The Influence of Geochemistry on Selenium in Soils and Plants of England and Wales

by

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ABSTRACT

Selenium deficiency is a widespread problem amongst grazing livestock in many areas of Britain. The pathway of selenium from soil to plants and hence to livestock is not clear and no simple relationships appear to exist between selenium concentration in soil and that in herbage. This study aimed to elucidate the soil factors influencing selenium accumulation by plants.

A field sampling programme was carried out in England and Wales, collecting herbage, topsoil and subsoil samples from 16 agricultural sites seasonally over a two year period. Seasonal variations in plant selenium concentrations were noted, with lower concentrations during the spring and summer due to increased plant growth rate. This seasonal difference was independent of seasonal soil contamination of pasture.

A significant correlation of r=0.41 was found between soil and herbage selenium concentrations; previous research had not found a significant correlation. Selenium concentrations in soil were closely associated with the organic matter content of the soil, but the association was not significant for herbage selenium. No correlations between soil sulphur and herbage selenium concentrations were found. The relationship between soil and herbage selenium concentrations and soil pH was complicated by the effect of organic matter on both selenium concentrations and soil pH. The lowest soil-plant uptake of selenium was found on an improved moorland farm site.

The field work was augmented by two greenhouse pot trials; i) the effect of fertilisers on the uptake of selenium in grass and clover species grown on vermiculite; ii) the influence of organic matter on selenium accumulation in *Lolium perenne* grown on soils from three of the field sites. Nitrogen fertilisation produced lower herbage selenium concentrations due to growth enhancement and dilution effects. No competition between selenium uptake and sulphur added in fertilisers was seen at the low concentrations of selenium and sulphur used in the experiments. The influence of phosphate fertilisers on selenium uptake was not clear. Selenite uptake was greater than selenate uptake in the plants grown on vermiculite, but those grown on soil accumulated selenate to a greater extent than selenite. Organic matter additions to soil increased the uptake of selenium in herbage; growth was also increased but no corresponding decrease in selenium concentration was found, in contrast to the results following nitrogen fertilisation.

Soil and herbage selenum analysis was compared using two methods, spectrofluorimetry and hydride generation-ICPAES. The spectrofluorimetric method was shown to have lower detection limits, slightly better precision and better agreement with the accepted international reference materials than the ICPAES method, especially for herbage selenium analysis. A 50% reduction in the detection limit and other minor improvements to the published spectrofluorimetric methods were also acheived.

The data obtained from the field samples were used to attempt a predictive model for selenium availability to pasture plants over a range of soils in England and Wales. The best model obtained from these data accounted for 36.6% of the variation in herbage selenium concentrations.

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LIST OF ABBREVIATIONS

AAS	Atomic absorption spectrometry
ADAS	Agricultural Development Advisory Service
DAB	Diaminobenzidine
DAN	2,3-Diaminonaphthalene
DDDIW	Double distilled deionised water
DIW	Deionised water
EDTA	Ethylenediaminotetraacetic acid
FAAS	Flameless atomic absorption spectrometry
ICPAES	Inductively coupled plasma atomic emission spectrometry
GSHPx	Glutathione peroxidase
MAFF	Ministry of Agriculture, Fisheries and Food
NAS	National Academy of Science (U.S.A.)
NBS	National Bureau of Standards
o-PDA	Ortho-phenylenediamine
ppb	Parts per billion (ng/g)
ppm	Parts per million (μg/g)
rpm	Revolutions per minute
u.v.	Ultra-violet
XFA	X-ray fluorescence analysis

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CHAPTER 1

GENERAL INTRODUCTION

1.1 INTRODUCTION

Selenium has been recognised as an essential trace element in animal nutrition for over 30 years (Schwartz and Foltz, 1957) and since this discovery selenium-responsive diseases have been noticed amongst livestock in many areas of the world, especially in New Zealand, Finland and parts of the U.S.A. The extent of selenium deficiency is still being assessed in many countries and large areas of agricultural land have been shown to produce livestock of marginal or deficient selenium status.

In Britain, selenium deficiency problems were first recognised in the Moray Firth area of Scotland where a locally occurring muscular dystrophy of calves was found to be preventable by treatment with selenium or vitamin E (Sharman, Blaxter and Wilson, 1959; Blaxter et al., 1961). Blaxter (1963) working in Scotland, found that the areas where selenium deficiency in livestock occurred were those with soils developed from a specific geological formation, the arenaceous sands of the Old Red Sandstone. He concluded that 10% of the total land area of Scotland was mildly deficient in selenium, and that the majority of this was rough grazing land.

Prior to this, areas of potential selenium deficiency had been identified by monitoring the response in health and weight gain to administered selenium in livestock grazing those areas (Hartley, 1961; Blaxter, 1963).

Geochemical surveys have since been used to identify areas where selenium deficiencies might exist and assessments of the extent of the problem in this country have brought an awareness of the economic importance of sub-clinical and clinical selenium deficiency to British agriculture.

Anderson, Berrett and Patterson (1979) surveyed blood glutathione peroxidase activity in 329 sheep flocks in England and Wales. Blood selenium concentrations in 47% of the grazing flocks were found to be lower than 0.075 μg/ml indicating inadequate herbage selenium levels to maintain the selenium status of the animals. On the basis of this survey, areas of north-east England, Wales and southern England have been classified as selenium deficient.

The total selenium content in soils was shown to be associated with the selenium content of the underlying rock in the U.S.A. (Lakin and Davidson, 1967; Rosenfeld and Beath, 1964) and this appears to be broadly true in all situations. However the selenium distribution in the soil may be modified due to soil forming processes and the biogeochemical cycling of selenium (Smith, 1983).

A survey of topsoil selenium concentrations in England and Wales was undertaken in the Applied Geochemistry Research Group of Imperial College to establish whether there was a geochemical basis for selenium deficiency in Britain (Thornton et al., 1983). This relationship had previously been recorded in the U.S.A. (Kubota et al., 1967) and New Zealand (Andrews, Hartley and Grant, 1968).

The results of this British survey suggested that the soil parent material is an important factor in determining the total selenium status of the soil. Soils derived from sands, sandstones and calcareous materials contained less selenium than those developed from finer grained sedimentary rocks, including clays and shales. Selenium was present in larger concentrations in soils than in their parent materials, and selenium distribution in the soil profile was influenced by its association with iron and organic matter. The study concluded that British soils developed on calcareous and coarse sandy parent materials were likely to have low total selenium contents, especially where the soils were acid, and it was thought unlikely that vegetation with selenium levels high enough to cause toxicity problems in grazing livestock would be found in Britain.

An earlier study of soils in England identified some areas with fairly high soil selenium levels (1.5 - 7.0 μg/g), although no signs of livestock selenium toxicity were found during the study (Webb et al., 1966).

Geochemical surveys, however, cannot fully predict areas which are likely to produce herbage containing insufficient selenium for livestock because no relationship has been found between total selenium in the soil and the selenium content of the plants growing on the soil, despite many studies involving soils of widely varying selenium content (Nye and Peterson, 1975; Hamdy and Gissel-Nielsen, 1976a; MAFF, 1983). Where the total soil selenium levels are high (>90 μg/g), a correlation has been found between extractable soil selenium and herbage selenium (Williams and Thornton, 1973). Consequently total soil selenium concentration is a very poor predictor of areas producing herbage of low or deficient selenium concentration, since the uptake of selenium into plants appears to be dependent upon the complex interrelationship of many soil, land-use and climatic factors of which total soil selenium is just one important aspect (MAFF, 1983). There are many reports in the literature of factors which influence the uptake of selenium into plants at high and low levels of selenium in field and experimental greenhouse conditions. The factors which have been shown to have strong correlations with plant selenium concentration include: pH (Geering et al., 1968); soil organic matter (Levesque, 1974a); parent material (U. S. NAS, 1974); soil texture, especially clay content (Hamdy and Gissel-Nielsen, 1977); iron concentration (Geering et al., 1968; Levesque, 1974a); sulphur concentration (Shrift, 1954a,b; Ferrari and Renosto, 1972); drainage and climate (Lakin and Davidson, 1967); plant species or sward composition (Davies and Watkinson, 1966); land-use and fertiliser applications (Gissel-Nielsen, 1971a); soil redox conditions and hence selenium speciation (Van Dorst, 1984); soil profile development (Smith, 1983) and season (Russell, 1987).

However, in general these controlling factors have been studied in isolation and no overall picture exists which can help to predict the availability of selenium from any soil in any situation.

For this research work it was therefore felt necessary to study as many of these interrelated factors as possible on a small group of widely different soils collected from field sites in England and Wales over a period of time.

1.2 AIMS OF THE RESEARCH

The overall aim of the research was to investigate the complex relationships of soil properties and land-use in order to identify soils which will produce herbage with a selenium concentration too low for the adequate nutrition of grazing livestock.

Thirteen field sites were chosen from contrasting soil types in five areas of England and Wales, and topsoil, subsoil and herbage samples have been collected from each of these areas for analysis every three months over a two year period.

As a supplement to the field work, two greenhouse experiments involving plant uptake of added selenium under various controlled conditions have been carried out in order to investigate the effects of sulphate fertilisers, nitrogenous fertilisers and soil organic matter on soil selenium availability and uptake by pasture species.

Selenium speciation is believed to have a strong effect on the bioavailability of the element, but there is very little information available on the ratio of species present in soils of low total selenium content. Some attempts were made to assess the contributions of different selenium species in the field soils although this analysis was often difficult as the selenium concentrations involved were close to the detection limit of the analytical methods available.

A comparison of two methods of selenium analysis (ICPAES and spectrofluorimetry) has been made during the course of the research including a discussion of the accuracy, precision and the advantages or disadvantages of each method for the trace analysis of selenium.

CHAPTER 2

SELENIUM IN THE GEOCHEMICAL ENVIRONMENT AND ITS IMPORTANCE FOR NUTRITION

2.1 SELENIUM: CHEMICAL AND PHYSICAL PROPERTIES

Selenium was first identified as an element in 1817 by Berzelius and, because of its similarity to tellurium named 35 years earlier, he named the element after the Greek word for the moon, Selene (Vokal-Borek, 1979).

Selenium belongs to Group VI A of the periodic table which also includes oxygen, sulphur and tellurium and these elements have many similar physical and chemical properties. Some of these properties are listed in Table 2.1.

All of the oxidation states of selenium listed in Table 2.1 are commonly found in nature except the +2 state, although some selenium compounds containing the divalent positive ion are known. The four common oxidation states of -2, 0, +4 and +6, are shared by both sulphur and tellurium which form many analogous organic and inorganic compounds.

2.2 SELENIUM GEOCHEMISTRY

Selenium is a ubiquitous trace element with an uneven distribution in the natural environment, however the concentrations are broadly dependent upon the local geology.

Geological, biological and industrial processes are all involved in the

Table 2.1 The physical properties of oxygen, sulphur, selenium and tellurium (Vokal-Borek, 1979)

Element	Usual valences	Atomic weight ^{12}C	Melting point (°C)	Boiling point (°C)	Covalent radius (Å)	Atomic radius (Å)	Ionic radius (Å) (ox. state)	Electro-negativity (Pauling)	First ionization potential (eV)
Oxygen	-2	15.99	-218.4	-183	0.74	—	1.40 (-2) 0.09 (+6)	3.5	13.61
Sulphur	+4, +6, +2, -2	32.06	119 (b)	444	1.04	1.27	1.84 (-2) 0.29 (+6)	2.5	10.36
Selenium	+4, +6, +2, -2 (a)	78.96	217 (c)	684	1.17	1.40	1.98 (-2) 0.42 (+6)	2.4	9.75
Tellurium	+4, +6, -2	127.60	415	1390	1.37	1.60	2.21 (-2) 0.56 (+6)	2.1	9.01

a) The Se^{2+} state has not been reported in nature b) Monoclinic c) Hexagonal

distribution, transportation and cycling of selenium, however natural geological and biological processes are probably almost entirely responsible for the present status of the element in the general environment.

2.2.1 The Selenium Cycle

The natural cycling of selenium in the environment has been widely discussed since Shrift (1964) first suggested an outline for the selenium cycle. Lakin and Davidson (1967) also proposed a geochemical selenium cycle and these have since been clarified as later research has provided evidence of the species of selenium, quantities of the element and the timescale involved in various pathways of the cycle. Mackenzie et al. (1979) estimated the quantities of selenium removed by rivers in soluble and suspended forms. Asher et al. (1977) reported that plants can convert selenite ions to selenate ions during selenium uptake and Sarathchandra and Watkinson (1981) demonstrated the oxidation of elemental selenium to selenite by a bacteria species. Other research has helped to elucidate many individual relationships and pathways within the overall selenium cycle which is outlined in Figure 2.1, although some pathways still need further substantiation.

2.2.2 Selenium in Rocks

Selenium is well dispersed throughout the earth's crust but only rarely does it occur in concentrations above a few hundred μg/g, except in some shales and sulphide minerals.

The concentration of selenium in igneous rocks is usually very low, often less than 0.1 μg/g and similar levels occur in metamorphic rocks. Sedimentary rocks have a more varied selenium concentration which is a result of the diverse conditions involved in their formation.

