the soil solution were much higher in MO-1 compared to FF-1.

The microcosm amended with 10 mM sodium sulfate also increased the molybdenum and uranium concentrations in solution. However on day 42 of MO-1 the molybdenum concentration was 1671.8 µg L\(^{-1}\) and the uranium concentration was 1501 µg L\(^{-1}\) which was significantly lower than the unamended anaerobic microcosm. Whereas, on day 42 of FF-1 the molybdenum concentration was 297.4 µg L\(^{-1}\) and the uranium concentration was 235.4 µg L\(^{-1}\) which was slightly higher than the unamended anaerobic microcosm. The microcosms amended with 10 mM sodium nitrate also showed an increase in molybdenum and uranium concentration in solution but was lower on day 42 compared to the unamended anaerobic microcosm and anaerobic microcosm amended with sodium sulfate. Molybdenum concentrations on day 42 were 1158 µg L\(^{-1}\) in soil MO-1 and 101.8 µg L\(^{-1}\) in soil FF-2. Uranium concentrations were 621.7 µg L\(^{-1}\) in MO-1 and 75.1 µg L\(^{-1}\) in FF-1. However, the microaerophilic microcosm conditions showed little effect on the uranium and
molybdenum concentration except for the molybdenum concentration from site MO-1 which increased from 473.3 µg L\(^{-1}\) to 918.3 µg L\(^{-1}\). Similar to selenium, the uranium oxyanion uranate was not detected in the IC analysis, however the molybdenum oxyanion molybdate was detected and increased in concentration from day 0 to day 42 in all microcosm conditions except the microaerophilic microcosm for soil FF-1, which was 0.

Figure 5.7. The concentration of Fe\(^{II}\) (mM) on day 0 and day 42 of the microcosm experiments under anaerobic, anaerobic + 10 mM sodium sulfate, anaerobic + 10 mM sodium nitrate and microaerophilic conditions. Concentrations are averages of triplicate samples and the standard error from soil MO-1 and FF-1.

Fe\(^{II}\) was observed in all microcosm solutions in both soil MO-1 and FF-1 by day 42 of the experiment. Figure 5.7 shows that the largest concentrations of Fe\(^{II}\) was observed in the anaerobically incubated microcosms with an increase of 24.9 mM in soil MO-1 and 13.8 mM in soil FF-2 from day 0 to 42. The increase in Fe\(^{II}\) concentration in solution indicates microbial dissimilatory reduction of Fe\(^{III}\). In soil FF-2, the amendment of the anaerobic microcosm with sodium sulfate did not slow down the rate of microbial reduction of Fe\(^{III}\) to Fe\(^{II}\) as there was also an increase of
However, the sodium sulfate amended microcosm for soil sample MO-1 showed an increase of 14.3 mM which was significantly lower than the unamended anaerobic microcosm. The amendment of the anaerobic microcosms with nitrate slowed down microbial reduction as nitrate was likely used as an alternative electron acceptor. However, there was still a small increase of 4 mM Fe\textsuperscript{II} in FF-1 and only 0.6 mM in soil MO-1 from day 0 to 42. Similarly, the microaerophilic microcosm slowed down the rate of reduction although a small increase of 3.3 mM Fe\textsuperscript{II} was observed in soil FF-1 and 3.8 mM in soil MO-1. Although site FF-2 was more reduced (Eh = 100 mV), the total iron was initially higher in site MO-1 with 21.9 mM in the anaerobic microcosm on day 0 compared to 2.5 mM in site FF-1 which explains why more iron was reduced to Fe\textsuperscript{II} over the 42 day experiment.

5.3.4. DNA Sequencing

The microbial community for sample MO-1 was determined on day 0 and day 42 for each microcosm condition using 16S DNA sequencing techniques. To compare the microbial community between the two agricultural sites, sample FF-1 was also analysed at day 0 from the microcosm kept under anaerobic conditions. Two samples of soil (B-1 and B-2) from the study area, just up slope from the contaminated soils with selenium and molybdenum concentrations of <5 mg kg\textsuperscript{-1} were also analysed for comparison, to determine if there were dramatic effects on the microbial communities due to the high Se, Mo and U concentrations. The diversity of observed phyla for the soil samples are shown in figure 5.8. Soil sample MO-1 contains a microbial community that remained stable during the microcosm experiments as there is only a small change in the relative abundance of phyla between day 0 and day 42 of the experiment in all microcosm conditions. On day 0 of the anaerobic microcosm the sample was dominated by 25.7% Proteobacteria (135 OTUs) of which 9.17% of obtained sequences were affiliated to the class Alphaproteobacteria (48 OTUs) shown in figure 5.9. This was followed by 18.6% Acidobacteria (96 OTUs) of which 16.7% of obtained sequences were affiliated to the class Acidobacteria (90 OTUs) and 9.5% Actinobacteria (69 OTUs) of which 4.87% of obtained sequences were affiliated with the class Thermoleophilia (30 OTUs). The largest change observed in the abundance of phyla from day 0 to day 42
of the anaerobic microcosm experiment was a 2% increase in *Firmicutes* associated with the class *Bacilli*. There was also a 2% increase in *Chloroflexi* of which 0.9% was associated with the class *Anaerolineae*.

Similar to the anaerobic sample, all other microcosm samples for MO-1 were dominated by the phyla *Proteobacteria* (25.6-28.5%), *Acidobacteria* (17.7-19.4%) and *Actinobacteria* (7.9-12%). The largest change in the abundance of phyla between day 0 and day 42 of the sulfate amended anaerobic microcosm was a 2.1% increase in *Proteobacteria* of which 1.9% was associated with the class *Epsilonproteobacteria*. There was also a 1.8% decrease in *Planctomycetes* of which 1.7% was associated with *Planctomycetacia*. This also occurred in the microaerophillic microcosm with a 2.6% increase in *Proteobacteria* with 2.4% associated with the class *Alphaproteobacteria*. There was also a 2.4% decrease in *Planctomycetes* associated with the class *Planctomycetacia*. However, the largest change in abundance was observed in the nitrate amended microcosms with a 3.3% decrease in *Actinobacteria* from day 0 to 42 of which 1.8% was associated with the class *Thermoleophila*. There was also a 2.3% decrease in *Proteobacteria* associated with both *Deltaproteobacteria* and *Alphaproteobacteria*.

On day 0 of the anaerobic microcosm from site FF-1 (945.2 mg kg\(^{-1}\) Se) the sample was dominated by 29.3% *Proteobacteria* (143 OTUs) similar to MO-1, however 12.4% of obtained sequences were affiliated to the class *Deltaproteobacteria* (41 OTUs). This was followed by 18.6% *Acidobacteria* (92 OTUs) of which 16.8% of obtained sequences were affiliated to the class *Acidobacteria* (85 OTUs) similar to MO-1. However, FF-1 was also dominated by 10.8% *Firmicutes* (17 OTUs), of which 10.3% was associated to the class *Bacilli* (15 OTUs) which differs from MO-1. Compared to sample MO-1, the main difference in phyla abundance was that FF-1 contained 6% more *Thaumoarcheota of which* 4.3% was associated to the Soil *Crenarchaeotic Group*. There was also 5.2% less *Acidobacteria* of which 4.6% was associated to the class *Acidobacteria*. 

123
Figure 5.8. The relative abundance of observed phyla present in anaerobic samples B-1, B-2, anaerobic soil microcosm sample FF-1 (T = 0) and all soil microcosm conditions for sample MO-1 (T = 0 and T = 42) which exceeded 1% abundance in soils.
Figure 5.9. The relative abundance of the most abundant classes of phyla present in anaerobic samples B-1, B-2, anaerobic soil microcosm sample FF-1 (T = 0) and all soil microcosm conditions for sample MO-1 (T = 0 and T = 42) which exceeded 1% abundance in soils.
Anaerobic sample B-1 (4.4 mg kg\(^{-1}\) Se), was dominated by 24.2\% Acidobacteria (82 OTUs) of which 22.1\% was associated with the class Acidobacteria (78 OTUs). This was followed by 21.5\% Actinobacteria (63 OTUs) of which 6.3\% was associated with the class Actinobacteria (18 OTUs). The sample also contained significant, 16.7\%, Proteobacteria (109 OTUs) of which 8.5\% was associated with Alphaproteobacteria (42 OTUs). Compared to anaerobic sample MO-1 (day 0), the largest difference in phyla abundance was sample B-1 contained 12\% more Actinobacteria which was mainly associated to the class Acidomicrobiia and Actinobacteria. There was also 9\% less Proteobacteria of which 3.7\% was Betaproteobacteria. Anaerobic sample B-2 (1.1 mg kg\(^{-1}\) Se), was dominated by 30\% Proteobacteria (134 OTUs) of which 14.5\% was associated with the class Alphaproteobacteria (48 OTUs). This was followed by 16.4\% Acidobacteria (91 OTUs) of which 16.1\% was associated with the class Acidobacteria (84 OTUs). The sample was also dominated by 13.9\% Actinobacteria (65 OTUs) of which 7.8\% was Thermoleophila (30 OTUs). Compared to anaerobic sample MO-1 (day 0), the main difference in phyla abundance was sample B-2 which was better drained and more oxidised, contained 6\% more Verrucomicrobia of which 5.9\% was Spartobacteria and 5.4\% less Planctomycetes associated with the class Planctomycetacia. Although the 3 most abundant phyla in sample B-1 and B-2 were the same as MO-1, both samples were less microbially diverse (403-470 total OTUs) compared to sample MO-1 and FF-1 (507-534 total OTUs).

5.4. Discussion

5.4.1. Selenium Concentration and Speciation

Examination of both soil cores revealed selenium was enriched compared to the average global soil concentration of 0.4 mg kg\(^{-1}\) and the trigger action value (TAV) for agricultural soil of 3-10 mg kg\(^{-1}\) (Kabata-Pendias, 2011). The most seleniferous horizon of soil was at a depth horizon of 10-20 cm in FF-1 and 20-30 cm in soil MO-1 (table 5.1). The bottom horizon contained the lowest concentration which suggests the source of selenium in both sites is not directly related to the underlying geology which was also observed in a study by Fellowes et al. (2013). The underlying geology is Visean limestone, and limestones rarely contain in excess of 0.1 mg kg\(^{-1}\)
selenium (Neal, 1995). The soil pH and Eh values were indicative of a slightly acidic and reducing soil environment which is consistent with the presence of the reduced inorganic selenium species selenide (Se\(^{-II}\)) and elemental selenium (Se\(^0\)) in the \(\mu\)-XANES linear combination fits for soil MO-1 (table 5.2). In anaerobic environments, selenium oxyanions selenate and selenite can be reduced by bacterial species such as *Geobacter sulfurreducens*, *Shewanella oneidensis* and *Veillonella atypica* to elemental Se\(^0\) nanospheres and metal selenide nanoparticles which are immobile and less bioavailable (toxic) (Pearce et al., 2008; Pearce et al., 2009).

Both soils were also rich in total organic carbon (TOC) which positively correlated with the selenium in the soil profile (figure 5.2). Organic matter is known to be strongly associated with selenium and stimulates the microbial reduction to these less bioavailable species (Zhang and Moore, 1996). Microfocus XAS maps of soil MO-1 in figure 5.3 showed areas of the soil where selenium was more dispersed within the soil material and the linear combination fits were improved with the addition of the organic selenium species SeMet. SeMet is a product of assimilatory reduction of selenium oxyanions and is a selenium amino acid which is bioavailable to plant and animals which poses a risk to health (Dungan and Frankenberger, 1999). Sequential extraction data also shows that selenium was present in soil FF-1 as mainly elemental selenium (Se\(^0\)) or organic selenium species (figure 6.3 in chapter 6).

Organic carbon is known to control the enrichment of molybdenum and uranium in natural environments by adsorption or chelation (Leventhal and Hosterman, 1982), and these two elements are also elevated within the soil cores. The molybdenum may be bound to organic matter as a result of nitrogen-fixing bacteria which release small complexing agents (Wichard et al., 2009). There was a strong positive correlation of uranium with selenium throughout both soil profiles (table 5.1) and the concentrations were far in excess (19.7-221.6 mg kg\(^{-1}\)) of the world soil average of 3 mg kg\(^{-1}\). Molybdenum concentrations also correlated positively with selenium and the concentration ranged from 9.6-1081.6 mg kg\(^{-1}\) which enormously exceeded the world soil average of 1.1 mg kg\(^{-1}\) and trigger action value of 5-10 mg kg\(^{-1}\) (Kabata-Pendias, 2011). Soil MO-1 contained the highest concentration of
molybdenum, although both sites can be defined as molybdeniferous (>0.3 mg kg\(^{-1}\)) (Mcgrath and Fleming, 2008), and represent some of the world’s naturally most molydeniferous soils. Microfocus XAS maps of soil MO-1 showed areas of high molybdenum were associated with iron (figure 5.3). The predominant molybdenum species identified was molybdenum trioxide (Mo\(^{VI}\)) which is the highest oxidation state. However, there were slight differences in the edge structure and is likely to be a similar oxidised species to MoO\(_3\). The XANES spectra also resembled molybdate sorbed onto ferrihydrate in acidic soils (Gustafsson and Tiberg, 2015) and would explain this association with iron.