Goldschmidt (1954) estimated that the concentration of selenium in igneous

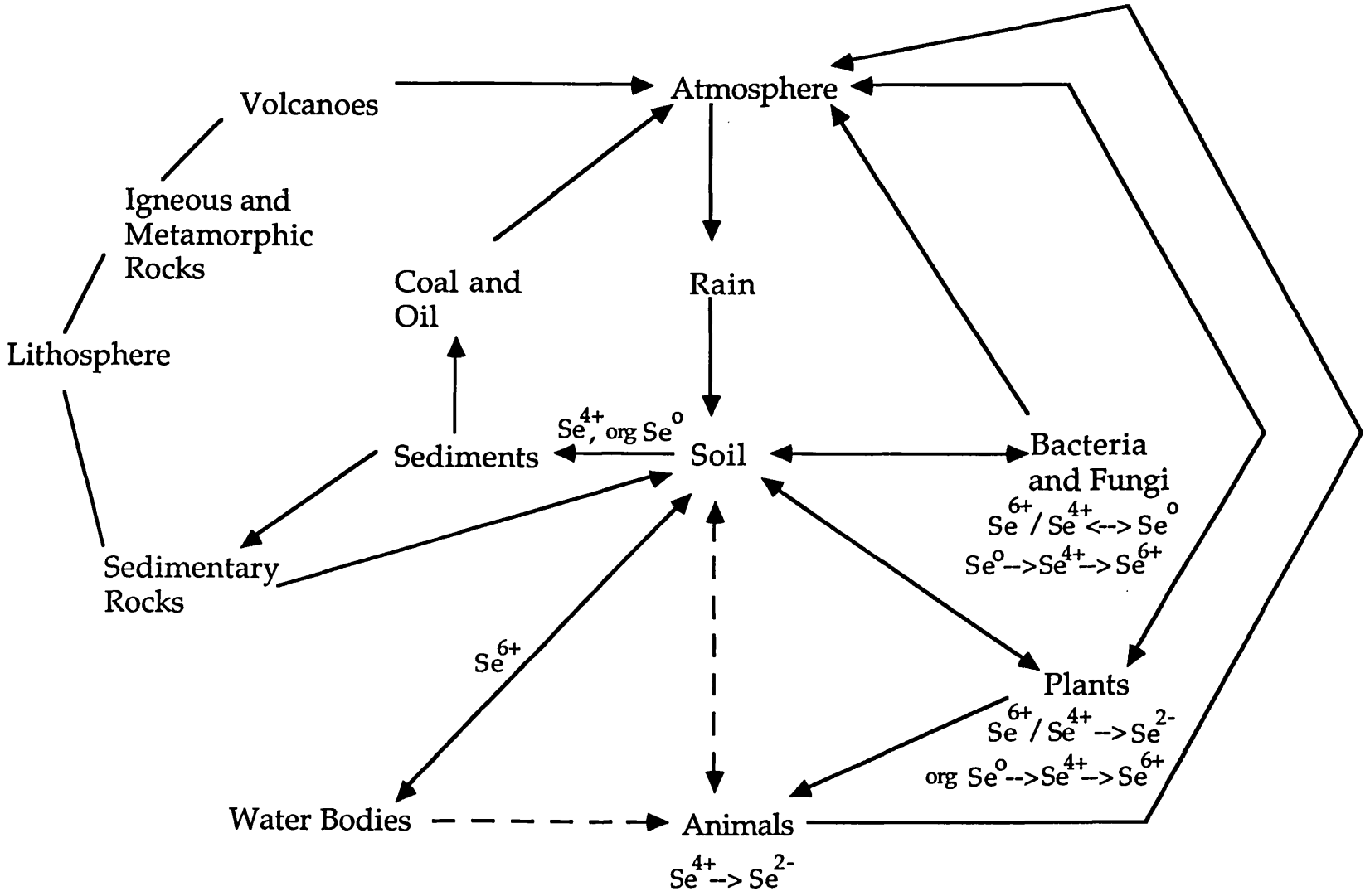


Figure 2.1 The selenium cycle (adapted from Shrift, 1964)

rocks was 0.09 μg/g. This figure was revised by Turekian and Wedepohl (1961) to give a value of 0.05 μg/g Se in the earth's crust. Oldfield (1974) concluded that the range of selenium concentrations in igneous rocks was 0.004 - 1.5 μg/g. Despite the generally low concentrations of selenium in igneous rocks, these are presumed to be the original source of selenium in the environment. The Cretaceous geological age has been suggested as being a period during which large quantities of selenium were brought to the earth's surface through volcanic activity (Byers et al., 1938).

Selenium is found in high concentrations associated with some sulphide ores, although rarely in concentrations greater than a few hundred μg/g (Lakin and Davidson, 1967), however it is not found in native sulphur derived from sedimentation processes. This is explained by the difference in oxidation potentials of the two elements, sulphur being more readily oxidised by weathering processes than selenium; this results in the two elements being separated in the environment after weathering.

Selenium tends to concentrate in sedimentary rather than magmatic rocks, and of the sedimentary deposits it is found mainly in oxidates such as vanadium-uranium ores, or in hydrolysates such as shales. Shales containing high levels of organic matter often have the greatest concentration of selenium. Sedimentary rocks are the principle parent materials of agricultural soils since they cover more than 3/4 of the earth's land surface (Jackson, 1964); the variable selenium content of sedimentary rocks accounting for much of the variation in selenium levels around the world.

Examples of the selenium content of various rocks and the range of concentrations commonly found are given in Table 2.2. In general, the selenium concentration of carbonate rocks is usually low, as is that of sandstones. Higher concentrations of selenium found in shales are assumed to be due to the concentration of organic matter during deposition.

Table 2.2 Selenium occurrence in various geological materials

Geological material	Selenium concentration µg/g	Reference
Earth's crust	0.05	Turekian, 1961
Igneous rocks	0.004 - 1.5	Oldfield, 1974
Shales	0.6	Bowen, 1966
Black shales	675	Lakin, 1972
Sandstones	0.01	Bowen, 1979
Limestones	0.03	Bowen, 1979
Phosphate rocks	1 - 300	U. S. NAS, 1976

Selenium has been found in high concentrations in some phosphate rocks (Robbins and Carter, 1970); this has occasionally been important when the rocks have been mined for use as phosphate fertilisers. These selenium enriched phosphates have been used to increase the selenium status of the soil in some cases and avoided for fear of toxicity problems in other areas. However the majority of phosphate fertilisers, although containing elevated levels of selenium, do not produce increased selenium uptake in plants (Gissel-Nielsen, 1971a).

2.2.3 Selenium in Natural Waters

Selenium is present in all natural waters as part of the geochemical cycle of the element. Schutz and Turekian (1965) analysed many sea water samples and their results are summarised in Table 2.3.

Table 2.3 Selenium in sea waters (Schutz and Turekian, 1965)

Location	Number of samples	Selenium concentration µg/g	
		Range	Mean
Caribbean	4	0.095 - 0.14	0.11
Western North Atlantic	8	0.069 - 0.13	0.096
Eastern North Atlantic	7	0.076 - 0.11	0.088
Western South Atlantic	2	0.070 - 0.080	0.075
Eastern Pacific	6	0.061 - 0.12	0.087
Antarctic	1	0.052	0.052
Long Island Sound	8	0.10 - 0.13	0.11

An average value of 0.09 µg/l is given as an estimate of selenium concentrations in major oceans. Chau and Riley (1965) measured the selenium concentrations of water in the English Channel and the Irish Sea and obtained values of 0.5 +/- 0.02 µg/l Se and 0.34 +/- 0.01 µg/l Se respectively.

The U.S. Department of Health Education and Welfare considered 10 µg/l selenium in drinking water to be a safe upper limit (U. S. NAS/NRC, 1976), however water is rarely a significant source of selenium in the diet, either for nutritional purposes or as a toxicant.

One notable and much publicised exception is the high level of selenium present in the San Joaquin Valley in California where selenium, leached from rocks which are naturally rich in selenium, is concentrated to dangerously high levels by the agricultural irrigation system used in the valley. The irrigation drainage waters are fed into Kesterton reservoir which has now accumulated a high salinity level and high levels of many trace elements (Se 3,800 µg/l, B 12,000 µg/l, Mo 5,000 µg/l). Many species of native fish have been eliminated and the high incidence of wildfowl mortality has been attributed to excessive selenium. The high level of selenium in the drainage water is believed to be derived from the marine sediment parent material in the fertile Panoche Fan which is an intensively cultivated and irrigated area of the valley. Analysis of fish taken from the drainage canals and reservoir have found tissue concentrations as high as 66 µg/g Se, which is over 100 times that of fish taken from other nearby areas receiving no agricultural drainage waters (Mikkelsen et al., 1986). In 1983 a study of wildfowl at the reservoir found that 20% of all nests contained deformed birds and 40% of all eggs contained dead embryos (U. S. Bureau of Reclamation, 1984). Elevated selenium concentrations were found in the eggs of all waterfowl nesting at Kesterton. Another recent study has also reported reproductive problems in the aquatic birds using the irrigation drain water ponds as their habitat (Ohlendorf et al., 1986), although the toxic signs reported in these birds resembled avian selenosis, the possible role of other water borne toxins such as As or Bo was also pointed out.

Some attempts to reduce the amount of irrigation drainage water entering the reservoir have been made and some selenium rich areas have been removed from agricultural production in order to prevent any further deterioration in the quality of the reservoir water.

2.2.4 Selenium in the Atmosphere

Soils, plants, micro-organisms, animals and volcanoes all contribute selenium to the atmosphere. All of these produce volatile forms of selenium; soils and volcanoes probably also contribute particulate matter containing the element to the atmosphere. The burning of fossil fuels also emits selenium into the atmosphere as selenium dioxide or in particulate materials in association with sulphur. Lakin and Byers (1941) found selenium in the range of 0.05 -10 μg/g in the atmospheric dust collected on air conditioning filters in 10 U.S. cities. Dams and De Jong (1976) suggest that the average concentration of selenium in air from natural sources should be less than 0.04 ng/m^3.

Volatilisation of organic selenium compounds from soils, higher plants and animals releases a significant amount of selenium into the atmosphere. The evolution of volatile selenium compounds from the soil is thought to be an entirely microbial process (Abu-Erreish et al., 1968; Doran and Alexander, 1976) and this is discussed in more detail in section 2.3.3.

It is probable that in the U. K., with its small land area and high urban and industrial populations, that the majority of atmospheric selenium would be anthropogenic.

2.2.5 Anthropogenic Sources of Selenium in the Environment

Elemental selenium is obtained during the electrolytic refining of copper from chalcopyrite ores. The majority of the industrial demand for selenium (1800 tonnes in 1987, Chemistry and Industry, 1988) is met from this source.

Selenium has many technological applications, some due to its photochemical properties, such as photocells, light sensors and xerography. Selenium is also used as iron selenide in alloy steels, as cadmium selenide for red and yellow pigments and in glass colouring, and as agricultural feedstuffs supplements.

As a result of these industrial applications, selenium is introduced to the environment. At present this is probably a localised and minor source of selenium in the general context but problems of toxicity to the workers in these industries and contamination of the immediate surroundings must always be considered.

The activity which releases most selenium to the atmosphere is the burning of fossil fuels, especially coal. It was estimated that 680 tonnes of selenium were emitted into the atmosphere as a result of coal consumption in the U.S.A. in 1970 (U.S. NAS/NRC, 1976). Anthropogenic emissions of selenium appear to have a short residence time in the atmosphere, similar to that of water. Weiss et al. (1971) measured the S : Se ratio in Greenland ice from a 250 year period and found that the sulphur levels had increased with industrialisation and the associated air pollution, but the selenium levels had remained stable suggesting that anthropogenic selenium is quickly lost from the atmosphere and does not travel far from the site of origin. High levels of selenium have also been found in fly ash from power-stations and this is occasionally used as an agricultural fertiliser. Gutenmann and Lisk (1976) found a range of selenium levels in fly ash from 1.2 - 16.5 μg/g Se. Furr et al. (1977) analysed cabbages grown on potted soil supplemented with several different fly ashes and found that the selenium levels in the cabbages were closely correlated with those in the respective fly ashes in which the plants were cultured. The ready bioavailability to animals of the selenium in plants grown on fly ash was demonstrated in a study (Stoewsand et al., 1978) in which Japanese quail were fed a diet containing 60% winter wheat that had been grown to maturity on either soil or a deep bed of fly ash. The levels of selenium in the tissues and also the eggs of the quail fed the wheat grown on fly ash were much higher than those of quail fed the wheat grown on soil.

2.3 SELENIUM IN SOILS

2.3.1 General

The total selenium content of the soil is closely related to that of the underlying parent material of the soil. However this initial direct relationship with the geology of the area is considerably altered by many external influences and in most cases the processes of weathering and soil formation serve to enrich the soil with selenium. The redox conditions of the soil, pH, soil drainage, climate and land use all affect the selenium content of the soil. Table 2.4 shows the reported concentrations of selenium in soils from various areas of the world.

Selenium concentrations are almost always higher in soils than in the rocks from which they are derived and higher in surface soils (0-15 cm) than in subsoils. In North Wales, soils of the Denbigh series have been found to contain selenium concentrations ten times higher than those in the Silurian parent material, averaging 0.46 μg/g Se for soils and 0.048 μg/g Se for rocks (Thornton, Smith and Van Dorst, 1985). This difference in concentration is probably a function of the biological cycling of selenium and its accumulation in organic material.