Field relations show that the selenium and molybdenum (and uranium) have very localised high concentrations that accumulated in organic-rich soils in the (local) lowest lying areas. The only possible natural source is the Carboniferous mudstones and shales which are known to contain high concentrations of these toxic trace elements (see table 6.3, chapter 6). The small stream adjacent to the MO-1 and FF-1 sites (figure 5.1) drains slightly higher ground (20 m) to the west which is comprised of the mudstones of the Clare Shales. Although this stream has been recently entrenched, all soil profiles in the low lying area had a very distinctive grey clay layer (>20 cm thick) beneath the brown/black soil profile (see supplementary report 8.1). This suggests these areas were at one time lacustrine, perhaps during the late/post glacial period (ca 13000BP). This impervious clay layer will have had the effect of an underlying impermeable barrier, inhibiting interaction with the underlying lithology and making an excellent environment for the development of an organic-rich, low Eh ‘sump’ which concentrated the toxic elements trickling in via the local hydrology, from the source area (Fellowes et al., 2013; Fleming and Walsh, 1957).

5.4.2. Microcosm Experiments

The transformation between organic and inorganic selenium species in soils is controlled not only by the redox potential and pH but also the microbial activity (Chasteen and Bentley, 2002; Coppin et al., 2009; Winkel et al., 2012). In soil solution, selenium can be present as either inorganic selenate and selenite or
soluble organic species which are bioavailable (Supriatin et al., 2016). In this study, the organic species SeMet (Se\textsuperscript{II}) has been shown to be the predominant soluble species present in the microcosm on day 0 as no selenium oxyanions were detected in the original soil material by XAS and in the soil solution by ion chromatography. Incubation of the anaerobic microcosms show selenium was rapidly removed from solution in both soil MO-1 and FF-2 (Figure 5.6). Therefore, the likely process of removal of selenium in the soil solution is shown to be microbial methylation of SeMet with the likely formation of volatile organic species such as dimethylselenide (Lenz and Lens, 2009). The SeMet could have been mineralised to inorganic forms but this would have produced soluble oxyanions, increasing selenium in solution, for which there is no evidence. A study by Martens and Suarez (1996) showed the majority of SeMet in soil is volatilized and therefore mineralisation is unlikely. The initial selenium concentrations in solution were lower in sample FF-1 due to the more reduced conditions and higher organic matter content, retaining the selenium in the soil.

The presence of high concentrations of Fe\textsuperscript{II} on day 42 of the anaerobic microcosm demonstrates microbial reduction of Fe\textsuperscript{III} is occurring in the soil (figure 5.7). Unlike selenium, the concentration of molybdenum increased in the soil solution which is not favoured by reducing conditions where insoluble Mo\textsuperscript{IV} complexes form (Lloyd, 2003; Tucker et al., 1996). However, the XAS analysis of the original soil indicated a similar species to molybdenum trioxide (Mo\textsuperscript{VI}) which is the most oxidised species but is relatively insoluble. The strong correlation with molybdenum and iron in the microfocus XAS maps (figure 5.3) and the low initial concentration of molybdenum in solution on day 0 of the microcosm experiment suggests sorption or coprecipitation of molybdenum with Fe\textsuperscript{III} (hydroxy)oxides (LeGendre and Runnells, 1975). Therefore, during reduction of Fe\textsuperscript{III} (hydroxy)oxides to Fe\textsuperscript{II}, molybdenum was released into solution and this resulted in the production of the soluble molybdate anion (Glass et al., 2012). The increase in uranium concentration in solution is also likely to be due to release of sorbed U\textsuperscript{VI} due to the reductive dissolution of Fe\textsuperscript{III} (hydroxy)oxides (Wu et al., 2010).
The selenium concentration decreased in the anaerobic microcosm amended with sodium sulfate, indicating the removal of bioavailable selenium from solution. Although sulfate is an electron acceptor, no sulfate reduction occurred during the experiment; bioreduction of Fe\textsuperscript{III} to Fe\textsuperscript{II} occurred indicating iron is preferentially used by the microbial community. The addition of sulfur has been shown to decrease soluble selenium and increase selenium bound to organic matter by lowering the pH (Liu et al., 2015; Winkel et al., 2015), however this study shows this does not occur with sulfate. Sulfate also had no effect on the mobilisation of uranium and molybdenum in soil FF-1, however a lower concentration of these two elements was observed in the soil solution of MO-1, compared to the anaerobic microcosm which could be due to the lower rates of Fe\textsuperscript{III} reduction.

In the microaerophilic microcosm, selenium was removed from solution although the rate was slower than the anaerobic microcosm (figure 5.6). The presence of Fe\textsuperscript{II} on day 42 suggests that the microbial activity and high total organic carbon content led to decreased oxygen levels over time (Fellowes et al., 2013). Microbial oxidation of Se\textsuperscript{0} to selenate and selenite oxyanions in soils occurs at relatively slow rates compared to reduction of oxyanions to Se\textsuperscript{0} (Losi and Frankenberger, 1997) and therefore did not occur in the microcosm experiment. As the reductive dissolution of Fe\textsuperscript{III} (hydroxy)oxides was lower in the microaerophilic microcosm, uranium was not mobilised and molybdenum concentrations remained low in solution which suggests low mobility in oxidised, well drained soils.

In the anaerobic microcosm amended with sodium nitrate, the soluble selenium species remained in the solution longer than the unamended anaerobic microcosm because high concentrations of NO\textsubscript{3}\textsuperscript{-} and NO\textsubscript{2}\textsuperscript{-} inhibit the microbial methylation of selenium (Thompson-Eagle and Frankenberger, 1990). Nitrate also inhibits anaerobic selenium transformations to less bioavailable species (Oremland et al., 1989) which has implications in agricultural land amended with nitrate fertilisers. There was also a significant decrease in the rate of Fe\textsuperscript{III} reduction to Fe\textsuperscript{II} which was also observed by Fellowes et al. (2013). In both the above cases nitrate acted as an alternative electron acceptor, shown by its decrease in concentration (denitrification) with time. Similar to the microaerophilic microcosm,
molybdenum and uranium release was inhibited due to the lower reduction rate of Fe\textsuperscript{III} (hydroxy)oxides. However, the use of synthetic groundwater may also affect the solubility of these elements in the soil microcosm experiments.

5.4.3. Microbial Community Analysis

To understand the microbial transformations of selenium and other toxic trace elements in the microcosm study, DNA sequencing techniques were used to analyse the microbial community in the soil. The most abundant phyla in all samples analysed from MO-1 were *Proteobacteria, Acidobacteria* and *Actinobacteria* which remained relatively stable during the microcosm study (figure 5.8). *Proteobacteria* have the biggest and most diverse phylogenetic lineage and play an important role in the global cycling of carbon, nitrogen and sulfur which explains the abundance in the soil samples (Kersters et al., 2006). *Acidobacteria* are diverse but are often indicative of oligotrophic soils and are thus abundant in soils with limited nutrient availability (Lin et al., 2011); the sample sites have not had fertiliser additions and are dairy pastures with inadequate nutrient status for growing crops. *Actinobacteria* are more tolerant than other bacteria to soils containing heavy metals and metalloids which may explain their elevated concentrations in in soils MO-1 and FF-1. However, the soil samples analysed were collected in the rhizosphere and are rich in organic matter which may also explain the abundance of *Actinobacteria* which have shown to play an important role in the decomposition of organic matter (Větrovský and Baldrian, 2018). Therefore, these soils appear to contain a normal microbial community despite elevated concentrations of toxic trace elements selenium, molybdenum and uranium.

In the anaerobic microcosm of MO-1 there was a small increase in the class *Bacilli* from days 0-42 of the experiment (figure 5.9). A number of *Bacilli* species which are anaerobic, rod-shaped bacteria, are capable of reducing selenium oxyanions (Switzer Blum et al., 1998). For instance, *Bacillus* sp. strain SF-1 uses selenate as an election acceptor causing microbial selenium reduction (Fujita et al., 1997). *Bacillus selenitireducens* can reduce selenite to elemental selenium (Switzer Blum et al., 1998) and the latter was extensively present in original soil material, as
shown by the XAS analysis. Bacilli were even more abundant in the anaerobic microcosm from soil FF-1 (the soil sample was more reduced than MO-1) and also contained higher concentrations of selenium in the solid soil material. However, the most abundant Bacilli in the soil were not identified to the genus or species level in this study. As the selenium concentration decreased in the soil solution and Fe\textsuperscript{II} increased over the 42 days in the anaerobic microcosm there was an increase in the class Anaerolineae which is one of the main microbial populations capable of anaerobic digestion (Xia et al., 2016).

In the anaerobic microcosms amended with sodium sulfate and the microaerophillic microcosms, there was a slight increase in the abundance of Proteobacteria over the 42 days (figure 5.8). The sulfate amended microcosm also increased in the class Epsilonproteobacteria which are mostly microaerophillic and chemoorganitrophic spiral shaped bacteria (Kersters et al., 2006). Epsilonproteobacteria can use sulfur compounds as both electron donors or acceptors (Yamamoto and Takai, 2011), although there was no observed change in the concentration of sulfate during the experiment. However, in the microaerophillic microcosm there was an small increase in the class Alphaproteobacteria (figure 5.9), in particular the order rhizobiales which are mainly aerobic, rod-shaped bacteria which occur near the roots of leguminous plants and fix atmospheric nitrogen in a symbiotic relationship which is important in agriculture (Kersters et al., 2006). In the anaerobic microcosm amended with nitrate there was a slight decrease in the most abundant classes Actinobacteria and Proteobacteria (figure 5.8). In the natural environment, most nitrate reducers belong to the phyla Proteobacteria, Actinobacteria, and Firmicutes due to the presence of membrane bound nitrate reductase (Bru et al., 2007; Palmer and Horn, 2012). Therefore, as the nitrate concentrations decreased over the 42 day experiment due to denitrification, this may have caused the nitrate reducers to also decrease.

Although no specific species of bacteria were identified which are involved in selenium reduction or methylation activity found in the literature, it is clear the highly seleniferous and molybdeniferous samples MO-1 and FF-1 had a more
diverse microbial community compared to the samples containing <5 mg kg\(^{-1}\) selenium (B1 and B2) which were better drained. A phylogenetically diverse microbial community is capable of using dissimilatory reduction of selenium for anaerobic growth (Nancharaiah and Lens, 2015) and therefore the wide variety of microorganisms present in soil FF-1 and MO-1 will have had the capability to cause elemental selenium (Se\(^0\)) and selenides (Se\(^{II}\)) to accumulate in original soil material.

5.5. Conclusions

In summary, this research shows both soil profiles collected in County Limerick were highly seleniferous and molybdeniferous with concentrations of selenium up to 945.2 mg kg\(^{-1}\) in soil FF-1 and concentrations of molybdenum up to 1081.6 mg kg\(^{-1}\) in MO-1. Selenium was present in soil MO-1 as a reduced phase which consists of the inorganic species elemental Se (Se\(^0\)) and selenide (Se\(^{II}\)), causing selenium to accumulate. However, the bioavailable organic species SeMet (Se\(^{II}\)), was also identified which is likely to be the predominant soluble phase. Molybdenum was present in the highest oxidation state (Mo\(^{VI}\)) and may be adsorbed or coprecipitated to an Fe\(^{III}\) (hydroxy)oxide mineral such as ferrihydrate. The base of the soil profiles contained the lowest concentrations of selenium and molybdenum indicating the origin is not the underlying lithology but surface drainage. Selenium correlated with the total organic carbon concentration which was elevated in the soil profile due to the presence of a post-glacial lake bed preventing further drainage and accumulating organic matter.

Selenium correlated positively with molybdenum and strongly with uranium within the soil profile, however microcosm studies show selenium behaves differently under changing physiochemical conditions to both elements. Soluble selenium decreased under anaerobic conditions which is likely to be microbial methylation of SeMet to more volatile forms as a detoxification mechanism. However, soluble uranium and molybdenum species (molybdate) increased which is caused by the reductive dissolution of Fe\(^{III}\) (hydroxy)oxides. Microaerophilic conditions and the presence of nitrate in anaerobic conditions slowed the rate of selenium removal
and the release of molybdenum and uranium into the soil solution. However, the presence of Fe$^{II}$ suggests microbial reduction still occurred but at a slower rate than the unamended anaerobic microcosm.

The microbial community was dominated by *Proteobacteria, Actinobacteria* and *Acidobacteria* which remained relatively stable and tolerant of changing physiochemical condition during the microcosm experiment. Samples MO-1 and FF-1 contained a more diverse microbial community compared to the samples containing selenium concentration >5 mg kg$^{-1}$ (B-1 and B-2). However further research is required to determine the specific microbial species involved in selenium and molybdenum cycling in the natural environment. This study shows that the bioavailability of selenium decreased in anaerobic conditions but the bioavailability of molybdenum increased which has implications for livestock health. This also represents a challenge for remediation of soils containing both elements. The microbial methylation and mineralisation of organic selenium species is a neglected areas of research which is important as SeMet is highly bioavailable and toxic. Further research needs to focus on the headspace gases from the microcosms to determine whether microbial methylation of selenium is occurring and producing volatile organic species or whether other soil processes are influencing selenium mobility.

5.6. References


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Selenium Fractionation and Speciation in Naturally Contaminated Agricultural Soils

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Abstract

Selenium (Se) is a potentially toxic trace element in the environment however, it is also essential for human and animal health. Naturally seleniferous soils occur in the low lying grassland of West Limerick and the source is believed to be the Clare Shale Formation which forms the lowest stratigraphical unit of the Upper Carboniferous basin. Soil cores were extracted for bulk chemical analysis and sequential extraction studies to fractionate selenium according to redox activity, solubility and exchangeability. Rock samples were collected from geological outcrops to determine the source of selenium in the soils. Selenium was strongly associated with the total organic carbon, with the exception of soil profile PK-1 which had the highest concentration of selenium at the base of the profile. The underlying geology of site PK-1 is the Clare Shale Formation which contained selenium concentrations up to 18 mg kg\textsuperscript{-1}. Weathered sediment from the Clare Shales has been transported from the hills in the west and into the poorly drained, low lying pastures causing selenium enrichment. The horizons of soil containing the highest selenium concentration were abundant in lipid moieties mainly as prist-1-ene which likely originates from chlorophyll and suggests selenium is associated with burial of plant material. Sequential extraction analysis showed selenium is mainly fractionated as insoluble elemental selenium (Se\textsuperscript{0}) in the most seleniferous.
soils. Although fractions of selenium associated to both recalcitrant organic matter and the fulvic and humic acids were also abundant. This study extends the knowledge of selenium speciation and fractionation in soils and the influence of organic matter and geology on selenium concentration.

**Keywords:** Selenium, sequential extraction, speciation, fractionation, organic matter

### 6.1. Introduction

Selenium is a potentially toxic trace element in the environment however, it is also essential for human and animal health. Globally, the concentration of selenium in soils is highly variable and ranges from 0.01 mg kg\(^{-1}\) to a highly toxic level of 1200 mg kg\(^{-1}\) (Fordyce, 2013; Winkel et al., 2015). Therefore, identifying the source of selenium in soils and the physiochemical conditions controlling the bioavailability is essential to avoid selenium toxicity related diseases. Selenium distribution in soils is controlled mainly by the weathering of Se-rich rocks and the transport via surface or groundwater (Dhillon and Dhillon, 2003; Fernández-Martínez and Charlet, 2009). Consequently, selenium toxic soils are often located in small geographical areas due to the influence of the underlying geology (Fordyce, 2013). However local conditions such as topography, soil redox and pH, organic matter content, soil texture and mineralogy also influence the concentrations in the soil. Inorganic selenium exists in the natural environment in several oxidation states (VI, IV, 0 and -II) but selenium can also occur as organic selenium compounds (-II), 50% of which are yet to be fully characterised (Fordyce, 2007). Selenium biogeochemistry is complex as several species can exist in one environment at the same time (Fernández-Martínez and Charlet, 2009) and each species has specific biogeochemical properties (Lenz and Lens, 2009; Qin et al., 2017).

In rocks, a significant amount of selenium is present as immobile selenides (Se\(^{\text{II}}\)) (Herbel et al., 2003). Weathering of selenium-rich rocks converts these inorganic selenides into oxidised species such as selenite (Se\(^{\text{IV}}\)) and selenate (Se\(^{\text{VI}}\)) which are more bioavailable and thus increased toxicity (Fordyce, 2007). Sedimentary rocks generally contain higher concentrations of selenium than igneous and metamorphic
rocks although concentrations in limestones and sandstones rarely exceed 0.1 mg kg\(^{-1}\). Selenium is particularly enriched in clay-bearing lithologies such as mudstone and shale and concentrations of up to 600 mg kg\(^{-1}\) have been reported in black shale formations (Fordyce, 2007). Selenium in black shales can be associated with organic matter and sulfide minerals such as pyrite (FeS\(_2\)) (Alloway, 2013). Selenium is found in pyrite as it can isomorphically substitute for sulfur due to their similarity in chemical structure (Neal, 1995; Presser and Swain, 1990; Strawn et al., 2002). Selenium is also found in sulfide deposits and as the minerals dzharkenite (FeSe\(_2\)) and klockmannite (CuSe) (Lenz and Lens, 2009; Qin et al., 2017). Elevated concentrations have also been identified in phosphatic rocks and coal measures (Fordyce, 2007). At present, there are 50 selenium bearing minerals identified, most of which are sulfides containing small concentrations of selenide (Se\(^{II}\)). However selenium may also occur in elemental form (Se\(^{0}\)) in volcanic emanations (Fordyce, 2007; Malisa, 2001). Nonetheless, the sequestration of selenium in rocks and the subsequent distribution in soils is still poorly understood (Zhu et al., 2014). Selenium is often strongly coordinated with organic matter in the terrestrial environment (Kulp and Pratt, 2004; Qin et al., 2012; Zhang and Moore, 1996) and a study on seleniferous soils by Fellowes et al. (2013) showed selenium was present predominantly in a recalcitrant, reduced organic phase within the most organic-rich horizon. In organic matter, selenium is generally associated with humic substances (Qin et al., 2012) in particular, the bioavailable organic selenium species selenomethionine (SeMet) has shown to be strongly associated with the fulvic acid fractions (Abrams and Burau, 1989). However, the association of selenium with organic matter may be indirect via adsorption onto iron oxides or clay minerals on the surface or matrix of the organic matter (Copin et al., 2009; Gustafsson and Johnsson, 1994). However, it is still debated whether the role of minerals or organic matter is more important in the primary and secondary accumulation of selenium in soils (Copin et al., 2009).

Following Se through natural biogeochemical cycles is challenging because it is often in too low concentrations to follow analytically. However, the extremely high concentration of Se in the soils of West Limerick, Ireland, provides an opportunity
to examine Se in a naturally formed accumulation. The source of selenium in the soils of County Limerick is thought to be the Clare Shale Formation which forms the lowest stratigraphical unit of the Namurian, Upper Carboniferous, Clare basin (figure 6.1). The Clare shales are pyrite-rich black shales and mudstone, rich in organic matter and were formed in the subsidising deep water basin (SEPMSTRATA, 2018) where anoxic conditions prevailed. The soils rich in selenium are often high in molybdenum and toxic concentrations occur in small geographical areas often in close proximity to deficient concentrations (Finch and Ryan, 1966; Fleming, 1961; Fleming and Walsh, 1956; Rogers et al., 1990). These soils pose a risk to livestock health grazing on the overlying pastures, and local farmers are well aware of the poisoned fields and the effects on their livestock.

The aim of this study was to examine selenium fractionation in seleniferous horizons of soil from four sites in County Limerick focusing on Se speciation and the influence of organic matter and geology on Se distribution. To achieve these aims the objectives of this research were to (i) determine the bulk inorganic and organic chemistry of four soil cores, and rock samples, (ii) undertake sequential extractions of the most seleniferous soil horizons to determine the various fractions of selenium in the soil, according to the redox activity, solubility and exchangeability, (iii) examine the organic composition of the soils to understand the association of Se with organic matter.

**6.2. Methodology**

**6.2.1. Field Site and Sampling Techniques**

The soil cores for bulk chemical analysis were collected from 4 grassland sites that are used for pastoral farming and have not been ploughed. The sites were selected from a wider study on selenium distribution (supplementary report 8.1) and are underlain by the Clare Shales, Visean Limestone, Waulsortian Limestone and the Rathkeale Formation (figure 6.1). Three adjacent soil cores were collected using a Van Walt corer at each site and transferred into tightly sealed polypropylene tubes. Samples for X-ray fluorescence (XRF) analysis were stored anaerobically. The soil samples selected for the sequential extraction scheme were taken from the most
seleniferous horizons, defined by XRF analysis, and taken from cores stored at 4°C and kept anoxic in oxygen free nitrogen to minimise microbial activity which may affect the selenium speciation. Rock samples were collected from geological outcrops surrounding the soil sampling locations and from Inishcorker Island in the Shannon Estuary where the Clare Shales are very well exposed in a 400 m section (supplementary figure 8.5).

Figure 6.1. Bedrock Geology map showing the location of soil cores FF-1, PK-1, TL-1 and TJ-1 (in red) and rock samples (in black), collected from outcrops of the Clare Shale Formation, the Rathkeale Formation, Shannon Group and the Shanagolden Formation.
6.2.2. Rock and Soil Characterisation

Rock samples were dried at 50°C to remove excess moisture, broken into small fragments (<10 mm) using a pestle and mortar and powdered to (<50 μm) in a ball mill. The soil samples selected for the sequential extraction were first frozen at -80°C and freeze dried to remove moisture but avoid changes to the organic matter composition and chemical speciation. The freeze dried soil was powdered by hand in a pestle and mortar, cleaned with hexane and passed through a metal sieve to avoid organic contamination. The powdered rock and soil samples were made into pellets using 12 g of sample and 3 g of wax and analysed by X-ray fluorescence (XRF) using a PANanalytical Axios sequential X-ray fluorescence spectrometer. The concentrations of elements (mg kg⁻¹) were determined by comparing the spectra peak intensities with known calibration standards using the pro-trace software and Omnian software was used to accurately calculate the major concentrations (wt%) which adjusts to matrix effects. The moisture loss (H₂O wt%), carbon dioxide (CO₂ wt%) and total organic carbon (TOC wt%) were calculated using the loss on ignition method (Nelson and Sommers, 1996). To determine the main mineralogical components, the soil was examined by X-ray diffraction using a Philips PW1730 and Bruker D8Advance fitted with a Göbel mirror.

6.2.3. Sequential Extraction Scheme

To determine the selenium speciation and distribution in the soils, a 5 step sequential extraction protocol modified after Bassil (2016) was applied to the most seleniferous horizon of all 4 soil cores (PK, FF, TL, TJ). At each sequential extraction step 40 ml of extracting solution was applied to the soil residue remaining from the previous step starting with 1.2 g of dry soil. The sequential extraction was repeated in triplicate for each soil sample.

1. Water Extraction
Deionised water was used to extract the soluble selenium which consists mainly of selenate (Se⁶⁺), selenite (Se⁵⁻) and mobile organic selenium (Se³⁺) (Kulp and Pratt, 2004; Matamoros-Veloza et al., 2014). In a glass bottle, 40 ml of 18.2Ω deionised
water was added to 1.2 g of freeze-dried, powdered sample. The bottle was capped and the contents mixed for 2 hours at room temperature using a vortex stirrer. The sample was decanted into a 50 ml polyethylene tube and centrifuged at 6000 rpm for 30 minutes to separate the supernatant from the residue. The supernatant was decanted and 10 ml of deionised water was added to the residue to wash the sample which was centrifuged again. The supernatants were mixed together to make 50 ml.

2. Phosphate Extraction
A phosphate buffer (KH$_2$PO$_4$/K$_2$HPO$_4$) adjusted to pH7 was used to extract the ligand exchangeable selenium which consists mainly of selenite (Se$^{IV}$) adsorbed onto oxides or clay minerals (Kulp and Pratt, 2004; Martens and Suarez, 1996; Matamoros-Veloza et al., 2014). The residue from step 1 was mixed with 40 ml of phosphate buffer (0.1 M) in the polyethylene centrifuge tube. The solution was decanted into a glass bottle and the same stirring and centrifuging protocol applied as the extraction in step 1.

3. Alkaline Extraction
Sodium hydroxide (NaOH) was used to extract base soluble selenium which consists of organic selenium (Se$^{II}$) and tightly bound selenite (Se$^{IV}$) (Martens and Suarez, 1996; Matamoros-Veloza et al., 2014) which are associated to the humic acid and fulvic acid fractions of organic matter (Wright et al., 2003). The residue from step 2 was mixed with 40 ml of sodium hydroxide (0.1 M) in the polyethylene centrifuge tube and decanted into a glass bottle. The bottle was capped and the NaOH solution was mixed in a vortex stirrer for 24 hours at room temperature. The same centrifuge protocol was applied as step 1 and 2 and the supernatants mixed to make a 50 ml volume. NaOH has shown to extract dissolved humic compounds (Kulp and Pratt, 2004) and therefore the supernatant from this sequential extraction step was used for organic analysis by pyrolysis GC/MS.
4. **Sulfur Extraction**

Sodium sulfite (Na$_2$SO$_3$) adjusted to pH 7 with hydrochloric acid (HCL) was used to extract elemental selenium (Se$^0$) without releasing organic selenium and selenides (Se$^{-II}$) (Kulp and Pratt, 2004). The residue from step 3 was mixed with 40 ml of sodium sulfite (1 M) in the polyethylene centrifuge tube. The solution was decanted into a glass bottle, capped and placed in an ultrasonic bath for 8 hours. During sonication, the elemental selenium is oxidised in the soil and is extracted as selenosulfate (Bassil et al., 2016). The same centrifuge protocol was applied as steps 1-3 and the supernatants mixed and diluted to 50 ml.