Lag and Steinnes (1978) found a strong correlation ($r = 0.83$) between the selenium content in the top humus layers of Norwegian soils and the annual precipitation. The selenium concentration in the soils was also higher in the coastal districts than inland and was associated with higher levels of chlorine, bromine and iodine in the soils. Furthermore the accumulation of selenium was not particularly related to the greater accumulation of organic matter under conditions of higher precipitation. These results indicated that much of the selenium was supplied to the soils through precipitation, although the origin of the selenium whether marine or anthropogenic, as air pollution, was uncertain.

In the British Isles the only reported occurrence of soils supporting seleniferous vegetation is in the counties of Limerick, Tipperary and Meath in Eire, where the high selenium content of the soils originates from the underlying

Table 2.4 Reported selenium concentrations in soils

Country	No. of samples	Soil selenium µg/g		Reference
		Range	Mean	
England and Wales	517	<0.01 - 4.66	0.48	Thornton et al., 1983
England and Wales	114	0.2 - 1.8	0.6*	Archer, 1980
England and Wales (black shale)	16	0.20 - 7.00	3.1	Webb et al., 1966
Norway - north	122	0.08 - 1.70	0.63	Lag and Steinnes, 1978
- east	117	0.07 - 1.35	0.42	Ibid.
Belgium	10	0.04 - 0.27	0.11	Robberecht et al., 1982
Sweden	24	0.16 - 0.98	0.39	Lindberg, 1970
Denmark	11	0.20 - 1.44	0.57	Bisbjerg, 1972
Finland	34	<0.01 - 0.96	–	Koljonen, 1975
S. W. Ontario, Canada	26	0.18 - 1.03	0.40	Whitby et al., 1978
U.S.A. - seleniferous soils	500	1.0 - 80	4.5	Trelease, 1945
U.S.A. - other soils	11	0.01 - 1.40	–	Lakin, 1967
U.S.A. - Montana	448	0.01 - 5.0	0.8	Williams et al., 1941
Mexico	28	0.1 - 9.2	0.5	Ibid.
New Zealand	62	0.08 - 10.4	0.6	Wells, 1967

* Median value

Carboniferous black shales and limestones (Walsh and Fleming, 1951). Selenium concentrations of up to 395 μg/g for topsoils and 1200 μg/g for subsoils have been reported (Fleming and Walsh, 1957). Levels of up to 64 μg/g Se were reported in Namurian shales and limestones from County Meath (Kiely and Fleming, 1969). Cattle and horses have been reported to suffer from selenium toxic symptoms in these areas (Fleming and Walsh, 1957). Webb et al., (1966) located non-toxic seleniferous soils (1.5 - 7.0 μg/g Se) in Derbyshire, U.K. associated with the Namurian and Visean Carbonifeous black shales. Areas in Devon, North Staffordshire and North Wales were also found to have some soils of high selenium content (0.2 - 5.0 μg/g Se) although no clinical or sub-clinical disorders were found in livestock. Marine black shales elsewhere in the U.K. also commonly contain higher than average amounts of selenium (Thomson, 1971).

Since the recognition of selenium deficiency symptoms in livestock in areas of Britain in the 1960's (Blaxter, 1963), it has been realised that deficiency of selenium is a far more widespread problem than toxicity for the livestock in this country.

The geochemical selenium status of Britain has been examined (Thornton et al., 1983) in order to realise the extent of selenium deficiency across the country. Samples (517) of surface soils (0-15 cm) collected over a period of several years representing the major soil forming parent materials in England and Wales, were analysed for selenium. The results of this survey are summarised in Table 2.5 and the mean value (0.48 μg/g Se) of the soils examined compared well with the world soil median value of 0.4 μg/g Se quoted by Bowen (1979) and the mean value of 0.53 μg/g Se found by MAFF (1983) in a study of selenium levels in 236 soils of England and Wales.

Table 2.5 **Selenium concentrations in some surface soils (0-15 cm) in England and Wales (Thornton et al., 1983)**

Parent material	Chalk	Limestone	Sandstone	Clay	Mudstone	Shale	Mineralised granite and shale	Peat	All soils
Number of samples	41	25	190	134	43	38	16	30	517
Selenium (µg/g) Range	0.11 - 143	0.13 - 0.86	<0.01 - 2.11	0.08 - 2.91	0.09 - 1.59	0.25 - 1.07	0.71 - 4.66	0.47 - 2.11	<0.01 - 4.66
Mean	0.33	0.38	0.36	0.43	0.45	0.64	1.14	1.20	0.48

This survey (Thornton et al., 1983) found that, in general, soils formed from calcareous and coarse sedimentary rocks contained less selenium than those from fine-grained sediments and from metamorphosed and mineralised parent materials. Peaty soils and soils from areas of sulphide mineralisation contained the highest selenium contents. Three broad groupings of parent materials which gave rise to soils low in selenium were recognised:

i) Coarse arenaceous formations on which light textured soils such as brown earths are developed, e.g. Old Red Sandstones and Cretaceous sands and sandstones of S.E. England;

ii) Ordovician and Silurian shales, slates and sandstones occurring in upland regions giving rise to brown earths, stagnogley soils and brown podzolic soils and, on higher ground, stagnopodzols and stagnohumic soils;

iii) Chalk and Jurassic limestones giving rise to redzinas and associated brown calcareous earths.

Although the selenium content of soils is essentially related to the parent materials, the importance of other soil forming processes cannot be forgotten. The effect of pH, organic matter, iron content, climate and drainage status can all modify the selenium status of a given soil considerably.

Soil profiles taken from soils occurring in the Denbigh upland and moorland showed the distribution of selenium in soils to be governed principally by its association with pyrophosphate extractable iron (iron extracted with 0.1 M potassium pyrophosphate) and organic matter (Smith, 1983).

Selenium as the selenite ion is absorbed on ferric oxides and concentrated in horizons of sesquioxide enrichment. This relationship was particularly strong in the O and Bs horizons of stagnopodzols. Podzolisation in British soils has been suggested as an important process in the redistribution of selenium within the soil profiles and away from the rooting zone (Thornton, Smith and van Dorst, 1985).

Applications of selenium to soils or plants is an indirect means of enriching selenium in animal fodder. Experiments conducted by Gissel-Nielsen (1976) show that the addition of selenite to the soil results in only moderately increased selenium accumulation in pastures and grain. Addition of selenate produces much higher accumulation but the results are

only short lived (Watkinson and Davies, 1967a,b). Recently a selenium 'prill' containing 1% sodium selenite has been introduced as a soil supplement in New Zealand with good results (Watkinson, 1983).

2.3.2 Chemical Forms of Selenium in Soils

Selenium in soils is thought to exist in several forms dependent on the nature and conditions of the soil; these forms include selenides (Se^{2-}), elemental selenium (Se^{0}), selenites (Se^{4+}), selenates (Se^{6+}) and organic forms. The actual selenium species which may exist in any one soil depends largely on the pH and redox conditions which prevail. Cary et al. (1967) suggested that the proportions of the various selenium compounds present in the soil are affected by soil type.

Selenites are considered to be the most prevalent species in acid to neutral, well drained soils (pH 4.5-6.5) and thus are the most important source of selenium to plants on these soils. It has been clearly established that the concentration of selenite in solution and thus its availability to plants is controlled by its association with ferric oxides (eg. goethite) as ferric oxide-selenite adsorption complexes (Geering et al., 1968; Howard, 1972; Hamdy and Gissel-Nielsen, 1977).

The equilibrium solubility of selenite in association with a ferric oxide - selenite adsorption complex increases with pH up to approximately pH 8, when the complex begins to decompose and the equilibrium solubility then increases more rapidly with pH such that there is almost complete desorption at pH 11 (Allaway et al., 1967). The increase of selenite in solution decreases the Eh of the selenite - selenate couple so that the formation of selenate in alkaline soils is favoured by the breakdown of the ferric oxide - selenite adsorption complex.

Adsorption of selenites on ferric hydroxide at pH 8 and below may remove more than 95% of the selenite ions from solution; the effect of the lowered selenite concentration being to broaden the range of redox potentials over which selenite will exist in solution (Howard, 1977).

Hamdy and Gissel-Nielsen (1977) investigated selenite adsorption by several soil clay minerals and also ferric oxide, but concluded that the latter is the more

effective adsorber, partly because the rates of adsorption are much quicker.

Neal and Sposito (1987a,b, 1989) have carried out several studies on the relative adsorption of selenite and selenate ions on alluvial soils from the San Joaquin valley. They concluded that iron oxide and clay mineral complexation of selenite was most likely to explain the adsorption of selenium on these soils. In contrast to selenite, selenate in solution was not significantly adsorbed at the concentrations found in the soil waters of the San Joaquin valley. However, Bar-Yosef and Meek (1987) have shown some evidence of selenate adsorption by kaolinite, especially in acid conditions (pH<4).

In alkaline soils (pH 7.5 - 8.5 or higher), the stability of these ferric oxide - selenite complexes is decreased and the selenite is oxidised by weathering and micro-organisms to the selenate ion, which remains soluble and is easily leached. Howard (1977) suggested that oxidation of selenite to selenate would not occur readily in normal surface oxidising conditions. However several species of bacteria and fungi can oxidise elemental selenium to selenite or selenate. Sarathchandra and Watkinson (1981) demonstrated the oxidation of elemental selenium to selenite and a trace of selenate in laboratory cultures by *Bacillus megaterium* isolated from soil. Also Geering et al. (1968) demonstrated that the oxidation of elemental selenium to selenite in the soil could probably proceed by both inorganic and organic pathways. Once present in the soil, selenate is not retained by colloids and is easily leached out of the soil. Thus accumulation of selenate may only be expected to take place in arid alkaline conditions such as those found in the Western U.S.A. (Rosenfeld and Beath, 1964).

Micro-organisms have been shown to be capable of reducing both selenate and selenite to elemental selenium (Falcone and Dickenson, 1963), although most of the evidence is for reactions in pure culture and the relevance to soil conditions is not clear (Peterson et al., 1981). Cary et al. (1967) suggested that the reduction to elemental selenium or selenide is responsible for the immobilisation of selenite added to a soil, since these forms are insoluble and therefore not available to the plant. Oldfield (1972) reported that in waterlogged acid soils, after the addition of selenite to soil, selenides and elemental selenium are formed. The reduction of selenate to selenite in soils and the reverse

reaction is slow (Geering et al., 1968). The rate of reduction of selenite to elemental selenium is independent of pH and varies between soils (Cary et al., 1967).

Fleming (1980) suggested that selenides, which are largely insoluble, do occur in soils of the semi-arid regions in association with pyrite where weathering is not greatly advanced. The low solubility of selenides would lead to their persistence in agricultural soils. It is theoretically possible that in very acid, reducing conditions, selenides could be formed by reduction of elemental selenium and Watkinson (1962) suggested that ferrous selenide may occur in some acid podzolic soils in New Zealand. However, the solubility of ferrous iron in acid conditions may lead to losses of iron from the soil, whilst selenide, being insoluble, would persist.

Little is known of the organic forms of selenium in the soil despite the fact that several workers have demonstrated associations between selenium and organic matter in the soil (Wells, 1967; Levesque, 1974a; Koljonen, 1975; Smith, 1983), and that selenium can accumulate in organic soils (Fleming and Walsh, 1957). Water soluble selenium can contain an organic selenium fraction but it is not clear if this is available to the plant. Nye and Peterson (1975) demonstrated that some selenium was linked to the organic fraction of a water soluble extract, while work by Levesque (1974b) suggested that associations between selenium and organic matter may require chelation with ferric iron and that these complexes could have a role in the mobilisation of selenium in the soil profile.

Although evidence exists for the presence of some or all of these selenium species in certain soils, little information is available on the relative abundance of each in any particular soil environment, and especially on the persistence and stability of the elemental and selenide forms. Elrashadi et al. (1987) have produced a theoretical study of the chemical equilibria of selenium in soils using thermodynamic data for 83 possible selenium minerals and solution species found in soils. This very comprehensive study highlights the importance of hydrogen selenites and hydrogen selenates in the soil solution. It would be useful for predicting the relative abundances of selenium species in soil solution, if it could be shown to produce values comparable to those measured in naturally occurring soils.

2.3.3 Selenium Volatilisation from Soils, Plants and Animals

Selenium forms highly volatile organic compounds and the release of these into the atmosphere by soil microbial activity, plants and animals probably removes a significant amount of selenium from the soil. The evolution of volatiles from the soil is thought to be an entirely microbial process (Abu-Erreish et al., 1968; Doran and Alexander, 1976), and several strains of fungi and bacteria have been shown to be capable of synthesising volatile selenium compounds from either inorganic selenium salts or organoselenium compounds (Fleming and Alexander, 1972; Cox and Alexander, 1974; Barkes and Fleming, 1974). Dimethylselenide is considered to be the primary volatile product (Francis et al., 1974) but dimethylselenone, dimethyldiselenide and hydrogen selenide have also been identified (Reamer and Zoller, 1980; Doran and Alexander, 1976); the form evolved depending upon the substrate for the synthesis.

The process is dependent upon many factors, but perhaps the most important is the availability of a suitable substrate; thus the availability of water soluble selenium influences the rate of methylation (Abu-Erreish et al., 1968; Zieve and Peterson, 1981) and it is therefore expected that alkaline soils, having an appreciable content of selenate, are more susceptible to volatile losses (Hamdy and Gissel-Nielsen, 1976b). Losses may be much less important on more acid soils where selenite or more reduced insoluble species predominate. Other factors influencing the process are those affecting microbial activity such as temperature, moisture status and the availability of organic matter for an energy substrate (Abu-Erreish et al., 1968; Doran and Alexander, 1976; Zieve and Peterson, 1981). Hamdy and Gissel-Nielsen (1976b) found that wetting and drying cycles, which are thought to advance the decomposition of organic matter, raised the levels of volatilised selenium. Zieve and Peterson (1981) found that more selenium was evolved from a soil collected in the spring than those collected in other seasons, and related this to an increase in the microbial population during this season, implicating seasonal differences in volatilisation in the field.

Among the higher plants the selenium accumulator *Astragalus racemosus* and the non-accumulator *Medicago sativa* are two of the many species which

have been reported to volatilise selenium, the former producing dimethyldiselenide and the latter dimethylselenide (Asher et al., 1967; Evans et al., 1968).

In animals, selenium becomes associated with glutathione peroxidase in the red blood cells (Flohe et al., 1973) and other tissues (Oh et al., 1974). Inside the tissues selenium can be reduced to selenide, become methylated or bound to proteins. Reduction of selenium to dimethylselenide or dimethyldiselenide leads to excretion via exhalation; further methylation produces the trimethylselenonium ions which are excreted via the urine. Exhalation of volatile selenium compounds from animals and man has generally only been detected when there is a high level of selenium intake.

2.4 SELENIUM IN PLANTS

2.4.1 Selenium Concentration in Plants

The concentration of selenium in plants is generally 10-40% of that found in soils (Koljonen, 1975), however, there is a striking difference in uptake of selenium between plant species.

Plants containing concentrations of 3 μg/g Se or above are considered potentially toxic, or seleniferous, to livestock (Bisbjerg and Gissel-Nielsen, 1969) and soils producing vegetation with this level of selenium are also considered seleniferous. Vegetation with a selenium content below 0.02 μg/g may produce deficiency symptoms in ruminants (U.S. NAS/NRC, 1971) and a level of 0.1 μg/g Se in the livestock feed has been suggested as the minimum requirement for normal growth (Walker, 1971).

Beath (1937) and Beath et al. (1939) divided plants into 3 categories according to their ability to accumulate selenium:

i) Primary selenium accumulators; These plants are found only on seleniferous soils and can accumulate thousands of μg/g of selenium. Species include members of *Astragalus* spp., *Stanleya* spp. and *Neptunium amplexicaulis.*

ii) Secondary selenium accumulators; These are not restricted in their distribution and contain a few hundred μg/g of selenium. Species include members of *Aster* spp., *Atriplex* spp. and *Grayia* spp.

iii) Non-accumulator plants; These contain only low concentrations of selenium (up to 30 μg/g) even when grown on seleniferous soils. Most grasses and cultivated crops are of this type.

Accumulator plants appear to have a capacity to absorb forms of selenium from the soil which are not available to other plants and convert them to biologically available forms including methylselenocysteine and selenocystathione (Beath et al., 1937). For this reason they are also known as converter plants. A few accumulator plants have been reported containing extremely high levels of selenium. *Neptunia amplexicauli* , a legume, often has a selenium content of several thousand μg/g and is an occasional cause of livestock poisoning in Australia. Several severe cases of toxic symptoms and death in humans after eating nuts of *Lecythis ollaria* containing more than 18,000 μg/g selenium have been recorded in South America and one mushroom species *Amanita muscaria* can accumulate 100-600 times more selenium than the plants growing in its surroundings (Shrift 1973).

Pasture species also vary in their ability to accumulate selenium with grasses reported to accumulate 2-4 times more selenium than clovers (Davies and Watkinson, 1966). Bisbjerg and Gissel-Nielsen (1969) found the following decrease in plant selenium concentrations on low selenium Danish soils:

crucifers > ryegrass > legumes > cereals.

Seasonal variation in selenium concentration is observed in crop plants, with maximum selenium concentration in the early spring and minimum levels in the summer months.

There is no evidence to suggest that selenium is an essential

micronutrient for plants except perhaps for a few accumulator species (Shrift, 1973).

2.4.2 Uptake of Selenium by Plants

Selenium uptake from soils into plants is highly variable and appears to be dependent upon many factors, most importantly the plant species and the soil conditions.

It has been known for many years that soils containing high levels of selenium do not necessarily produce toxic vegetation (Lakin et al., 1938). Selenium rich soils in Hawaii (up to 15 μg/g) which are also highly ferruginous do not produce seleniferous vegetation. Soils from the U.S.A. and Puerto Rico also contain up to 4 μg/g of selenium in their iron-rich horizon but do not support seleniferous vegetation. In both cases the selenium is assumed to be in the form of insoluble ferric oxide-selenite adsorption complexes.

Studies by Epstein (1955), Leggett and Epstein (1956), Ulrich and Shrift (1968), Shrift and Ulrich (1969) and Ferrari and Renosto (1972) using excised barley roots and *Astragalus* spp. suggested active uptake of selenate by roots and that selenate has a common binding site with sulphate. Working with plants given selenate solution, Asher, Butler and Peterson (1977) and Gissel-Nielsen (1979) reported selenate as the major transport species of selenium in the xylem, suggesting a similarity between sulphate and selenate transport routes. Ulrich and Shrift (1968) suggested that the uptake of selenite is a passive diffusion process, and this has been substantiated by Asher, Butler and Peterson (1977). Although some selenite may enter the root by diffusion, it appears that it must be oxidised to selenate, or another form, before being transported through the plant, as very little selenite has been identified in plant xylem exudates (Peterson et al., 1981).

Selenium is translocated to all parts of the plant, newly formed leaves containing more than older ones (Rosenfeld and Eppson, 1962).

In plant selenium uptake experiments, soils spiked with selenate always produce vegetation with a higher selenium content than those spiked with

selenite (Bisbjerg and Gissel-Nielsen, 1969), due to the greater solubility of the selenate ion in soils. Experiments on plant selenium uptake from solution culture, however have shown inconsistent results for the relative uptake of selenate and selenite ions.

Beath, Gilbert and Eppson (1937) reported that elemental selenium was accumulated by *A. bisulcatas*, *A. pectinatus* and also by wheat although it is now generally accepted that this form of selenium has a limited availability to plants from soils (Bisbjerg and Gissel-Nielsen, 1969; Carter, Brown and Robbins, 1969).

An extract of organic selenium from the selenium accumulator *A. racemosus* was rapidly accumulated by plants grown in culture solution (Trelease and Disomna, 1944; Trelease and Greenfield, 1944). The high availability of organic forms of selenium to plants may account for the increased uptake of selenium by plants from soils with a high organic matter content.

Most higher plant species appear to metabolise inorganic selenium to organic selenium compounds and a projected pathway has been outlined by Burnell (1981). Accumulator species have been shown to synthesise selenocystathionine (Peterson and Butler, 1967; Peterson and Robinson, 1972) and methylselenocysteine and its γ-glutamyl peptide (Shrift and Virupaksha, 1963; Chen, Nigam and McConnell, 1970). Non-accumulators are also reported to synthesise selenomethionine (Butler and Peterson, 1967) and Se-methyl-selenomethionine (Peterson and Butler, 1962). Many organoselenium compounds have been identified in plants and some of these are listed in Table 2.6.

Information on the precise biochemical pathways of selenium in the plant is sparse but it appears that the end products of selenium assimilation, during which selenium is necessarily reduced to selenide, are predominantly the seleno-amino acids (Peterson et al., 1981). The accumulation of high levels of selenium without any harm to the accumulator species is achieved by synthesising and storing non-protein amino acids and therefore excluding selenium from functional enzyme systems. Selenosis of plants occurs when non-accumulator species assimilate high concentrations of selenium, in which case the high proportion of seleno-amino acids, which substitute for their sulphur analogues in proteins, has deleterious effects.

Compound	Formula	Source
Dimethylselenide	CH_3SeCH_3	Fungi
Dimethyldiselenide	$CH_3SeSeCH_3$	Fungi, *Astragalus*
Se-methylselenocysteine	Cy^*SeCH_3	Se accumulators e.g., *Astragalus* and *Stanleya*
Selenomethionine	$CyCH_2SeCH_3$	Bacteria, Fungi, *Lemna*
Selenocystine	$CySeSeCy$	*Lolium* , *Trifolium*
Selenocystathionine	$CyCH_2SeCy$	Se accumulators e.g., *Astragalus* , *Morinda* *Stanleya* , *Neptunia*
Selenohomocystine	$CyCH_2SeSeCH_2Cy$	*Lecythis*
Selenomethionine selenoxide	$CyCH_2Se(=O)CH_3$	*Astragalus*
Se-propenylselenocysteine selenoxide	$CySe(=O)CH=CHCH_3$	*Allium*

* Cy = $-OOC-\underset{\displaystyle NH_3^+}{\underset{|}{CH}}-CH_2-$

Table 2.6 Organoselenium compounds found in plants (Girling , 1984)

The availability of selenium to plants is dependent on the pH of the soil, with greater uptake occurring at higher pH's. This is explained by the increasing selenite concentration in the soil solution at raised pH's when in equilibrium with a ferric oxide-selenite adsorption complex (section 2.3.2), and also that at higher soil pH levels soluble selenates may be formed. Studies into liming soils to increase their pH and the effect of this on selenium uptake have shown increased selenium uptake with liming (Cary and Allaway, 1969; Gissel-Nielsen, 1971b), but the increased uptake from soils of low or normal selenium level was small. In both these studies there was also an effect due to the soil texture; the heavier soils with the greater adsorption capacity retaining more selenium than the lighter soils. It appears that the real effect of increasing soil pH on the uptake of selenium by plants on normal to low selenium soils is small and would produce negligible increases in the selenium content of pasture.

Competitive antagonism may take place between sulphate and selenate uptake by plants, due to the similarity between the chemistry of selenium and sulphur. The addition of sulphate was not found to decrease the uptake of selenium by plants from seleniferous soils in the U.S.A., primarily because the soils already contained high levels of gypsum (Frank and Painter, 1937). However, nutrient solution studies demonstrated that sulphate depressed selenium uptake when selenate, but not selenite was the source of selenium (Hurd-Karrer, 1938). This effect has subsequently been proved to be successful in suppressing selenium uptake from toxic soils elsewhere (Ravikovitch and Margolin, 1959; Fleming, 1980; Williams and Thornton, 1972). Ferrari and Renosto (1972) also reported that the uptake of sulphate by barley roots is competitively inhibited by selenate. Sulphate has been found to counteract selenate toxicity in micro-organisms and higher plants, the mechanism of selenite toxicity however appears to be different to that of selenate (Shrift, 1958).

The possible effects of sulphur additions to soils sustaining vegetation potentially deficient in selenium is of greater concern in this country. Sulphur fertilisation is not common in Great Britain, however the application of some fertilisers, notably superphosphate and ammonium sulphate, necessarily involves the addition of sulphate to the soil. There is some qualitative evidence that

sulphur additions, as superphosphate, can reduce the plant selenium levels in soils of low selenium status. Walker (1971) reported that farmers in Central Alberta suspected that sulphur fertilisation increased the incidence of White Muscle Disease, however trials showed that the decrease in plant selenium levels was only significant when the sulphur fertilisation produced an increase in plant growth. Similar dilution effects were observed in New Zealand soils when the addition of superphosphate was compared with that of mono-calcium phosphate, which contains no sulphur (Davies and Watkinson, 1966).

Cary and Gissel-Nielsen (1973) demonstrated that sulphate additions had only small effects on the solubility of soil selenate and negligible effects on that of selenite, and thus assumed that any change in plant uptake of selenium with sulphate addition was due to competitive interaction during plant absorption. This assumption has not yet been clarified and the primary effect of sulphate addition as fertilisers appears to be that of growth stimulation and consequent dilution of the selenium content of the plant.

The effect of other fertilisers on plant selenium uptake has also had some consideration. Carter et al. (1972) found that phosphate additions increased plant selenium contents of plants grown on six out of fourteen soils, for both native and added selenium, and they considered that the effect might be sufficient to induce adequate levels of selenium in marginal pastures. Other work with N, P, S and Se additions to soils has shown that the effects of phosphate depended upon the level and interaction with other nutrients (Gissel-Nielsen, 1974). Cary and Gissel-Nielsen (1973) found that the addition of phosphate did not increase the stability, in dilute $CaCl_2$, of added selenite, so it has been suggested (Fleming, 1980) that the addition of phosphate may increase the selenium uptake by promoting plant root growth.

Fleming (1962) reported that applications of superphosphate fertiliser, which contains high levels of sulphate, to toxic soils in Ireland decreased the selenium accumulation in the herbage, however, the effects of the phosphate may be masked by those of the sulphate.