5. **NaOCl Extraction**

Sodium hypochlorite (NaOCl) was used to oxidise organic matter and extract selenium associated with recalcitrant forms of organic matter (Wright et al., 2003; Zhang and Moore, 1996). The residue from step 4 was mixed with 40 ml of 5% sodium hypochlorite in the polyethylene centrifuge tube. The solution was decanted into a glass bottle, capped and mixed in a vortex stirrer at room temperature for 24 hours. The same centrifuge protocol was applied as steps 1-4 and the supernatants mixed make a volume of 50 ml. Wright et al. (2003) found this step can efficiently extract FeSe and represents metal selenides mixed within the organics. After the extraction, one residue from each sample was analysed by XRD and only duplicates digested in the next step.

6. **Refractory Selenium**

An acid digestion was performed to mineralise the refractory selenium which contains unextractable mono and diselenide minerals. The residue from step 5 and the raw soil was dried at 60°C and weighed into PFA vessels. The soil was mixed with 3 ml of concentrated nitric acid (HNO$_3$) and 3 ml of hydrochloric acid (HCl) and capped overnight. The next day, 1 ml of hydrofluoric acid (HF) was added to the PFA vessels and left for one hour before microwave digestion. The vessels were sealed tightly and digested at 800 W (100%), with a 10 minute ramp to a temperature of 120 °C and held for 50 minutes. Once depressurized, the solution was poured into 120 ml PTFE beakers and heated on a hot plate until the HF evaporated. The
residue was dissolved in 2% ultra-pure HNO₃, reheated again and the remaining residue washed with 2% ultra-pure HNO₃ to make a volume of 50ml.

6.2.4. Post-Extraction Chemical Analysis

To determine the elemental concentrations of the sequential extracts the solutions were first filtered through a 0.45 µm filter and diluted by 1:100 (100 µl sample in 9.9 ml of 2% nitric acid). The replicate samples were analysed with reagent blanks using an inductively coupled plasma mass spectrometer (ICP-MS) Agilent 7500cx equipped with a pressurized octopole collision/reaction cell with a H₂ flow under standard conditions. The aqueous extracts were introduced by a nebulizer with an argon plasma gas flow. The extracts were analysed for selenium (³²Se) with a detection limit of 50-100 ppt. The speciation of selenium was determined in the first 3 steps of sequential extraction scheme with deionised water, phosphate buffer and NaOH by coupling the ICP-MS to a High Pressure Liquid Chromatography (HPLC) system. The mobile phase was delivered with 10 mM ammonium citrate diluted in deionised water according to the chromatographic conditions. Standard solutions (1000 mg L⁻¹) of the selenium species, DL-selenomethionine (SeMet), L-selenocystine (SeCys), sodium selenite (Na₂SeO₃) and sodium selenate (Na₂SeO₄) were prepared using ultra-pure 18.2 Ω deionised water apart from SeCys which prepared in 0.2% HCl. To measure the precision of the chemical data, the mean and standard error was calculated for the replicate samples. The recovery rate for selenium was determined by comparing the sum of selenium concentrations from the extracts with the raw soil sample.

6.2.5. Organic Analysis

The total dissolved organic carbon (TDOC) was measured in the aqueous extracts using a Shimadzu TOC-C CPN analyser. The filtered sample (15 ml) was injected into the sample port and heated at 680°C to measure the total carbon (TC) and acidified in a quartz medium to measure the inorganic carbon (IC). The CO₂ released was measured using an infrared detector and the TDOC was calculated by subtracting the IC from the TC in mg L⁻¹. The NaOH fraction which contained the highest TDOC and the original soil sample were analysed further using pyrolysis gas
chromatograph mass spectrometry (Py-GC/MS) to characterise the complex organic components associated with selenium.

Between 2 mg and 4 mg of freeze dried and powdered soil sample was placed into a quartz tube and 2 μl of 0.05 mg ml^{-1} 5α-androstane diluted in hexane was added and dried. The sample was a heated (20°C per ms) to 600°C using a CDS 5200 series pyroprobe pyrolysis unit. The pyrolysis products were flushed from the pyrolysis probe and injected into the GC/MS via a heated transfer line using helium as the carrier gas with a split ratio of 20:1. The oven was set to a maximum of 320°C programmed at 40°C for 3 minutes then 6°C/min to 320°C for 10 min. The injector temperature was set to 350°C, the MSD transfer line was set to 280°C, the MS source at 230°C (max 250°C) and the MS quadrupole to 150°C (max 200°C). The organic moieties were analysed by the GC/MS scanning a range of m/z 50-700 with ionisation energy of 70 eV and identified on the basis of the retention times. The resulting chromatograms were analysed using MSD Chem Station software and comparison with data in the literature. The pyrolysis moieties were organised into groups of aromatic compounds, phenols, polysaccharides, lignins, nitrogen containing compounds and lipids (n-alkenes and n-alkanes) according to (Vancampenhout et al., 2009).

6.3. Results

6.3.1. Chemical Characterisation of the Soil Cores

X-ray florescence was used to determine the major and trace element composition and distribution of selenium in 4 soil profiles with different underlying lithologies (figure 6.1) collected from dairy pastures in County Limerick. Table 6.1 shows soil core PK-1 was enriched in selenium which increased with the depth of the shallow soil profile. Concentrations ranged from 26 mg kg^{-1} in the top 0-10 cm to 110 mg kg^{-1} in the bottom mineral-rich layer at 30-40 cm. High selenium concentrations at the base are evidence that the source is the underlying Clare Shales which are black, organic rich, Carboniferous deep water shales and mudstones (figure 6.1). The concentration of arsenic and uranium was also elevated in the soil profile but the distribution differed from selenium. The highest uranium concentration was 30.7
mg kg\(^{-1}\) and the highest arsenic was 12.7 mg kg\(^{-1}\) both at a depth horizon of 20-30 cm. This horizon also contained the highest concentration of copper of 189.0 mg kg\(^{-1}\) and vanadium 189.0 mg kg\(^{-1}\). Major analysis showed there was a strong negative correlation of selenium with SiO\(_2\) \((R = 1)\) and Al\(_2\)O\(_3\) \((R = 0.91)\) which decreased with the soil profile depth. The concentration of SiO\(_2\) was 34.5 wt% and Al\(_2\)O\(_3\) was 6.2 wt% at 0-10 cm depth. The total organic carbon (TOC) concentration was most enriched at 20-30 cm depth with 24.2 wt% and had a poor correlation with selenium throughout the profile (figure 5.2).

As discussed in chapter 5, in soil core FF-1 the selenium concentration was highest at a depth horizon of 10-20 cm with 945.2 mg kg\(^{-1}\). In contrast to PK-1, the lowest concentration was at the base of the soil profile with 121.3 mg kg\(^{-1}\), which suggests the source is not the underlying Carboniferous Visean Limestone (figure 6.1) but from surface drainage; with the Clare Shales to the west as the potential source. However, the selenium concentration at the base was higher than in soil core PK-1. There was a positive correlation with arsenic \((R = 0.99)\), uranium \((R = 0.93)\) and to a lesser extent molybdenum \((R = 0.52)\) with selenium (table 6.1). All these elements peaked at depth horizon of 10-20 cm, the same as selenium with a concentration of 45.3 mg kg\(^{-1}\) arsenic, 221.6 mg kg\(^{-1}\) uranium and 252.7 mg kg\(^{-1}\) molybdenum. This horizon also contained the highest concentration of vanadium 620.2 mg kg\(^{-1}\) and manganese 387.7 mg kg\(^{-1}\). Major analysis showed that there was a poor correlation with SiO\(_2\) and Al\(_2\)O\(_3\). SiO\(_2\) decreased with the depth of the soil profile from 40.6 wt% at 0-10 cm to 1.2 wt% at 50-60 cm and Al\(_2\)O\(_3\) decreased from 5.8 wt% to 0.2 wt%. However, the CaO concentration increased with the soil profile depth from 3.7 wt% to 50 wt% and there was a distinctive grey calcareous clay layer at the base. As shown in figure 6.2, the TOC also correlated with selenium \((R = 0.69)\) and peaked at a depth horizon of 10-20 cm with 29.3 wt% which may be causing the toxic trace elements to accumulate in the profile.

As discussed in chapter 4, in soil core TL-1 the selenium concentration was highest at the same depth horizon as PK-1 (30-40 cm) with 171 mg kg\(^{-1}\). However, this was not the base of the profile and the concentration decreased to 61.9 mg kg\(^{-1}\) at 40-50 cm. Similar to soil FF-1 there was a positive correlation for arsenic \((R = 0.90)\),
uranium ($R^2 = 0.93$) and molybdenum ($R^2 = 0.97$) with selenium (table 6.2). The arsenic concentration peaked at 54.6 mg kg$^{-1}$, uranium at 13 mg kg$^{-1}$ but only 2.4 mg kg$^{-1}$ molybdenum, all at a depth horizon of 30-40 cm with selenium. Major analysis showed a poor correlation of CaO with selenium concentration which increased with the soil profile depth similar to FF-1 again with a grey calcareous clay layer at the base of the core. The concentration was 3.1 wt% at the top compared to 18.8 wt% at the base of TL-1. The SiO$_2$ concentration did not vary significantly with a range of 57.1-40.4 wt%. However, there was a strong positive correlation of TOC with selenium with an ($R^2 = 0.96$) with a concentration of 15.1 wt% in the seleniferous horizon similar to FF-1 which supports an important role of organic matter in retaining selenium. The underlying geology is dark Carboniferous Limestone of the Waulsortian Formation (figure 6.1).

In soil core TJ-1 the selenium concentration in the top 0-10 cm of the profile was similar to PK-1 with 23.9 mg kg$^{-1}$, however the soil profile was much deeper and the concentration decreased to 0.20 mg kg$^{-1}$ in the bottom 60-70 cm of the core (table 6.2). This suggests the source is not the underlying Rathkeale Formation which consists of dark-grey argillaceous limestones and shaly mudstones; this is supported by the absence of selenium in rock samples analysed (see table 6.4). Positive correlations were observed with selenium and other potentially ‘toxic’ trace elements arsenic ($R^2 = 0.98$), uranium ($R^2 = 0.86$) and molybdenum ($R^2 = 0.70$) as observed in soil FF-1 and TL-1. The arsenic concentration peaked at 28.4 mg kg$^{-1}$ also at a depth horizon of 0-10 cm. However, the highest uranium concentration was 35.2 mg kg$^{-1}$ and molybdenum 33.70 mg kg$^{-1}$ both at a depth horizon of 20-30 cm. Major analysis showed CaO had a good negative correlation ($R^2 = 0.76$) with selenium and increased with depth reaching a concentration of 28.5 wt% at 60-70 cm. There was a poor correlation with SiO$_2$ and Al$_2$O$_3$ which did not change significantly with the depth of the soil profile. As observed in soil FF-1 and TL-1, there was a strong correlation with TOC ($R^2 = 0.89$) with a concentration of 14.6 wt% at a depth horizon of 0-10 cm (figure 6.2).
Table 6.1. X-ray fluorescence data showing the concentration of major and trace elements in soil core PK-1 and FF-1. $R^2$ = the correlation of selenium with other elements within the soil profile where a value closer to 1 is the better fit to the regression line. TOC was calculated by the loss on ignition method. Highest Se horizons in bold.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Depth (cm)</th>
<th>Se mg kg$^{-1}$</th>
<th>Mo mg kg$^{-1}$</th>
<th>As mg kg$^{-1}$</th>
<th>U mg kg$^{-1}$</th>
<th>V mg kg$^{-1}$</th>
<th>Mn mg kg$^{-1}$</th>
<th>Cu mg kg$^{-1}$</th>
<th>CaO wt%</th>
<th>Fe$_2$O$_3$ wt%</th>
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Table 6.2. X-ray Fluorescence data showing the concentration of major and trace elements in soil core TL-1 and TJ-1. \( R^2 \) = the correlation of selenium with other elements within the soil profile where a value closer to 1 is the better fit to the regression line. TOC was calculated by the Loss on Ignition method. Highest Se horizons in bold.

<table>
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<th>Sample</th>
<th>Depth (cm)</th>
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<th>Mo mg kg(^{-1})</th>
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<th>V mg kg(^{-1})</th>
<th>Mn mg kg(^{-1})</th>
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Figure 6.2. The relationship between selenium concentration (mg kg\(^{-1}\)) and the TOC (wt\%) concentration with the depth of the soil profile in cores PK-1, FF-1, TL-1 and TJ-1.
6.3.2. Chemical Characterisation of Rock Samples

To assess the influence of different parent rocks on the soil geochemistry, rock samples collected from 8 different outcrops were analysed by X-ray fluorescence. Five of the outcrops analysed were of the Clare Shales Formation which is implicated as the source of selenium in the soils of County Limerick (Finch and Ryan, 1966) and is the underlying geology of site PK-1 which had an increase in selenium with soil profile depth (table 6.1). The average selenium concentration from the Clare Shale samples collected was 4.6 mg kg\(^{-1}\). The highest concentrations were observed in the outcrop at Inishcorker (I) which is a small island in the Shannon Estuary (figure 6.1) where the succession is well exposed and is more than 200 m thick; Se concentrations in samples ranged from 0-18 mg kg\(^{-1}\) as shown in table 6.3. Sample I2A with the highest selenium concentration also contained 12.2 mg kg\(^{-1}\) arsenic, 6.2 mg kg\(^{-1}\) uranium and 43.7 mg kg\(^{-1}\) molybdenum which were elevated in the soil profiles analysed. Outcrop M which is from a location where the Clare Shales form part of the hills to the west that overlook soil sampling locations FF-1 and PK-1, also ranged from 0-11.5 mg kg\(^{-1}\). The highest selenium sample also contained 13.6 mg kg\(^{-1}\) arsenic, 9.3 mg kg\(^{-1}\) uranium and 37.8 mg kg\(^{-1}\) molybdenum and therefore this formation is, indeed, likely to be the source of toxic trace elements in the surrounding soils. Major analysis shows the SiO\(_2\) concentration was high in all samples and ranged from 46.0-92.1 mg kg\(^{-1}\) (typical of fine grained clastics) and the CaO concentration was low ranging from 0-1.8 mg kg\(^{-1}\). TOC concentrations of the shale samples ranged from 0-1 wt% and did not correlate with selenium.

As a comparison, 2 outcrops were sampled from the Rathkeale Formation which is the underlying geology of site TJ-1 (figure 6.1) which had a decrease in selenium with the soil profile depth. As expected, the rock samples contained no detectable selenium and low molybdenum concentrations (0.7-1.0 mg kg\(^{-1}\)). However, arsenic ranged from 0-13.2 mg kg\(^{-1}\) and uranium ranged from 4.6-9.4 mg kg\(^{-1}\) (table 6.4).
Table 6.3. X-Ray fluorescence data showing the concentration of major and trace elements of rock samples collected from outcrops of the Clare Shale Formation from locations I, F, O, D and M. TOC was calculated by the loss on ignition method.

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<th>Mo mg kg⁻¹</th>
<th>As mg kg⁻¹</th>
<th>U mg kg⁻¹</th>
<th>V mg kg⁻¹</th>
<th>Mn mg kg⁻¹</th>
<th>Cu mg kg⁻¹</th>
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Table 6.4. X-Ray Fluorescence data showing the concentration of major and trace elements in rock sample collected from the Rathkeale Formation (R and P) The Shannon Group (B) and the Shanagolden formation (Q). TOC was calculated by the loss on ignition method.

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</tr>
<tr>
<td>Shanagolden</td>
<td>Q-1</td>
<td>0.0</td>
<td>0.5</td>
<td>0.2</td>
<td>3.9</td>
<td>2.7</td>
<td>327.6</td>
<td>4.8</td>
<td>52.9</td>
<td>0.3</td>
<td>6.6</td>
<td>0.6</td>
<td>0.1</td>
</tr>
</tbody>
</table>
The remaining rock samples were collected from geological formations surrounding the soil sampling locations which included the Shannon Group, a Carboniferous mudstone, sandstone and siltstone and the Shanagolden Formation, a Carboniferous black, well bedded skeletal limestone which contained no selenium. The Shanagolden Formation contained lower concentrations of the potentially toxic trace elements with 0.5 mg kg\(^{-1}\) molybdenum, 0.2 mg kg\(^{-1}\) arsenic and 3.9 mg kg\(^{-1}\) uranium (table 6.4). Samples from the Shannon Group contained even lower concentrations of molybdenum with 0-0.4 mg kg\(^{-1}\). Concentrations of arsenic ranged from 4.9-7.6 mg kg\(^{-1}\) and uranium concentrations ranged from 4.2-7.1 mg kg\(^{-1}\). Both are unlikely to be the source of selenium in the Limerick area, although more samples need to be analysed.

6.3.3. Sequential Extraction Scheme

The most seleniferous horizon of the soil cores PK-1 (30-40 cm), FF-1 (20-30 cm), TL-1 (30-40 cm) and TJ-1 (0-10 cm) were sub-sampled from the second soil core stored anaerobically to allow analysis of selenium fractionation using a sequential extraction protocol. Figure 6.3 shows the abundance of selenium in each soil fraction which is the percentage of selenium present compared to the total concentration in the extracts. The most abundant selenium fraction in soil sample PK-1, FF-1 and TL-1 was the sulfite fraction which represents elemental selenium (Se\(^{0}\)). Table 6.5 shows the selenium concentration in the sulfite fraction was highest in soil FF-1 with 61.3 ± 3.2 mg kg\(^{-1}\) which was 42% of the total selenium in the soil sample. This is consistent with the soil core chemical data, as the selenium concentration was highest in soil FF-1 and suggests the majority of selenium is present as an immobile phase. Sample TL-1 contained 42.7 ± 2.5 mg kg\(^{-1}\) selenium in the sulfite fraction which was 37% of the total selenium in the soil sample. Sample PK-1 contained 17.4 ± 0.9 mg kg\(^{-1}\) selenium in the sulfite fraction which was 35% of the total selenium abundance.

The second most abundant fraction in soil FF-1 and PK-1 was the NaOCl fraction which represents selenium associated with recalcitrant organic matter and suggests reducing soil conditions are prevalent. In FF-1 the selenium concentration in the
NaOCl fraction was highest with 46.5 ± 5.6 mg kg\(^{-1}\) which was 32% of the total selenium and in sample PK-1 the selenium was 15.5 ± 1.1 mg kg\(^{-1}\) which was 31% of the total selenium. The second most abundant fraction in sample TL-1 was the alkaline extract (25%) which represents base soluble selenium associated with the fulvic and humic acid fractions of organic matter; however this was closely followed by the NaOCl fraction (24%) associated with recalcitrant organic matter. In TL-1 the concentration of Se in the alkaline fraction was 28.6 ± 2.1 mg kg\(^{-1}\) and in the NaOCl fraction it was 27.7 ± 2.2 mg kg\(^{-1}\).

Table 6.5. The average concentration of selenium measured in triplicate ± the standard error in the sequential extracts for soil samples PK-1, FF-1, TL-1 and TJ-1 determined by ICP-MS.

<table>
<thead>
<tr>
<th>Extracting Solution</th>
<th>PK-1 (\text{Se mg kg}^{-1})</th>
<th>FF-1 (\text{Se mg kg}^{-1})</th>
<th>TL-1 (\text{Se mg kg}^{-1})</th>
<th>TJ-1 (\text{Se mg kg}^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>H(_2)O</td>
<td>1.7 ± 0.2</td>
<td>1.1 ± 0.6</td>
<td>9.2 ± 0.4</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Phosphate</td>
<td>2.0 ± 0.2</td>
<td>8.4 ± 0.1</td>
<td>4.4 ± 0.4</td>
<td>3.5 ± 0.4</td>
</tr>
<tr>
<td>Alkaline</td>
<td>12.5 ± 1.1</td>
<td>27.3 ± 2.6</td>
<td>28.6 ± 2.1</td>
<td>13.4 ± 0.6</td>
</tr>
<tr>
<td>Sulfite</td>
<td>17.4 ± 0.9</td>
<td>61.3 ± 3.2</td>
<td>42.7 ± 2.5</td>
<td>3.0 ± 0.4</td>
</tr>
<tr>
<td>NaOCl</td>
<td>15.5 ± 1.1</td>
<td>46.5 ± 5.6</td>
<td>27.7 ± 2.2</td>
<td>2.8 ± 0.6</td>
</tr>
<tr>
<td>Acid Digestion</td>
<td>0.9 ± 0.1</td>
<td>1.6 ± 0.0</td>
<td>2.6 ± 0.2</td>
<td>2.3 ± 0.2</td>
</tr>
</tbody>
</table>

Figure 6.3. The abundance of selenium (%) in the H\(_2\)O, phosphate, alkaline, sulfite, NaOCl and acid digestion extracts in soil samples PK-1, FF-1, TL-1 and TJ-1 calculated from the total amount of selenium in the extracts.
The alkaline fraction was the most abundant fraction in soil TJ-1 with a selenium concentration of $13.4 \pm 0.6 \text{ mg kg}^{-1}$ which was 53% of the total selenium in the sample. The high abundance of selenium in this fraction is likely because it was present at the surface at a depth horizon of 0-10 cm which is high in organic matter. The second most abundant fraction in sample TJ-1 was the phosphate fraction which represents ligand exchangeable selenite desorbed from oxides or clays. The concentration of selenium in this fraction was $3.5 \pm 0.4 \text{ mg kg}^{-1}$ and was 14% of the total selenium in the sample. The $\text{H}_2\text{O}$ extract was the least abundant for soil samples FF-1 and TJ-1 which represents soluble selenate and selenite. Whereas the least abundant in sample TL-1 and PK-1 was the acid digestion extract which represents refractory selenium. However, the recovery of selenium from the whole sequential extraction compared to the total selenium concentration in the raw soil sample ranged from 70-82%. Therefore, there may have been loss of material which could affect the abundance of selenium in each fraction.

**Selenium Speciation**

The speciation of selenium was determined in the first three extracts of the sequential protocol. The abundance of selenium species was determined from the total selenium concentration determined in the extracts by ICP-MS. Figure 6.4 shows the most abundant selenium species present in all four samples was selenite (SeIV). Table 6.6 shows that the highest selenite concentrations were observed in the alkaline fraction which suggests selenite is strongly associated with the humic and fulvic acid fractions of organic matter. The selenite concentration was highest in the alkaline fraction of sample TL-1 with $22.2 \pm 1.1 \text{ mg kg}^{-1}$, followed by FF-1 with $16.9 \pm 1.1 \text{ mg kg}^{-1}$, TJ-1 with $9.0 \pm 0.5 \text{ mg kg}^{-1}$ and PK-1 with $1.4 \pm 0.0 \text{ mg kg}^{-1}$. Most of the remaining selenite was in the phosphate extract desorbed from oxides or clays. Selenate was only detected in the $\text{H}_2\text{O}$ extract of soil sample TL-1 with $9.4 \pm 0.1 \text{ mg kg}^{-1}$. This suggests TL-1 contains a small concentration of mobile selenium species. Selenocystine could not be quantified in the soil extracts due to concentrations present that were below the detection limits.
Table 6.6. The average concentration of selenite and selenate measured in duplicate ± the standard error in the sequential extracts for soil samples PK-1, FF-1, TL-1 and TJ-1 determined by ICP-MS.

<table>
<thead>
<tr>
<th>Extracting Solution</th>
<th>PK-1</th>
<th>FF-1</th>
<th>TL-1</th>
<th>TJ-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂O</td>
<td>1.4 ± 0.0</td>
<td>1.1 ± 0.0</td>
<td>1.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Phosphate</td>
<td>1.5 ± 0.1</td>
<td>6.9 ± 0.2</td>
<td>2.4 ± 0.1</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>Alkaline</td>
<td>8.0 ± 0.3</td>
<td>16.9 ± 1.1</td>
<td>22.2 ± 1.1</td>
<td>9.0 ± 0.5</td>
</tr>
</tbody>
</table>

Figure 6.4. The abundance of selenite, selenate and unidentified selenium calculated from the total selenium concentration in the H₂O, phosphate and alkaline extracts in soil samples PK-1, FF-1, TL-1 and TJ-1.
The selenomethionine concentration could not be quantified as the standard changed species during the analysis and two peaks were identified as shown in supplementary figure 8.6. However, the abundance of 'unidentified' selenium was calculated by subtracting the selenite and selenate concentration from the total selenium values shown in figure 6.4. In sample PK-1 and FF-1 the highest abundance of unidentified selenium was found in the alkaline fraction, whereas in sample TL-1 and TJ-1 the highest abundance was in the phosphate fraction.

**Total Dissolved Organic Carbon**

Table 6.7. The average concentration of TDOC measured in triplicate ± the standard error in the sequential extracts for soil samples PK-1, FF-1, TL-1 and TJ-1.