Little is known of the effects of nitrogenous substances on the uptake of selenium by plants. Cary and Gissel-Nielsen (1973) noted that nitrogen application may reduce the uptake of selenium but that this is probably due to differences in uptake at the plant root or growth influenced dilution, rather than an effect on the solubility of selenium in the soil.

2.5 SELENIUM IN ANIMAL NUTRITION

2.5.1 Selenium Toxicity

The first report of a selenium disorder in livestock appears to be from Marco Polo. In 1295, when travelling in Western China his animals lost their hooves, and his descriptions suggest that they were suffering from an excess of selenium. Two chronic livestock disorders are known to be associated with plants growing on seleniferous soils; alkali disease and blind staggers (Rosenfeld and Beath, 1964). Alkali disease is characterised by liver cirrhosis, emaciation, deformation and loss of hooves, loss of hair and lack of vitality, and is caused by animals ingesting vegetation with selenium levels of 10-30 μg/g over periods of weeks or months. Alkali disease has more often been reported in cattle, however chronic selenium toxicity of this type has also been described in sheep (WHO/Environ. Health Criteria, 1987). The symptoms of blind staggers include eye lesions, wandering in circles and eventual death due to respiratory failure, and is caused by the ingestion of limited numbers of selenium accumulator plants over a period of weeks or months. Acute selenium poisoning, although rare, can also occur due to the ingestion of toxic quantities of selenium from highly seleniferous plants, and death often occurs within a few hours of ingestion.

Selenium has been found in toxic amounts in wheat and plants growing in many areas world-wide including; areas of the Great Plains and Rockies, (western U.S.A.), South America, Hawaii, Ireland, Canada, Australia and New Zealand

(Rosenfeld and Beath, 1964).

More recently, selenium toxicity has been encountered in the San Joaquin valley in California (section 2.2.3). High levels of selenium have accumulated in the Kesterton reservoir from agricultural drainage waters, and selenium poisoning has been observed in wildfowl using the reservoir as their habitat and breeding ground (Mikkelsen et al., 1986).

2.5.2 Selenium Deficiency Diseases in Animals

The first recognition of selenium as an essential trace element in animal nutrition was when Schwarz and Foltz (1957) discovered that selenium prevented dietary liver necrosis in rats fed a vitamin E deficient diet. Since this realisation, four enzyme catalysed reactions, two in bacteria and two in mammals, have been shown to involve a selenium-containing protein (Stadtman, 1974). One of these reactions in mammals is catalysed by glutathione peroxidase (GSHPx) and its activity in blood and tissues of rats has been shown to be dependent upon the selenium content of the diet (Rotruck et al., 1973; Reddy and Tappel, 1974; Chow and Tappel, 1974; Hafeman et al., 1974; Smith et al., 1974). Furthermore it has been proved (Rotruck et al., 1973) that many of the nutritional effects of selenium can be explained by the action of GSHPx. In both cattle (Flohe et al., 1973) and sheep (Oh et al., 1974) erythrocyte GSHPx has been shown to contain four atoms of selenium per molecule of enzyme.

Once the essential nature of selenium had been realised several selenium responsive diseases were recognised in farm animals. In sheep and cattle, White Muscle Disease and the sub-clinical 'ill thrift' have been attributed directly to selenium deficiency, with vitamin E having an associated role in these diseases. Selenium deficiency has also been suggested as one cause of low fertility rates in livestock (Russell, 1987).

Of the selenium deficiency disorders in livestock, the most outstanding is White Muscle Disease (WMD). In New Zealand about 30% of the lambs and calves can be affected. The symptoms of WMD are poor growth of hair, general depression of body growth rate, skeletal and cardiac muscle damage, the muscle often being calcified (Rosenfeld and Beath, 1964; Hartley and Grant, 1961; Oldfield, 1972).

Several factors including the lack of available selenium in soils, intensive farming and fertilising practices, altering ground water status by drainage or irrigation, minimal grain and concentrate feeding to the animals can all contribute to the incidence of selenium responsive diseases. The selenium status of grazing livestock has been shown to decrease at high stocking rates (Langlands, 1982) and hence farm management can be a very important factor in eliminating deficiency problems (Russell, 1987). In New Zealand the alteration of pasture from mainly *Agrostis capillaris* (bent grass) to mostly clover caused outbreaks of WMD (Grant, 1965). Years of superphosphate dressing of pasture in Western Australia apparently led to the development of WMD (Gardiner and Gorman, 1963).

Selenium-vitamin E responsive diseases are still prevalent in farm animals in various areas of the world and consequently there has been considerable interest in the relationship between erythrocyte GSHPx and blood selenium concentrations. The positive correlation between these two was described by Allen et al. (1975) and Boyd (1975) for cattle and by Wilson and Judson (1976) and Thompson et al. (1976) for cattle and sheep. Erythrocyte GSHPx measurement is now commonly used as a measure of the selenium status of livestock.

In New Zealand where selenium deficiency is a widespread problem in livestock, pastures associated with selenium responsive ill-thrift in sheep contained 0.008-0.030 μg/g selenium (Hartley, 1967). A level of selenium in foodstuffs of 0.02 μg/g has been quoted as a critical level, below which deficiency symptoms are observed in ruminants (U.S. NAS/NRC, 1971). Ehlig et al. (1968) and Walker (1971) suggested a selenium concentration of 0.1 μg/g (dry weight) in animal feed as a requirement for normal growth, whereas more than 3-5 μg/g Se in forage can result in toxicity (Bisbjerg and Gissel-Nielsen, 1969). The selenium

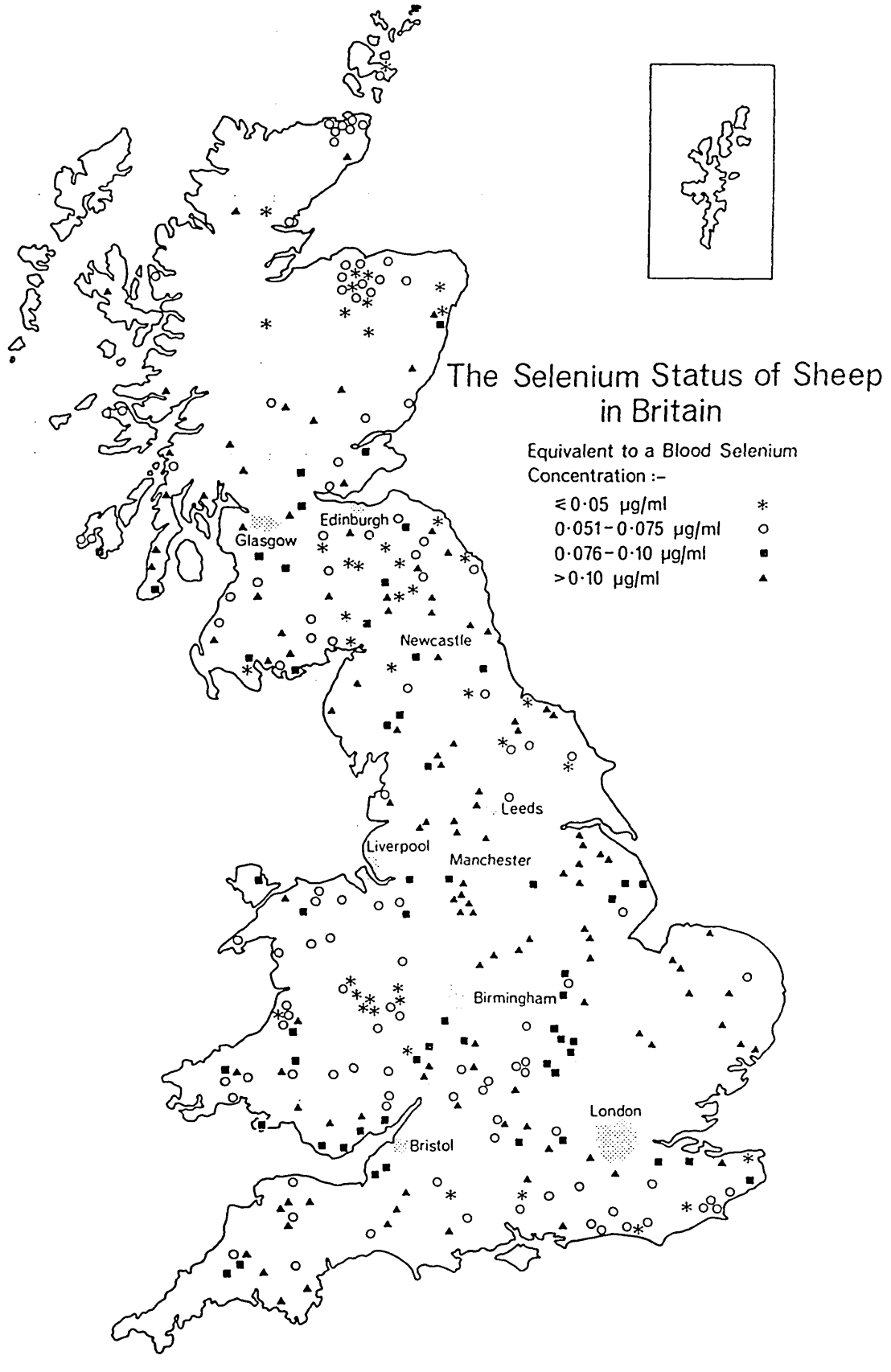
requirement of animals thus falls in a rather narrow range and this contributes to the problem of maintaining an adequate selenium status in livestock in areas of low to normal soil selenium concentrations.

White muscle disease in livestock has become more prevalent in Britain over the last two decades. This is primarily due to modern farming practices producing selenium and vitamin E deficiency at the end of the winter feeding period. One of the first reports of WMD in Britain came from the Moray Firth in Scotland (Blaxter, 1963).

A survey of erythrocyte glutathione activity (GSHPx) in 329 flocks of sheep in England, Wales and Scotland indicated that in 47% of the grazing flocks examined, herbage selenium levels were unable to maintain the selenium status of the animals at blood selenium concentrations of 0.075 μg/ml or above (Anderson et al., 1979). The results of this survey are shown in Figure 2.2. A large proportion of the flocks sampled in Scotland together with many from Wales and the Welsh Border counties, southern and south-eastern England and a few elsewhere were considered to be selenium deficient. The authors concluded that such a high proportion of deficient stock could be related to the increase in white muscle disease and other selenium responsive disorders in Britain at that time.

In this country, measures to increase the selenium status of livestock include winter feeding with 'concentrates' containing selenium salts amongst other trace elements. This form of selenium supplementation is now widely used in the U.K. Selenium injections or slow release glass bullets in the rumen (Carlos et al., 1985) have also been used for animals suffering from deficiency.

Figure 2.2 **The selenium status of sheep in Britain (Anderson, 1979)**



2.5.3 Soil Ingestion

Soil as a source of trace elements in animal diets has long been recognised. Thornton (1974) estimated that the soil ingested by cattle in England during winter ranged from 140-1400 g/day and suggested that such a large intake of soil could provide the animal with a substantial part of its trace element requirements. Russell (1987) found a wide range of soil ingestion from contaminated herbage in sheep (0.5% to 31% of the diet), which varied with season and rainfall rates. The mean value of soil contamination of herbage was around 5%. At peak soil contamination, grazing livestock ingested in excess of 100 g of soil per day from pasture as well as significant amounts by direct ingestion from wormcasts and plant roots.

The availability of many trace elements, including selenium, from the ingested soil has been studied. However the evidence seems to suggest that the selenium status of sheep is not affected by the selenium content of ingested soil (Brebner, 1987). Soil selenium was apparently unavailable to sheep under controlled feeding conditions, although the reason for this was not clear. The sheep were in a selenium- sufficient state at the beginning of the experiments and it has been shown that animals only respond to selenium when they are deficient in selenium. Other explanations were that the selenium forms insoluble complexes with iron and other heavy metals (such as As, Cd, Hg, Ag and Cu) and is therefore rendered unavailable for absorption; that the actual levels of selenium entering the animal with the soil are low for most soils in this country and the soil may not contribute very much to the selenium content of the diet; and also that the ingested soil may inhibit the absorption of dietary selenium (Brebner, 1987). The majority of selenium in animal diets, apart from additional feed supplements, is therefore still considered to be derived from plant material. The pathway of selenium from the soil and into the plant is the area where most uncertainties lie and which is of greatest importance in the availability of selenium to the grazing livestock of any particular area.

2.5.4 Selenium Nutrition in Human Populations

Selenium is also an essential trace element in the human diet and problems associated with its deficiency have been encountered in China and suggested in Finland and New Zealand. Other areas of the world have low selenium levels in the soils and feedstuffs although no human selenium responsive diseases have been noted outside of China. Due to the varied diet of the majority of people and the transport of food from one area to another, deficiencies or excesses of selenium in the diet are rarely traced to geochemical levels of selenium, unlike livestock which rely on a supply of food from a very limited area. However problems have occurred in the past and are still being recognised, usually in remote, rural areas where the population may depend entirely upon locally grown produce.