<table>
<thead>
<tr>
<th>Extracting Solution</th>
<th>PK-1 TDOC mg g⁻¹</th>
<th>FF-1 TDOC mg g⁻¹</th>
<th>TL-1 TDOC mg g⁻¹</th>
<th>TJ-1 TDOC mg g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂O</td>
<td>1.0 ± 0.0</td>
<td>1.8 ± 0.1</td>
<td>2.5 ± 0.2</td>
<td>2.2 ± 0.1</td>
</tr>
<tr>
<td>Phosphate</td>
<td>3.0 ± 0.1</td>
<td>5.9 ± 0.2</td>
<td>9.4 ± 0.7</td>
<td>8.0 ± 0.2</td>
</tr>
<tr>
<td>Alkaline</td>
<td>39.6 ± 0.9</td>
<td>70.6 ± 2.3</td>
<td>92.4 ± 3.9</td>
<td>64.0 ± 2.8</td>
</tr>
<tr>
<td>Sulfite</td>
<td>4.9 ± 0.3</td>
<td>7.9 ± 0.6</td>
<td>6.4 ± 0.4</td>
<td>4.0 ± 0.4</td>
</tr>
<tr>
<td>NaOCl</td>
<td>16.2 ± 6.8</td>
<td>31.8 ± 1.7</td>
<td>27 ± 1.0</td>
<td>21.5 ± 0.6</td>
</tr>
</tbody>
</table>

Figure 6.5. The abundance of TDOC in the H₂O, phosphate, alkaline, sulfite and NaOCl extracts in soil samples PK-1, FF-1, TL-1 and TJ-1 calculated from the total amount of TDOC in the extracts.
The total dissolved organic carbon (TDOC) was analysed in the sequential extracts from steps 1-5 before the acid digestion protocol to determine whether selenium is associated with the organic fractions. Figure 6.5 shows that the TDOC abundance in all 4 soil samples was highest in the alkaline fraction which was expected as this is the fraction selenium associated with the fulvic and humic acid fractions of organic matter. Within the alkaline fraction the highest TDOC concentration was in sample TL-1 with 92.4 ± 3.9 mg g⁻¹ which was 67% of the total observed in the sample (table 6.7). Sample TL-1 was sampled at 30-40 cm depth and indicates an organic rich horizon at depth. This was followed by sample FF-1 with a TDOC of 70.6 mg g⁻¹ which was 60% of the total sample. Sample TJ-1 was 64.0 ± 2.8 mg g⁻¹ which was 64% of the total and PK-1 was 39.6 ± 0.9 which 61% of the total. In the alkaline fraction the concentration of TDOC appeared to positively correlate with the selenium concentration (R² = 0.69) of the samples. The NaOCl extract was also elevated in TDOC which is the fraction of selenium associated to recalcitrant organic matter. The highest concentration was in sample FF-1 with 31.8 ± 1.7 mg g⁻¹ which was 27% of the total TDOC in the sample. TL-1 had 27 ± 1.0 mg g⁻¹ which was 20% of the total TOC, TJ-1 had 21.5 ± 0.6 mg g⁻¹ which was 33% of the total and PK-1 was 16.2 ± 6.8 mg g⁻¹ which was 25% of the total. PK-1 had the lowest overall TDOC concentration as it was sampled at the bottom of a soil profile in mineral rich layer. Minor amounts of TDOC was released in the phosphate and sulfite fraction and the lowest abundance was in the H₂O fraction (<2.2 mg g⁻¹).

6.3.4. Organic Analysis

The original soil material collected from the seleniferous horizon and the alkaline extract which contained the highest TDOC values (39.6-92.4 mg g⁻¹) were analysed by Py-GC/MS to determine the abundance of organic moieties. The pyrolysis products were first identified and the peak areas calculated to determine the abundance. All identified organic moieties are provided in supplementary table 8.6-8.7. The pyrolysis moieties were separated into 7 compound categories based on chemical similarity: aromatics, nitrogen containing compounds, polysaccharides, phenols, lignin and lipids (figure 6.6). The pyrolysis moieties for soil sample PK-1 which was collected at the base of the profile at 30-40 cm depth was dominated by
48% lipids, followed by 17% lignin. In soil sample FF-1 which was the most seleniferous and organic rich sample collected at 10-20 cm depth was also dominated by 31% lipids however this was followed by 24% phenols and 23% aromatics. Soil sample TL-1 which was collected from 30-40 cm depth was similar to FF-1 and dominated by 29% lipids, followed by 22% phenols and 20% aromatics. Sample TJ-1 which had the lowest selenium concentration contained the highest abundance of aromatics with 41% which was followed by 34% lipids.

Figure 6.6. The relative composition (%) of aromatics, nitrogen containing compounds, polysaccharides, phenols, lignin markers and lipids in the original soil material from cores PK-1, FF-1, TL-1 and TJ-1 and the dried alkaline extracts from step 3 of the sequential extraction protocol for each soil.

The high abundance of lipid moieties in all samples is due to the presence of n-alkenes/n-alkanes doubles and also prist-1-ene. Figure 6.7 shows the gas chromatograms of the pyrolysates with the 8 largest peaks labelled. For samples PK-1, FF-1 and TL-1 the largest peak was prist-1-ene and there was a positive correlation of prist-1-ene with selenium concentration ($R^2 = 0.84$). The prist-1-ene peak was largest in FF-1 which had the highest TOC (29.3 wt%) and highest selenium concentration (945 mg kg$^{-1}$) in the original soil core material. The highest peak in TJ-1 was the aromatic compound toluene. The aromatic compound styrene
was also a dominant peak in the chromatogram of TJ-1. The high abundance of phenols in samples FF-1 and TL-1 was influenced by the large peaks in phenol and ¾ methylphenol. In contrast, PK-1 which had a lower abundance of phenols and aromatics had large peaks in the lignin markers 4-methoxyphenol (guaiacol) and 4-vinylguaiacol. The abundance of the pyrolysis moieties changed in the alkaline extracts, which are the fraction of base soluble selenium associated with the fulvic acid and humic acid fractions of organic matter. Compared to the original soil material, there was an increase in the abundance of aromatic and phenol moieties but a decrease in the abundance of lipid moieties (figure 6.6). As a result, all soil extracts were dominated by aromatics (37-47%) and phenols (28-48%). Soil TJ-1 had the largest changes in abundance with a 29% increase in phenols and a 29% decrease in lipids. However FF-1 had the largest change in the abundance of aromatics with a 25% increase. Although polysaccharides were present in the original soil samples they were absent in the extracts from soil samples TL-1 and PK-1 and <1% in samples FF-1 and TJ-1. There was also a decrease in the abundance of lignin markers.

The largest peak in the extracts was the aromatic compound toluene with the exception of soil extract PK-1 which was the lipid prist-1-ene followed by the aromatic compound toluene. The prist-1-ene peak was smaller in the extracts compared to the original soil material although there was still a positive correlation with selenium concentration ($R^2 = 0.69$). The prist-1-ene peak was largest in soil extract TL-1 which had the highest TDOC with 92.4 ± 3.9 mg g⁻¹ and highest selenium concentration with 28.6 ± 2.1 mg kg⁻¹ compared to the other soil samples.
Figure 6.7. Chromatograms showing the peaks in pyrolysis moieties for soil samples PK-1 and FF-1 and the alkaline extract from both soils with the 8 largest peaks labelled. 1- Toluene, 2 – Styrene, 3 – Phenol, 4 - 4-methoxyphenol (guaiacol), 5 - ¾ methylphenol, 6 - 4-vinylguaiacol, 7 - Prist-1-ene, 8 - Androstane Standard.
Figure 6.8. Chromatograms showing the peaks in pyrolysis moieties for soil samples PK-1 and FF-1 and the alkaline extract from both soils with the 8 largest peaks labelled. 1- Toluene, 2 – Styrene, 3 – Phenol, 4 - 4-methoxyphenol (guaiacol), 5 - ¾ methylphenol, 6 - 4-vinylguaiacol, 7 - Prist-1-ene, 8 - Androstanone Standard.
6.3.5. X-ray Diffraction Analysis

X-ray diffraction was used to analyse the mineralogy of the raw soil sample and the soil residue from the NaOCl step before the acid digestion to determine if any minerals were released with selenium during the sequential extraction protocol. In sample PK-1 the dominant mineral in the original soil material was calcite followed by quartz and minor amounts of dolomite, muscovite and clinochlore. Soil FF-1 had the same mineralogy which suggests they originate from the same source; however there was a dominance of quartz over calcite. In samples TL-1 and TJ-1 there was also a dominance of quartz with minor amounts of muscovite and clinochlore. In the soil residue for PK-1 and FF-1, there was a decrease in calcite and dolomite during the extraction protocol. The mineral siderite was also identified in the soil residue which was not observed in the original soil analysis. However, the soil residues from soils TL-1 and TJ-1 remained relatively unchanged from the original soil material.

6.4. Discussion

6.4.1. Selenium Concentrations in Soil and Rocks

All soil profiles collected in West Limerick were highly seleniferous and concentrations exceeded the world soil average of 0.4 mg kg\(^{-1}\) and the trigger action value (TAV) for agricultural land of 3-10 mg kg\(^{-1}\) (table 6.1 and 6.2) (Kabata-Pendias, 2011). Selenium was positively correlated with the total organic carbon in all soil profiles except for PK-1 where selenium was most concentrated (110.2 mg kg\(^{-1}\)) at the base of the soil profile. Soils high in organic matter can accumulate selenium as it increases microbial activity, coupled with the presence of electron donors it stimulates the reduction of bioavailable selenium species to insoluble reduced species that accumulate (Zhang and Moore, 1996). A positive correlation of selenium with carbon was also observed in a study by Fellowes et al. (2013) on the seleniferous soils of County Meath, Ireland.

In soil profile PK-1 the underlying geology is the Clare Shale Formation which comprises black shales and mudstones believed to be the source of selenium and molybdenum in the soils of County Limerick (Finch and Ryan, 1966). Samples collected from outcrops of the Clare Shales confirmed the presence of selenium
concentrations which significantly exceeded the average crustal abundance of 0.05 mg kg\(^{-1}\) (table 6.3) (Kabata-Pendias, 2011). Concentrations of 18 mg kg\(^{-1}\) were found at Inishcorker Island (table 6.3), however, a number of samples contained no detectable selenium which shows concentrations can vary in different beds of the same geological formation (Dhillon and Dhillon, 2003). The Clare Shale samples collected from the hills to the west of the seleniferous soil sampling locations contained up to 11.5 mg kg\(^{-1}\) Se. Therefore, chemically weathered sediment from these hills will have been transported via groundwater and streams into the agricultural sites leading to selenium enrichment in the organic-rich horizons (Finch and Ryan, 1966; Fleming, 1961). The mineralogy of the soils in the seleniferous horizons consists mainly of quartz and minor amounts of muscovite and clinochlore, consistent with a fine grained clastic source, rather than from the underlying carbonates. However, sites PK-1 and FF-1 also contained calcite and dolomite which may be the influence of the underlying carbonates (Braithwaite, 1993).

The highest concentrations of selenium were in the soil cores collected from site FF-1 with 945.2 mg kg\(^{-1}\) and site TL-1 with 171 mg kg\(^{-1}\) (table 6.2 and 6.3). Both sites are low lying and poorly drained which are typical of seleniferous soils in Limerick (Finch and Ryan, 1966). The underlying geology of both sites is limestone which typically contains low concentrations of selenium within the range of 0.03–0.08 mg kg\(^{-1}\) (Fordyce, 2013). Both soil profiles also have a distinctive grey calcareous clay layer at the base of the soil profile, which resembles a lacustrine clay that has likely prevented selenium from leaching out of the system (McGrath and Fleming, 2008). The lowest concentrations of selenium were observed in soil TJ-1 with 23.9 mg kg\(^{-1}\) at the surface (0-10 cm) which decreased with soil profile depth. The underlying bedrock is the Rathkeale Formation which consists of limestones and mudstone and analysis of samples detected no selenium which supports the hypothesis that selenium originates from an external source into the sediment at the surface.

In all sites the most seleniferous horizons of the soil were also enriched with uranium with concentrations ranging from 13.0 - 221.6 mg kg\(^{-1}\) compared to the global soil average of 3 mg kg\(^{-1}\) (Kabata-Pendias, 2011). Molybdenum concentrations were also high in soil profile FF-1 with 252.7 mg kg\(^{-1}\), however
concentrations in all soil profiles were still considered molybdeniferous (>0.3 mg kg\(^{-1}\)) (McGrath and Fleming, 2008). Similar to the soils, the Clare Shale samples were enriched with uranium (table 6.3) and were higher than the crustal abundance of 2 mg kg\(^{-1}\) (Kabata-Pendias, 2011). This relates to Clare Shales forming in anoxic deepwater basins were seawater containing soluble U\(^{VI}\) ions are reduced to insoluble U\(^{IV}\) leading to enrichment (Lüning and Kolonic, 2006). Molybdenum was also enriched compared to the average crustal value of 1.5 mg kg\(^{-1}\). Molybdenum is known to be enriched in black shales in the oxidation states between Mo\(^{IV}\) and Mo\(^{VI}\) (Helz et al., 1996).