Epidemiological studies in Finland suggested an inverse relationship between the selenium content in crops and the incidence of multiple sclerosis in the population (Palo et al., 1973). Other Finnish (Westermarck, 1977) and Swedish (Masironi and Parr, 1976) studies have shown some links between areas of low selenium and higher rates of cardiovascular diseases. The Finnish government has considered supplementing the national drinking water with selenium in order to maintain adequate selenium levels in the population since the majority of Finland has soils which are deficient in selenium. Selenium, in the form of sodium selenite, is now added to fertilisers in Finland and used country-wide on cereal crops and grassland in an attempt to increase the selenium content of Finnish foods (Koivistoinen and Huttanen, 1985).

The U.S. NAS/NRC (1980) has recommended 50 μg/day selenium as a safe and adequate intake for adults. There are some areas in China where the daily selenium intake was found to be 10 μg/day. The unusually low intake of selenium has led to the investigation of its relationship with occurrence of two selenium-responsive diseases, Keshan and Kaschin-Beck diseases in China (Xu and Jiang, 1985). Keshan disease is a cardiomyopathy particularly affecting young women and children. Kaschin-Beck disease affects the bones and joints and occurs mainly in children. These selenium deficiency diseases appear to be unique in

man and are attributed in part to the almost complete reliance upon local crops which are very low in selenium.

Keshan disease is an endemic cardiomyopathy of unknown cause in Keshan county, Heilongjiong Province and other areas. The disease is prevalent in a wide belt across China from the southwest to the north east and this corresponds to areas of low soil selenium and the occurrence of selenium-responsive diseases in livestock. The low levels of selenium in the soils of the area are made worse by weathering, oxidation and leaching of selenate.

Sodium selenite supplementation in the diet has been found to be effective in reducing the incidence of the disease, however selenium deficiency may not be the only cause of the disease. A viral infection is one possibility, with selenium deficiency and poor protein nutrition reducing the immunity of the population. However, at present the aetiology of the disease remains unknown, but selenium deficiency is assumed to be one causative factor.

The geographical incidence of Kaschin-Beck disease is similar but not always coincidental with that of Keshan disease. Kaschin-Beck disease is an endemic multiple osteoarthropathy. The growing centre of the bone undergoes dystrophy which results in dwarfism and shortness of fingers and toes. Bone enlargement and disfiguration of the joints also occurs (Mo, 1987). The cause of this disease is unknown, however selenium deficiency is again almost certainly involved.

Selenium in association with vitamin E has also been suggested as a protective agent against cancer (Shamberger, 1970) and oxygen induced tissue damage (Diplock, 1981) due to their combined anti-oxidant properties.

Disorders in man have also been reported due to high inorganic selenium ingestion. The health effects of high selenium intake on populations living in seleniferous areas of the U.S.A. have been widely studied and symptoms include chronic arthritis, gastro-intestinal disorders, discolouration of the skin and teeth and loss of hair and nails (Schroeder, Frost and Balassa, 1970). A report of a localised intoxication of selenium in China has been reported (Yang et al., 1983) with symptoms of hair loss, scalp irritation, brittle nails, skin lesions and occasional ulcerations, and abnormalities of the nervous system. This outbreak of

selenium toxic symptoms has later been attributed to the use of a seleniferous coal in an area of China which normally has high levels of selenium in the soil and occasional reports of selenosis (WHO/Environ. Health Criteria, 1987).

Diplock (1987) has discussed the role of selenium as a trace element in human health, and the WHO has recently produced a comprehensive survey of the literature pertaining to selenium in human health, reviewing all aspects of selenium toxicity, deficiency, biochemistry and human exposure to selenium (WHO/ Environ. Health Criteria , 1987).

CHAPTER 3

FIELD SURVEY, DESCRIPTION OF FIELD SITES, GEOLOGY AND SOIL CLASSIFICATION

3.1 INTRODUCTION

Field surveys undertaken in Britain prior to this research have identified some areas of the country which may produce selenium deficiency in the grazing livestock and these have also been used to estimate the extent of areas with low soil selenium levels.

These surveys have generally taken the form of geochemical studies such as that of Thornton et al., (1983), where the relationship between the total soil selenium concentrations and the selenium content of the underlying parent material was investigated, or studies of the selenium status of animals. For example Russell (1987) examined the selenium content in the diet of grazing ruminants provided by the local herbage and associated soil ingestion and the effect of this on the blood glutathione peroxidase levels of the animals.

Apart from an inconclusive study by MAFF (1983), the relationship between the soil selenium concentrations and the uptake of selenium into pasture plants in this country has not been widely studied with respect to selenium deficiency, and many reports have outlined this as an area requiring further research.

Many of the soil factors which affect the availability of selenium to plants have been studied in the field and the laboratory, but usually in isolation and generally at high levels of total selenium concentrations. The results of these studies were discussed in detail in Chapter 2.

3.2 PROJECT DESIGN

The field work was intended to provide preliminary information towards a predictive equation for soil selenium availability in the permanent pasture of England and Wales, and the sampling programme was therefore designed with this primary objective.

For this research it was decided to study a limited number of widely different soils on a seasonal basis, and to analyse the collected soils and herbage for a wide range of chemical and physical characteristics.

The choice of sites was given careful consideration in order to obtain soils with sufficient variation in those soil and climatic conditions which are thought to affect selenium uptake by plants. Certain overall requirements were kept in mind while deciding on the areas to use and finally local soil maps were utilised to find a particular field or farm site which fulfilled the necessary conditions. The co-operation of the farmers was also one important criterion in the choice of the sites, however this was always obtained on request. All the sites studied had to be in areas of the country where the rearing of livestock is an important aspect of the local agriculture and especially areas where sheep rearing predominates. Many of the upland areas of England and Wales are used extensively for sheep grazing and so areas of Derbyshire and Wales were considered suitable for the study.

Soils were chosen which reflected the general levels of soil selenium in this country, concentrating especially on the areas of low to marginal selenium status. However some sites where the selenium level is higher were also chosen in order to provide the full range of selenium concentrations normally found in England and Wales.

An area of Derbyshire has soils derived from marine black shales, which contain elevated levels of selenium, and one site was chosen from this area. For comparison, another site was chosen from the same area of Derbyshire but the soil here was a brown earth developed over limestone which is the predominant soil type of the whole area, and which has an average selenium concentration.

Romney Marsh on the Kent-Sussex coast is an area with one of the highest density of sheep flocks in the country, and the area also has some problems with

selenium deficiency in the sheep. Consequently one farm on the Marsh was chosen for sampling and the field used in this case has been left virtually unchanged, not reseeded or treated with nitrogenous fertilisers, herbicides or pesticides, only given a phosphate dressing every few years. The field has been designated a site of special scientific interest (S.S.S.I.) by the Nature Conservancy Council because it is such an unusal example of original and unaltered permanent pasture which was once typical of Romney Marsh.

The other factors which were considered important in this study included pH, organic matter content and drainage status. In order to eliminate the strong influence of parent material selenium content on soil selenium content, an area in North Wales was studied which is entirely underlain by a relatively uniform parent material of Silurian rocks or drift material derived from these Silurian rocks. The various soil types which are found in this area are due to climatic, drainage and land-use differences as they are all derived from similar parent materials. This region is centred around the village of Llansannan in Clwyd and includes the Hiraethog moorland areas. Eight sites have been chosen from this region from farmland and peat moorland, and these eight soils vary widely in drainage status, organic matter content and pH, the moorland soils being relatively acidic. One of these eight sites (Site 2), a poorly drained gleyed soil, was drained and covered with other soil and gravel as part of improvements on the farm during the sampling programme and therefore could not be used for the whole two years. A replacement site (Site 8) of the same soil series bordering a small stream was found for the remainder of the sample collections, however the results from the two sites should not be directly comparable and have been treated separately.

Soils derived from sandstones are often low in selenium and some areas of South Wales, near Brecon, where the soil parent material is the Old Red Sandstone have had problems with selenium deficiency symptoms in the livestock. The high rainfall of this area may also suggest that the leaching of selenium could be a problem. As an example of the soils from this area, samples were collected from an experimental farm, Bronydd Mawr, in the Brecon Hills.

The fields chosen from all the sites mentioned above were under

permanent pasture, or rough grazing in the case of the moorland sites, and the areas used for sampling within the fields were always well away from field boundaries, overhead wires, paths or trees.

In addition to the twelve sites outlined above, access was allowed to the long-term liming experiment at Woburn belonging to Rothamsted Experimental Station. This field has been divided into small blocks and treated with different levels of lime since the 1960's. The individual plots have consequently been maintained at different pH levels for over 27 years and the soil has had time to equilibrate. In all other aspects apart from pH, the soils from the plots should be alike. The interest in these soils was therefore to investigate the effect of pH alone on the uptake of selenium from the soil when all other soil and climatic factors remained the same. However it was not always possible to collect soil and herbage from these plots as the ongoing experiments could not be disturbed and so a full two year seasonal sampling was unfortunately not obtained from this site, and hence the seasonal changes could not be effectively monitored.

At each site chosen for study, samples of topsoil, subsoil and herbage were taken every three months during a two year period, as described in Chapter 4, and rainwater samples were also collected in each area during one year.

3.3 FIELD SITE DESCRIPTIONS

A brief description of each site sampled during the two year collection period is given below and the site numbers given here will be referred to later in this work. Table 3.1 provides a summary of the sites sampled and the selenium concentration of the soil at each site.

a) North Wales

Eight sites were sampled in this area near Llansannan, Clwyd. Although all sites are underlain by the same Silurian parent material, the soils vary widely in organic matter content and drainage status, from well drained sandy soils to waterlogged peat moorland. The selenium content of the soils is average to marginally deficient.

The majority of farms in this area are small upland farms with lower stocking rates than are possible in lowland areas. The pastures on many of the farms have been improved by reseeding and addition of lime, phosphate and nitrogen. The main problem of sheep farming in the uplands is the environment during the winter, in particular control of nutrition and poaching of waterlogged ground (MAFF, 1981a). To alleviate this the winter housing of sheep for lambing is recommended in this area, with controlled feeding of silage and concentrate feeds. The moorland area of the Hiraethog is used as rough grazing land for many free ranging flocks of sheep which remain outside all year and receive minimum feed supplements except during snow cover when hay is frequently provided.

Site 1 Melai Farm on Moel Unben

This is a large, well-kept farm, the farm buildings are situated in the Nant Melai valley and the field sampled covers one side of a small steep hill known as Moel Unben. The area of the sampling grid lay 50 m from the field gate along the ridge of the hillside. The soil is freely drained due to the slope and is rather shallow and stony. Subsoil samples could not be obtained from this site.

Site 2 Ty Uchaf

This is predominantly a dairy farm also situated near the Nant Melai but on lower land. The area sampled was a small, marshy patch uphill and behind the farm buildings which drains the gently sloping land above the farm. A small stream is formed slightly lower down the hillside than the area sampled. Both the topsoil and subsoil were waterlogged and gleyed all year round.

After one year of sampling the area was badly disturbed by drainage improvements on the farm and could not be used for further sampling. Another poorly drained site (Site 8) was later chosen as a replacement.

Site 3 Nant y Garreg

This farm is situated on high land just south of the Hiraethog moorland and some of the pasture fields are improved moorland. The field sampled lay uphill from the farm almost on the brow of the hillside above the valley, and the area of the grid was 10 m uphill and 20 m to the right from the gate. The drainage is moderate due to the slight slope of the field.

Site 4 Pencraig Fawr

This farm lies in the sheltered Afon Aled valley near the village of Llansannan and the field sampled is opposite the farm house, relatively flat and moderately well drained. The grid area lay 15 m from the gate and 10 m to the left.

Site 5 Hiraethog

This is the first of three sites sampled on the peat moorlands of the Hiraethog, all with different drainage conditions. The sampling grid for Site 5 was chosen from a well drained area on the brow of a small hill to the north of Aled Isaf Reservoir where an iron pan underlies the shallow peat soil.

Site 6 Hiraethog

This site lies south-west of Site 5 at the foot of the small hill. The area is very poorly drained and remains saturated all year round despite being drained by a tiny stream. The peat soil here is very thick with no noticeable difference between the topsoil and subsoil.

Site 7 Hiraethog

The area sampled for Site 7 lay in a slightly different area of the moorland by the east side of Llyn Aled. A shallow slope with a moderately drained peat soil was chosen for the sampling area as a comparison between the well drained and poorly drained sites 5 and 6.

Site 8 Plas Panton

This site was sampled as a replacement for site 2. The small farm has a stream running through it and an area was sampled at the side of this stream, in a field which is under permanent pasture. The subsoil was waterlogged and gleyed all year round but the topsoil was only waterlogged in the winter and spring. The subsoil especially was rather stony presumably due to material deposited by the stream.

b) Brecon, South Wales

One site was sampled in this area near Trecastle, Brecon. The mineral soil is derived from Old Red Sandstone and is sandy , fairly well drained and low in organic matter. The selenium content of the soil is rather low.

Site 9 Bronydd Mawr

An example of the soils in the Brecon area was taken from a field on the experimental farm Bronydd Mawr. This is an extemely large farm which is unusual for the area but is the result of the acquisition of land from surrounding farms. The field used as Site 9 lies almost at the end of the track running uphill from the farm buildings. The field sampled has had no experimental treatments, only a normal level of fertilisation. The land is very bleak and windswept with a high precipitation level, and the red sandy soil, although fairly well drained, was almost always wet during sampling. The grid area lay 10 m into the field from the gate and 10 m to the left.

c) Derbyshire

Two very different sites were sampled in two areas of Derbyshire. The first was a farm in Tissington, near Hartington, where the soil is derived from marine black shales and consequently rather high in selenium. The second site was from a farm above Taddington, near Bakewell, where the soil is a brown earth developed on a limestone parent material with average selenium levels. Upland conditions are found at both sites and farming conditions are similar to those in North Wales although the winter temperatures and precipitation rate are usually lower.

Site 10 Shaw Farm, Tissington

Part of this farm lies over marine black shales and has soils with a high selenium level and this is the area of interest. The field sampled was the second field on the left from the track leading from the farmyard, and the sampling grid was 25 m into the field from the second tree uphill from the gate. The soil is freely drained and small fragments of the black shales are often visible in both the topsoil and subsoil.

Site 11 Taddington Fields Farm, Taddington

This farm is situated on high ground above the village of Taddington and lies over limestone parent material. The field sampled was the second behind the farmhouse to the east and the sampling grid was 15 m from the lower gate (east) and 10 m up the slope (north). The soil is a well drained brown earth.

d) Romney Marsh

One site was sampled from the marsh itself. The soil is a silty soil of marine origin with rather poor drainage and very low in selenium.

Sheep farming on the marsh is intensive and the sward is grazed very close to give a short dense pasture all year round. Sheep remain outside all year even for lambing and are usually fed locally grown hay and occasionally concentrates. It

is a common practice to send the the ewes to the surrounding uplands and even as far as Hampshire and West Sussex during the summer and it is possible that this practice has in the past alleviated some of the potential deficiency problems that might arise if the animals were to remain on the marsh all year.

Site 12 Brookgate's Farm, Romney Marsh

This farm is heavily stocked with sheep and all the land is under permanent pasture. The field sampled lies between the house and the road and is an N.C.C. designated S.S.S.I. since it is unaltered by nitrogen fertilisation, reseeding or other treatments and is maintained in this unaltered state. The sampling grid lay 20 m into the field away from the road sign to the left of the gate. Both the topsoil and subsoil are fine silty soils, moderately drained and stoneless.

e) Woburn, Rothamsted Experimental Station

Rothamsted Experimental Station has allowed access to their long term liming experiment in Woburn. Four sites are used here from within the same area of one field but all maintained at different pH levels. The records of liming this area of the field date back to the 1960's and the soils have had time to equilibrate to the different pH's. The soil is sandy, low in organic matter and with a relatively low selenium level. It was not always possible to obtain soil and herbage samples from these sites since cropping experiments are continuously being carried out.

Site 13-16 Woburn Experimental Farm, Husborne Crawley.

The four sites sampled were plots from the long-term liming experiment in Stackyard Field, area C.

Site 13 High Lime- plot 35

Site 14 Medium Lime- plot 44

Site 15 Low Lime- plot 46

Site 16 No Lime- plot 41

Table 3.1 **The soil type, geology and total soil selenium concentrations of the sites sampled**

Site No.	Area	Soil Type	Parent material	Total Soil Se* μg/g
1	N. Wales	Brown earth	Silurian Shale	0.329 ± 0.057
2	N. Wales	Stagnogleyic brown earth	Silurian Shale	0.183 ± 0.036
3	N. Wales	Brown podzolic soil	Silurian Shale	0.434 ± 0.069
4	N. Wales	Brown earth	Silurian Shale	0.200 ± 0.030
5	N. Wales	Ferric stagnopodzol	Silurian Shale	0.323 ± 0.056
6	N. Wales	Stagnohumic gley soil	Silurian Shale	0.717 ± 0.203
7	N.Wales	Stagnohumic gley soil	Silurian Shale	0.755 ± 0.149
8	N. Wales	Stagnogleyic brown earth	Silurian Shale	0.125 ± 0.011
9	Brecon	Brown earth	Old Red Sandstone	0.134 ± 0.081
10	Derbyshire	Non-calcareous pelosol	Marine Black Shale	1.363 ± 0.084
11	Derbyshire	Brown earth	Limestone	0.330 ± 0.042
12	Romney Marsh	Calcareous alluvial soil	Silt Alluvium	0.125 ± 0.085
13	Woburn	Brown earth	Devonian Sandstone	0.195 ± 0.054
14	Woburn	Brown earth	Devonian Sandstone	0.179 ± 0.062
15	Woburn	Brown earth	Devonian Sandstone	0.169 ± 0.037
16	Woburn	Brown earth	Devonian Sandstone	0.157 ± 0.052

* The mean selenium concentration of 8 seasonal samples +/- 95% confidence limits

3.4 AREA GEOLOGY AND SOIL SURVEY CLASSIFICATIONS

A brief outline of the local geology at each sampling site is described here and maps of the geology and soil types of each area are included for information. A list of the Soil Survey classifications for the soil found at each site is given in this section and other site details are also provided.

3.4.1 Area Geology

a) North Wales

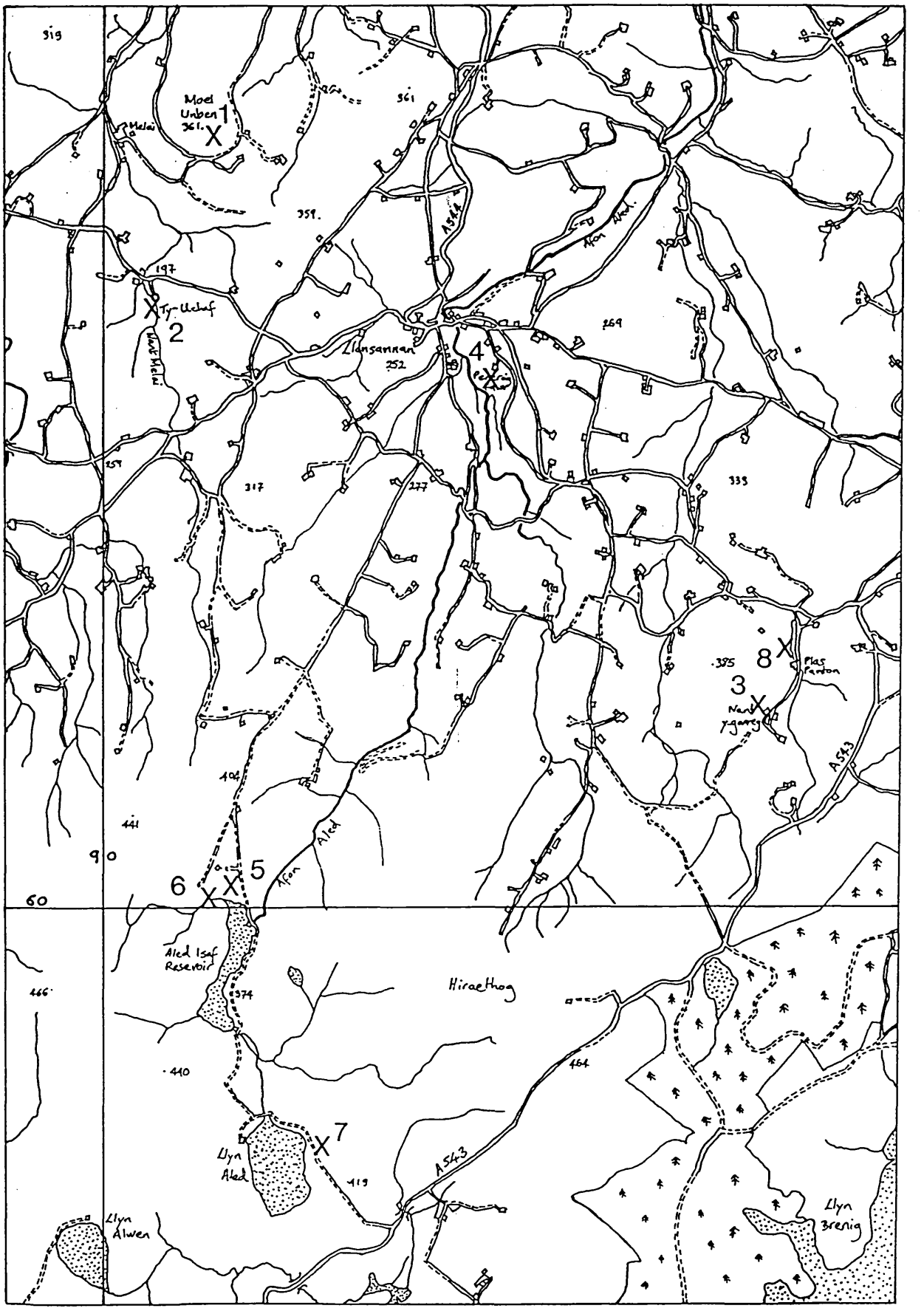
Within the study area there is only one well defined physical region, controlled by the geology, formed on the oldest rocks in the area. The Denbigh Upland and Moors founded on Silurian rocks lie mostly between 150-300 m a.m.s.l. and there is a gradual increase in elevation to the south where the moorlands average 350-425 m. The Upland is separated into hill-blocks by narrow, steep sided flat floored valleys where most of the villages are situated. The river Elwy has its source in the moorlands and drains the major part of the Upland.

The whole area is underlain by Lower Palaeozoic Silurian rocks, which in North Wales consist of a thick series of non-calcareous marine sediments. The Silurian system has been divided into three series, however, only the Ludlow and Wenlock series are found in the area. These series comprise mudstones and shales alternating with flags, sandstones and grits.

Drift cover in the area is quite extensive being predominantly a till produced by the local Pleistocene ice and can be up to 37 m thick. The till consists of boulder clay which is grey-brown in colour and generally an incoherent silty clay loam. It derives from the Ludlow and Wenlock rocks only and is therefore difficult to distinguish from the in-situ weathering products of the rocks themselves (Boswell, 1949. Ball, 1960). Other recent deposits include valley and river accumulations of sands and gravels, again mainly developed from the Silurian rocks and drift derived from them.

Ordnance Survey, geology and soil survey maps of this area are shown in Figures 3.1-3.3.

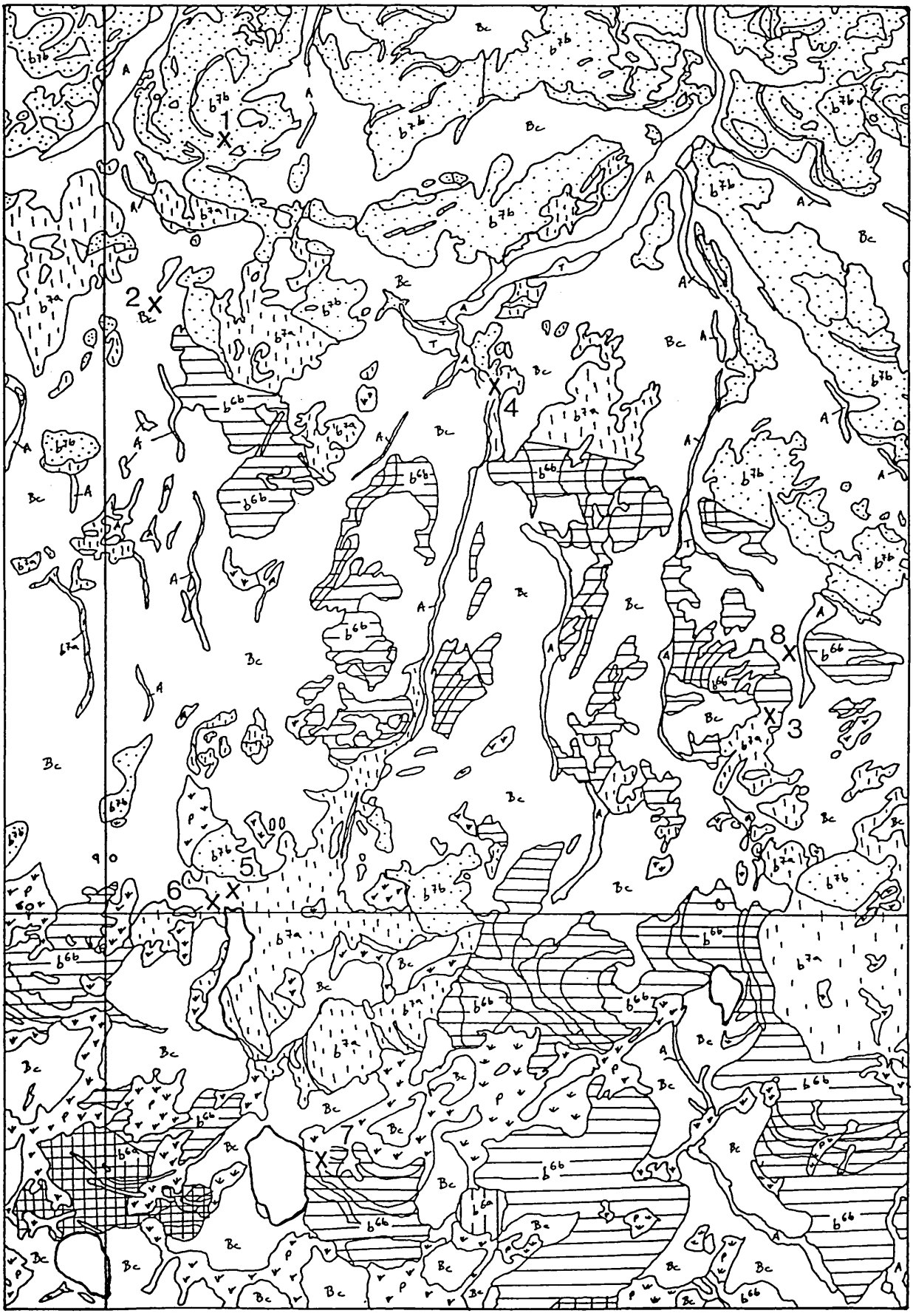
Figure 3.1 The Ordnance Survey map (1 : 50,000 O. S. Landranger 116, Denbigh) of the area around Llansannan, Clwyd (Sites 1-8)