6.4.2. Selenium Fractionation and Speciation in Soil

Selenium was most abundant in the fraction of soil associated with elemental selenium (Se\(^0\)) in sites PK-1, FF-1 and TL-1 (figure 6.3). Elemental selenium is formed by selenium reducing microbes and is prevalent in anaerobic environments (Kulp and Pratt, 2004; Oremland et al., 1989). Elemental selenium accumulates in soil as it is insoluble and not available for plant uptake (Fordyce, 2007). Soil sample FF-1 had the highest fraction of elemental selenium (61.3 ± 3.2 mg kg\(^{-1}\) Se) which explains the accumulation of highly seleniferous concentrations in the soil core. Elemental selenium is likely to be present as Se nanoparticles which can be formed by the reduction of the bioavailable oxyanions selenate and selenite by bacterial species such as Geobacter sulfurreducens, Shewanella oneidensis and Veillonella atypical (Pearce et al., 2008; Pearce et al., 2009). The dominance of elemental selenium in soil TL-1 and FF-1 supports the XAS data discussed in chapters 4 and 5. Selenium was also abundant in the fractions associated with recalcitrant organic matter (24-32%) and the fulvic and humic acid fraction of organic matter (19-25%). Within the fulvic and humic acid fraction selenium was present as both selenite (Se\(^{IV}\)) and ‘unidentified’ Se species attributed to organic selenium species (Se\(^{II}\)) which are formed by the assimilatory microbial reduction of selenium oxyanions (Kulp and Pratt, 2004). Organic selenium and selenite (Se\(^{IV}\)) are soluble and bioavailable for plant uptake (Girling, 1984) and hence the observed problems with livestock health.
However, in soil sample TJ-1 selenium was most abundant only in the fulvic and humic acid fractions of organic matter and was present mainly as selenite (Se\textsuperscript{IV}) (figure 6.4). 33% was present as ‘unidentified’ selenium which again, is likely attributed to organic selenium compounds (Se\textsuperscript{II}) (Kulp and Pratt, 2004). The high abundance of the bioavailable selenite oxyanion has likely caused selenium to be removed by plants from the soil profile more readily than soil FF-1, TL-1 and PK-1 which were dominated by insoluble elemental selenium and explains the lower selenium concentration in soil profile TJ-1 (23.9 mg kg\textsuperscript{-1}). Selenium was also present in soil TJ-1 in the fraction associated with ligand exchangeable selenite desorbed from oxides or clays (figure 6.3). The lower concentration of selenite in this fraction suggests sorption is greatest to fractions of soil high in organic matter. This was also observed by Coppin et al. (2009) with >400 mg kg\textsuperscript{-1} selenite in the organic fraction compared to <200 mg kg\textsuperscript{-1} in the mineral fraction of soil.

6.4.3. Selenium Associated to Organic Matter

The soil organic matter from the seleniferous horizons of soil cores PK-1, FF-1 and TL-1 were most abundant in lipid moieties which included n-alkanes, n-alkenes and prist-1-ene (figure 6.6). The origin of n-alkanes and n-alkenes is possibly aliphatic biopolymers such as cuticle material in plants or soil microorganisms (Saiz-Jimenez and De Leeuw, 1986; van Bergen et al., 1998). Prist-1-ene was the highest peak in the chromatograms (figure 6.7 and 6.8) which is an isoprenoid alkene and the primary origin is believed to be the phytol side chain of chlorophyll (Larter et al., 1979). Prist-1-ene frequently occurs in shale, although the incorporation into sediment is still poorly understood; the thermal history since the Carboniferous precludes its preservation in the Clare Shale (SEPMSTRATA, 2018) although according to Ishiwatari et al. (1991) prist-1-ene is often found in the pyrolysis products of kerogen as a result of long term diagenesis of fractions of sedimentary organic matter containing chlorophylls. Prist-1-ene appeared to positively correlate with selenium in the soils and was highest in sample FF-1 which contained 945.2 mg kg\textsuperscript{-1} Se and lowest in TJ-1 which contained 23.9 mg kg\textsuperscript{-1} Se. Therefore selenium may be associated with the humification of plant matter which contains chlorophyll. The soil organic matter from the seleniferous horizon of soil core TJ-1 was most
abundant in aromatic moieties. Aromatic compounds are derived from tannins, polyphenols and proteins (toluene) in soil organic matter (Vancampenhout et al., 2009) and toluene was the largest peak in the chromatogram.

In soils TL-1 and FF-1 there was also a high abundance of phenols which can be derived from multiple sources but they most likely originate from lignin through the oxidation of side chains (Lobe et al., 2002; van Bergen et al., 1998). In soil PK-1 there was also a high abundance of lignin markers which is a marker for plant residue (Lobe et al., 2002) and is associated to the ‘slow pool’ of organic matter (Dungait et al., 2008). The presence of lignin at 30-40 cm depth in core PK-1 may indicate burial of plant matter. The lignin was present in all the soil samples mainly as methyloxyphenol (guaiacol) which is indicative of vegetation in soil organic matter (Ashton, 2014; Saiz-Jimenez and De Leeuw, 1986) and 4-vinyl-guaiacol which is produced from lignin oxidation of non-woody vascular plant tissues (Dungait et al., 2008; van der Hage et al., 1993). The soils were collected from grass pastures which may explain the presence of 4-vinyl-guaiacol in the seleniferous horizons. TJ-1 contained the lowest abundance of lignin markers which may indicate faster decomposition rates in the soil which was sampled from the surface (0-10 cm) (van Bergen et al., 1997).

The sequential extraction scheme showed the fraction of selenium associated with the fulvic acid and humic acid fraction of organic matter accounted for 18-54% of the total Se concentration depending on the soil sample (figure 6.5) also contained the highest concentration of total dissolved organic carbon (TDOC). These soils differ from those described by Wright et al. (2003) who observed a higher amount of organic carbon released in the fraction associated with recalcitrant organic matter. Humic and fulvic acids mainly consist of aromatics (carbon rings) mixed with weak aliphatic (carbon chains) (Derrien et al., 2017; Pettit, 2004). Therefore Py-GCMS of the extracts showed an abundance of aromatic moieties and toluene was the largest peak in all samples except PK-1. The prist-1-ene peak still remained in the extracts and was highest in soil TL-1 which had the highest selenium concentration and total dissolved organic carbon concentration of the 4 extracts. Therefore, selenium is likely associated to aliphatic biopolymers from plants and
not to aromatic compounds in organic matter which supports the conclusion in Bassil et al. (2016).

6.5. Conclusions

In summary, this work has identified areas of County Limerick, Ireland with highly seleniferous soil concentrations which varied with soil profile depth and contained up to 945.2 mg kg\(^{-1}\) Se. Selenium was strongly associated with the total organic carbon, with the exception of soil profile PK-1 which had the highest concentration of selenium at the base of the profile. The underlying lithology of site PK-1 is the Clare Shale Formation which is the source of selenium and other potentially deleterious elements such as molybdenum and uranium in the soils of the Limerick area. The Clare Shale samples contained selenium concentration ranging from 0-18 mg kg\(^{-1}\) showing variation in different geological beds. Over time, chemically and physically weathered sediment from the Clare Shales has been transported from the hills in the west and into the poorly drained, low lying pastures which are underlain mainly with limestone. The most seleniferous horizons of the soil profiles were abundant in lipid moieties, in particular prist-1-ene which likely originates from chlorophyll and suggests selenium is associated with burial of plant material. Soil profile TJ-1 had the lowest selenium concentration (23.9 mg kg\(^{-1}\)) and contained the lowest abundance of prist-1-ene and instead was dominated by aromatic moieties.

Sequential extraction analysis has showed that selenium is fractionated in the 3 most seleniferous soil profiles predominantly as insoluble elemental selenium Se\(^0\) causing the trace element to accumulate. However, selenium was also abundant in both the recalcitrant organic matter fraction and the fulvic and humic acid fraction which may contain organic selenium compounds. In contrast, soil profile TJ-1 which only contained selenium in the top horizons of soil was associated predominantly with the fulvic acid and humic acid fractions of organic matter. This fraction contained high concentrations of the soluble species selenite which is bioavailable to plants and therefore uptake may have caused the lower soil selenium concentrations compared to the 3 other sites. This work shows how the organic
matter content, topography, drainage and local geology exert a huge influence on the fractionation of selenium in the soils of West Limerick. Also this study deals with a complex natural ‘real’ system and it is the finding here that require further examination (as opposed to experimental systems). For instance future works needs to focus on the speciation of selenium in shale samples and the behaviour during weathering to understand the processes leading to transport prior to enrichment.

6.6. References


Oremland, R.S., Hollibaugh, J.T., Maest, A.S., Presser, T.S., Miller, L.G., Culbertson, C.W., 1989. Selenate Reduction to Elemental Selenium by Anaerobic Bacteria in Sediments and
Chapter 7 – Conclusions and Further Work

7.1. Conclusions

Seleniferous soil locations were identified in dairy pastures in West Limerick, Ireland using data on soil selenium concentrations (Fleming, 1961; McGrath and Fleming, 2008), reports of selenium toxicity in livestock (Fleming and Walsh, 1956; Rogers et al., 1990) and with the guidance of TEAGASC, The Irish Agriculture and Food Development Authority. Soil samples collected from 5 agricultural sites showed selenium distribution was inhomogeneous and concentrations in the most seleniferous site ranged from 1.0 to 1265.8 mg kg\(^{-1}\) within the same field. Most of the selenium toxic soils were also enriched with molybdenum and concentrations ranged from 2.8 to 1627.5 mg kg\(^{-1}\) in the most molybdeniferous site. Pastures containing a mixture of perennial grass species and white clover (Trifolium repens) which are considered non-accumulators contained concentrations of both selenium and molybdenum which are toxic to livestock health. The uptake and translocation of selenium was largest in white clover species with concentration of 296.15 mg kg\(^{-1}\) in the leaves. Therefore, pastures overlying seleniferous soils should avoid seed mixtures containing Trifolium repens. Selenium was present in the seleniferous horizon of selected soil cores as a reduced inorganic phase predominantly as elemental selenium (Se\(^0\)) which caused accumulation in the organic rich horizons of soil. However, the presence of the reduced organic species SeMet (Se\(^{II}\)) caused the uptake in plants as it is highly bioavailable. The selenium accumulator species Brassica oleracea accumulated up to 971.72 mg kg\(^{-1}\) selenium and 1000.35 mg kg\(^{-1}\) molybdenum in the leaves and shows promising capabilities for phytoremediation.

To further understand the terrestrial cycling of selenium the research focus moved onto the rhizosphere and the activity of soil microorganisms which influence selenium speciation and mobility. The predominant soluble phase in the soil was likely the organic selenium species SeMet (Se\(^{II}\)) as no oxyanions were detected in the soil solution. Microcosm studies of 2 seleniferous soils showed anaerobic conditions decreased the solubility of selenium and thus the bioavailability which may indicate microbial methylation of organic species is occurring. However,
anaerobic conditions increased the solubility of molybdenum and uranium which were also elevated in the soil. Molybdenum was strongly associated to iron and was released into solution as molybdate by the reductive dissolution of Fe\textsuperscript{III}(hydroxy)oxides in anaerobic conditions. Molybdate is bioavailable to plants which highlights a challenge for remediation of soils containing high concentrations of both selenium and molybdenum using microorganisms. Microaerophillic conditions and the addition of nitrate to the anaerobic microcosms did not increase the solubility of the elemental selenium (Se\textsuperscript{0}) and selenides (Se\textsuperscript{II}) already present in the soil, however the rate of Fe\textsuperscript{III} reduction was hindered. The microbial community was dominated by *Proteobacteria*, *Actinobacteria* and *Acidobacteria* which remained relatively stable and tolerant of changing physiochemical condition during the microcosm experiment.

Sequential extraction techniques were used to understand the fractionation of selenium in 4 soil profiles overlying different Carboniferous lithologies. All soils were highly seleniferous but concentrations in the most enriched horizons varied from 23.9–945.2 mg kg\textsuperscript{-1}. Selenium was positively correlated with the total organic carbon in 3 of the soil profiles which were all low lying and poorly drained. The remaining soil profile was well drained and contained the highest selenium concentration at the base of the soil profile below the organic rich horizons. The underlying geology of this site was the Clare Shale Formation which contained concentrations of selenium ranging from 0–18 mg kg\textsuperscript{-1}. The Clare Shale Formation forms part of the hills to the west of the seleniferous soils and therefore weathering of the rocks and transport of sediment has caused enrichment of selenium and other deleterious elements in the low lying, poorly drained soils which supports McGrath and Fleming (2008). The most seleniferous horizons of the soil profiles were abundant in lipid moieties, in particular prist-1-ene which originates from chlorophyll and suggests selenium is associated with the burial of plant material. Sequential extraction techniques showed the 3 most seleniferous soil profiles contained selenium mainly in the fraction of soil associated with insoluble elemental selenium (Se\textsuperscript{0}) causing the trace element to accumulate. Whereas the soil profile with 23.9 mg kg\textsuperscript{-1} Se at the surface was associated predominantly with
the fulvic acid and humic acid fractions of organic matter. This fraction contained high concentrations of the soluble species selenite (Se\textsuperscript{IV}) which is bioavailable to plants leading to subsequent removal from soil.

The research presented in this thesis extends the knowledge of the biogeochemical cycling of selenium in the natural environment. The findings show selenium distribution in soils was very inhomogeneous and is influenced largely by the organic carbon content, redox conditions, speciation and geology. The uptake of both selenium and molybdenum in pastures pose a risk to livestock health however *Brassica oleracea* plants show potential for phytoextraction which can be used to supplement deficient areas.

### 7.2. Further Work

To understand the spatial distribution of selenium in soils in more detail and help predict future outbreaks of livestock selenosis, a more systematic sampling approach needs to be applied on <10 m\textsuperscript{2} scale as this research showed selenium toxic areas are in very close proximity to deficient areas. Using fixed sampling grids will ensure a more detailed and accurate description of localised selenium distribution. These maps can be used by farm managers at TEAGASC to ensure safe farming practices in the future.

*Brassica oleracea* plants showed potential for phytoextraction of selenium toxic soils however, the mechanisms which allow selenium to accumulate in the plant tissues in such high concentrations are still poorly understood. High resolution imaging is required to highlight the principle storage sites of selenium and the distribution in sub cellular layers that could not be identified by µ-XRF techniques in this thesis. Techniques such as STXM (scanning transmission soft X-ray microscopy), and TEM (transmission electron microscopy) can provide the high resolution required. Techniques such as NanoSIMS (secondary ion mass spectrometry) could also be used to analyse both selenium and the analogue sulfur, to determine how these elements compete for plant uptake and provide more insight into uptake pathways.
Microcosm studies suggest microbial methylation of organic selenium species may be occurring in the anaerobic conditions. To confirm the presence of volatile organic selenium species such as dimethylselenide (DMSe), future experiments should include analysis of the head space gases in the microcosm bottles by connecting to a gas chromatograph. There is a lack of research on the methylation of organic selenium species such as selenomethionine (SeMet) which is highly bioavailable and volatilisation is another potential method of remediating the seleniferous soils.

As selenium-rich sediment from the Clare Shales has likely caused enrichment in the soils, speciation analysis using a combination of XAS and sequential extraction techniques is required on seleniferous rock samples which will help understand the processes occurring during weathering and transport to the soils. EXAFS analysis on the seleniferous soil horizons can also provide more information on the coordination environment. Finally, the drainage history in West Limerick since the soils developed (15,000 BP) needs to be studied in detail to understand the transport of selenium in the environment.

7.3. References

Table 8.1. X-ray fluorescence data showing the concentration of major and trace elements in soil samples collected at 10-20 cm depth at sites MO and PK. LOI = loss on ignition.

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Table 8.2. X-ray fluorescence data showing the concentration of major and trace elements in soil samples collected at 10-20 cm depth at site FF. LOI = loss on ignition.

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Table 8.4. X-ray fluorescence data showing the concentration of major and trace elements in soil core TL-2. TOC was calculated by the Loss on Ignition method.

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<th>Mo mg kg⁻¹</th>
<th>As mg kg⁻¹</th>
<th>U mg kg⁻¹</th>
<th>V mg kg⁻¹</th>
<th>Mn mg kg⁻¹</th>
<th>Cu mg kg⁻¹</th>
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<th>SiO₂ wt%</th>
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Table 8.5. X-ray fluorescence data showing the concentration of major and trace elements in soil core TJ-2. TOC was calculated by the Loss on Ignition method.

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<th>Cu mg kg⁻¹</th>
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<th>Fe₂O₃ wt%</th>
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Figure 8.1. µ-XRF maps showing iron in a section through a soil pellet with the selected points of interest (POI) for Fe K-edge XANES analysis labelled 1-4 in soil FF-1 (left) and labelled 1-3 in soil MO-1 (top). The µ-XRF is on a ‘pseudo-temperature’ scale with high Fe counts in red and areas with no counts in dark blue.
8.2. Normalised Se $K$-edge XANES spectra of the tall fescue root from site TL-1 showing selenium species in selected POI’s compared to the SeMet Standard.

8.3. Photographs of the Se non-accumulator plants (pasture species), tall fescue grass with necrosis of the leaves and white clover.
8.4. Photographs of the Se accumulator species *Brassica olerace* (cabbage and kale). Note the hispi cabbage have necrosis of the leaves.

8.5. Photograph of an outcrop of the Clare Shale Formation at Inishcorker Island in the Shannon Estuary where selenium was up to 18 mg kg\(^{-1}\).
8.6. HPLC-ICP-MS spectra showing the SeMet standard with an unknown peak caused by oxidation or reduction.

8.7. HPLC-ICP-MS spectra showing the SeCys, $\text{Se}^{\text{IV}}$, SeMet and $\text{Se}^{\text{VI}}$ standards. The signal for SeMet is poor due to the change in species during the sample run.
Table 8.6. The pyrolysis moieties identified in the seleniferous soil horizon from core PK-1, FF-1, TL-1 and TJ-1 with approximate retention time and mass. Label A = aromatics, N = nitrogen containing compound, Ps = polysaccharide, Ph = Phenol, L = Lignin marker, Lp = Lipids.

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Table 8.7. The pyrolysis moieties identified in the seleniferous soil horizon from core PK-1, FF-1, TL-1 and TJ-1 with approximate retention time and mass. Lp = Lipids (Alkenes and alkanes).
Report on investigations into the seleniferous soils of West Limerick, Ireland

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And analytical support from Paul Lythgoe¹ and Alastair Bewsher¹ (XRF) and Karen Theis (plant digestions).

With access and assistance from West Limerick farmers, especially James and John O’Flynn, Tom Larkin and TJ Daley.
Introduction

This report documents the investigation of the reported areas of high natural selenium values in the agricultural soils of West Limerick, Ireland. It is a collaboration between the University of Manchester, Teagasc (Newcastlewest) and farmers in West Limerick.

The aim of the investigation was to determine the nature, source and extent of the contamination, and to inform the natural cycling of the selenium, with a particular focus on:

a) major and trace element chemistry of contaminated soils
b) analysis of potential source rocks
c) the uptake of the selenium into the pasture vegetation
d) the speciation of the selenium in soils and plants
e) the microbial characteristics of the soils and their behaviour in amended microcosm experiments.
f) determination of the distribution of the contamination through the soil profile
g) and, in specific locations, determine the lateral extent of the contamination.

Wider aims of the study were to determine the extent of the contamination and likely mechanisms that led to the development of this natural contamination and to provide input into the management of the toxicity issues by local farmers.

The main resources applied to the problem were a NERC funded PhD (Shauna McLoughlin), and input and support from TEAGASC and the local farmers. Following initial field sampling, further small suites of soil samples were collected and analysed as part of undergraduate and MSc environmental science projects at the University of Manchester.

The report here provides supplementary information to the chapters in the main body of the thesis. A full report will incorporate components from the three chapters. Background details are provided within the thesis.

The area investigated is in the west of Co. Limerick, Ireland, between Foynes on the Shannon Estuary and Newcastle west. Dairy cattle farming is the major agricultural industry, in a large network of pastureland fields, forming part of the so called ‘Golden Vale’ in Munster. To the west of the area is a north-south trending ridge comprising Carboniferous mudstone and sandstones of the Shannon Group. To the east of this ridge are the overlying Clare Shales (mudstone and units of muddy Visean carbonate and further mudstone/shale dominated units. These latter units underlie the Golden Vale.
Report Figure 1. Sampling sites in West Limerick, Ireland. (FF = O’Flynn’s Farm; MO = Michael O’Donovan’s Farm; PK = Patrick Keily’s Farm; TL = Tom Larkin’s Farm; TJ = TJ Daley’s farm; B = Ballinvulla area).

Report Figure 2. The Geology of West Limerick and detail of the geology of the key sites. Sites FF, MO and B are on Visean Limestone, PK on Clare Shale, TL on Waulsortian Limestone and TJ the Rathkeale formation. Adapted from Geological Survey of Ireland 1:100,000 Bedrock Map. 2018. Contains Irish Public Sector Data (Geological Survey) licensed under a Creative Commons Attribution 4.0 International (CC BY 4.0) licence.
Ballegny Beg Area

The area around the O’Flynn farm had been identified as one with high Se contents in the soil by Walsh and Fleming working for the Department of Agriculture in the 1950s (Fleming and Walsh, 1956; and references therein). This area was one of the main focuses of the current research.

The area comprises low relief with drainage into a small basin, with a developing stream running to the east. Sites FF-1 and MO are either side of that stream, the former in largely waterlogged ground. Site FF-2 is in an adjacent field to the west, 30m from the nascent stream. There are low angle slopes to the north, south and west.

Report Figure 2. Google Earth image of the Ballegney Beg area showing the three sites and the position stream of the stream draining the area.

The farmer records a history of selenium poisoning in his dairy cows in specific fields which is ameliorated by rapid rotation. Affected animals are treated with sulfur; molybdenosis was also a perceived issue.

At site FF1, the soil was wet and organic-rich near the surface, with a brown layer overlying a distinct porous carbonate-rich clay layer. At Site MO the soil was brown and only 15 cms before a stiff impervious clay layer was reached.

Report Figure 3. View of the Ballyegny Beg area, looking northeast from the relatively higher ground towards the former marshy/lacustrine area containing the sites. The approximate contact of the Clare Shales and the Visean Limestone forms the ridge in the foreground.

Report Figure 4. Field site FF-1, in relatively lower lying, boggy ground, separated by a small stream from site MO-1 (Left). Soil pit showing dark, organic-rich soil overlying light grey, carbonate-rich clay (Right).

Report Figure 5. Field site MO-1, in lower, boggy ground, separated by a small stream from site FF-1 (Left). (Right) Site FF-2.
Churchyard area (Tom Larkin).

Following reports of selenium poisoning in the fields near Clounagh, the soils were tested and found to have elevated values of selenium. A follow-up study showed a restricted area of a field owned by Farmer Tom Larkin was contaminated. There is a gentle upslope towards the village and a disused railway line forms the southern border to the field. Historically the contaminated area was a small lake and marsh. The soil was relatively deep but at 50 cms, a stiff impervious light grey clay layer was encountered.

Report Figure 6. Google maps image of the site TL, showing the sampled field.

Report Figure 7. The Larkin field, looking east (above) and core sampling (right).
Churchyard Area (T J Daley)

An area recognised by the local farmers for its selenium toxicity is adjacent to stream near Clounagh Churchyard. A well-drained field between two drainage channels has a low lying area which had significant Se contents but as soon as a small ridge was encountered, the soils were Se-free.

**Report Figure 8.** Google map image of site TJ, showing the field, drainage, slope and Se values in soil.

**Report Figure 9.** View of the TJ field site looking southeast, from the top of the slope. The field is highlighted by the orange dashed line.
Report Figure 10. Soil profiles in site TJ. A dark, organic-rich layer overlying a light impermeable clay layer (left and above). A pit profile, showing a lighter soil layer overlying a darker layer, and the impermeable grey clay (right).

Report Figure 11. The TJ field site showing field sampling, the rich grassland and the main stakeholders.
Lisgordan - Ballymakeely (Patrick Keily)

Reports of selenium poisoning in certain fields near Lisgordan led to sampling at four locations. The area slopes down from well drained ground at Ballymakeely farm to wet fields with marsh flora in the relatively lower ground. The soil collected from the marshy area had over 600 ppm Se and a deep soil profile; an impervious clay was encountered beneath the soil. Upslope the soil was very thin (10 cms) before Clare Shale regolith was reached and this soil contained no detectable selenium.

Report figure 12. Google Earth image of site PK showing the marshy area (shaded) (left) and the sampling sites with the soil Se values (right).

Report figure 13. Marshy ground at site PK and the line of the slope to the west (left). Soil trench showing dark brown soil overlying impervious light grey clay (right).

Report figure 14. Upslope sampling site at site PK, showing lush grasses. (left). The very shallow soil exposed in a pit (right).
Ballinvulla area.

This location was chosen because it had a history of selenium poisoning in livestock that had been turned over to cereals 20 years ago. Three fields have been cultivated (Reidy) but surrounding fields remained as managed grazing land or rough grazing. Samples were collected from both areas and are presented in Report Table 1.

Analysis of the samples from the cereal fields showed almost all of the soils were below detection levels. One sample with 10ppm Se was an anomaly in the cereal fields. Soil pits showed that the soil that had developed above a light grey, impervious clay was shallow (15cm). Samples from the adjacent fields, in contrast, yielded significant selenium. In the rough pasture, the core showed values of 6-49ppm Se.

Figure report 15. Cereal field at Ballinvulla (left). A soil pit (centre) and the 15 cm soil above in the cereal fields above the impervious clay layer (right).
Table Report 1. Selected elemental concentrations in soils from the Ballinvulla area.

References