Key to Figure 3.2

P	Peat
T	River terrace
A	River alluvium
Bc	Boulder clay
b^{7b}	Elwy group, silty mudstones with sandstones
b^{7a}	Upper Nantglyn Flaggs, mudstones
b^{6b}	Lower Nantglyn Flaggs, mudstones
b^{6a}	Denbigh Grits group, mudstones and sandstones

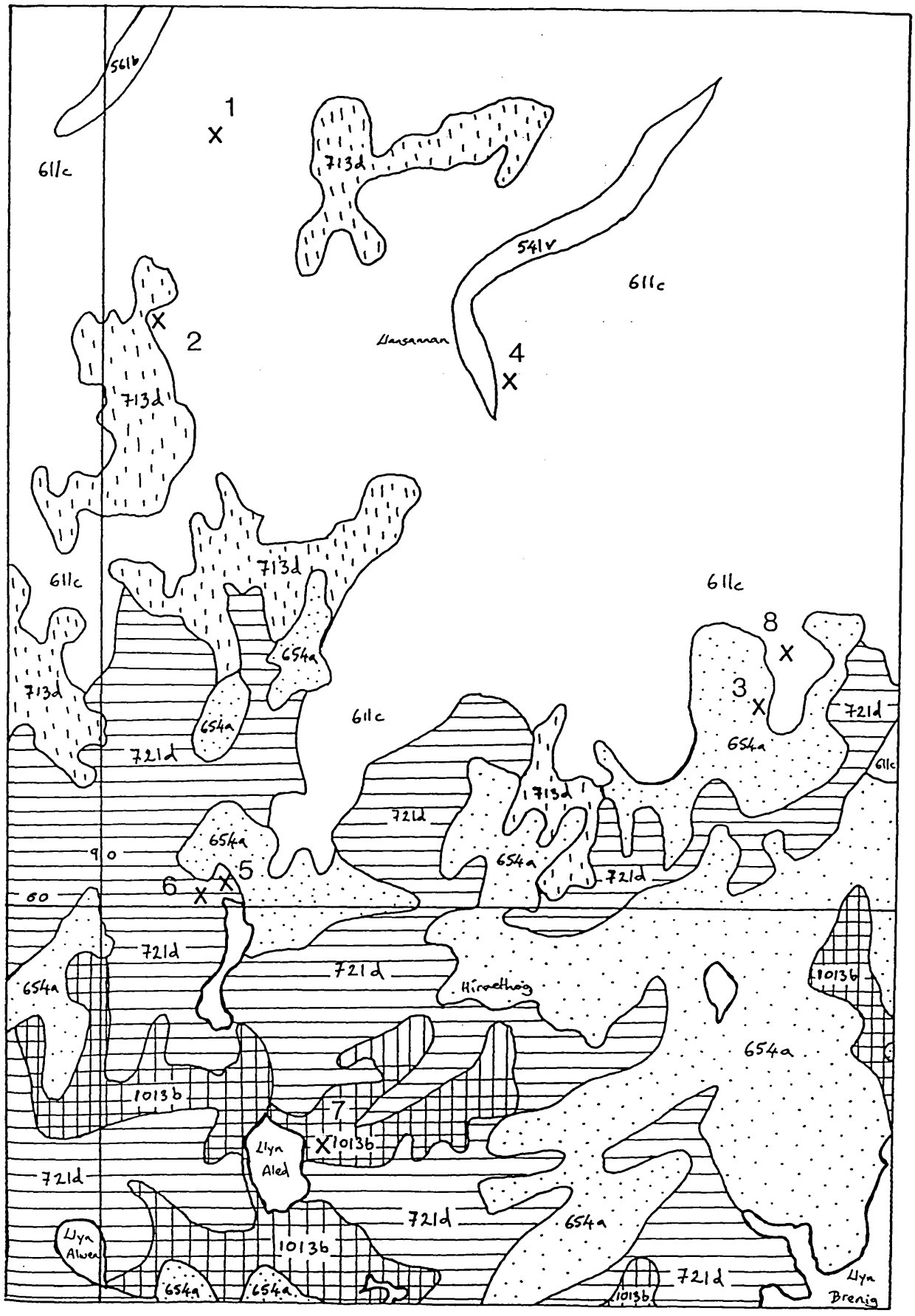
Figure 3.2 The solid and drift geology map (1: 50,000 Geological Survey, sheet 107, Denbigh) of the area around Llansannan, Clwyd (Sites 1-8)



Key to Figure 3.3 (Soil Survey of England and Wales, Legend for the 1: 250,000 soil maps of England and Wales)

541v Rheidol
561b Teme
611c Manod
654a Hafren
713d Cegin
721d Wilcocks 2
1013b Crowdy 2

Figure 3.3 The soil map (1: 50,000 taken from the 1: 250,000 Soil Survey map of England and Wales, sheet 2, Wales) of the area around Llansannan, Clwyd (Sites 1-8)



b) Brecon

The site studied lies on the Brecon Beacons in South Wales. These hills are formed of a relatively uniform outcrop of Devonian Old Red Sandstone, overlying older Silurian rocks which are not exposed in the site area.

Ordnance Survey, and Soil Survey maps of this area are shown in Figures 3.4-3.5.

Figure 3.4 The Ordnance Survey map (1: 50,000 O. S. Landranger 160, Brecon Beacons) of the area near Sennybridge, Powys (Site 9)

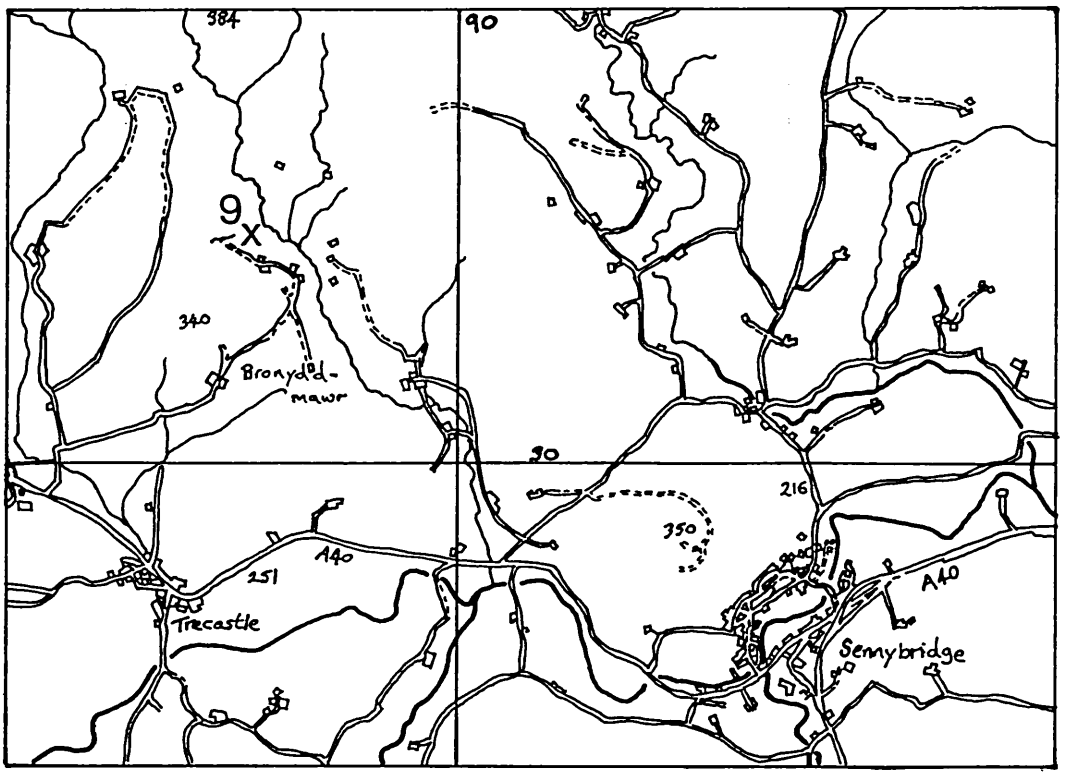
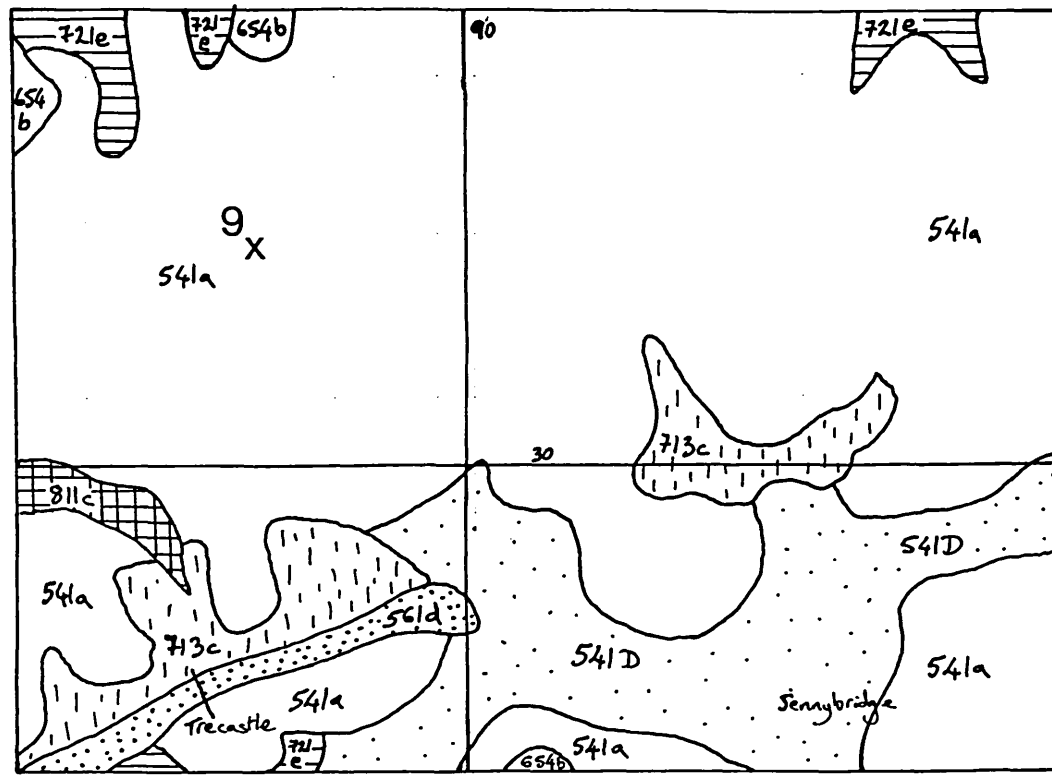


Figure 3.5 **The soil map (1: 50,000 taken from the 1: 250,000 Soil Survey of England and Wales, sheet 2, Wales) of the area near Sennybridge, Powys (Site 9)**



Key to Figure 3.5 (Soil Survey of England and Wales, Legend for the 1: 250,000 soil maps of England and Wales)

541a Milford
541D Oglethorpe
561d Lugwardine
654b Lydcott
713c Fforest
721e Wenallt
811c Hollington

c) Derbyshire

The Pennines consist of a dissected plateau with summits ranging up to about 700 m a.m.s.l. The rock strata comprising the Pennine Uplands are mainly Carboniferous limestones and Millstone Grits, with some coal measures. In Derbyshire the Carboniferous limestones are exposed over 180 square miles, and surrounded on virtually all sides by Millstone Grits. The Carboniferous limestone forms the Dales, with the older limestone rocks exposed to the west. The Millstone Grits are found primarily in the north and some Dolomotised limestone is found around Matlock.

The commonest rock types found in the study area are standard, well-bedded limestones. These limestones are mainly massive and thick-bedded, and give rise to an elevated plateau intersected by deep dales. Some of the limestone beds are shaly, some are dolomitic and some are locally silicified. The sequence of beds is interupted by both intrusive and extrusive igneous rocks and much of the limestone is heavily mineralised.

Shales, formed with the Millstone Grits, are soft and have been rapidly eroded, and therefore have tended to form valleys. Shales are found in the Derwent Valley, and some in the south near Belper and Froghall.

The standard limestones contain occaisional reef knolls, but the margins of the limestone dome are fringed with reef-limestone.

Ordnance Survey, geology and Soil Survey maps of this area are shown in Figures 3.6-3.11.

Figure 3.6 The Ordnance Survey map (1: 50,000 O. S. Landranger 119, Buxton) of the area around Tissington, Derbyshire (Site 10)

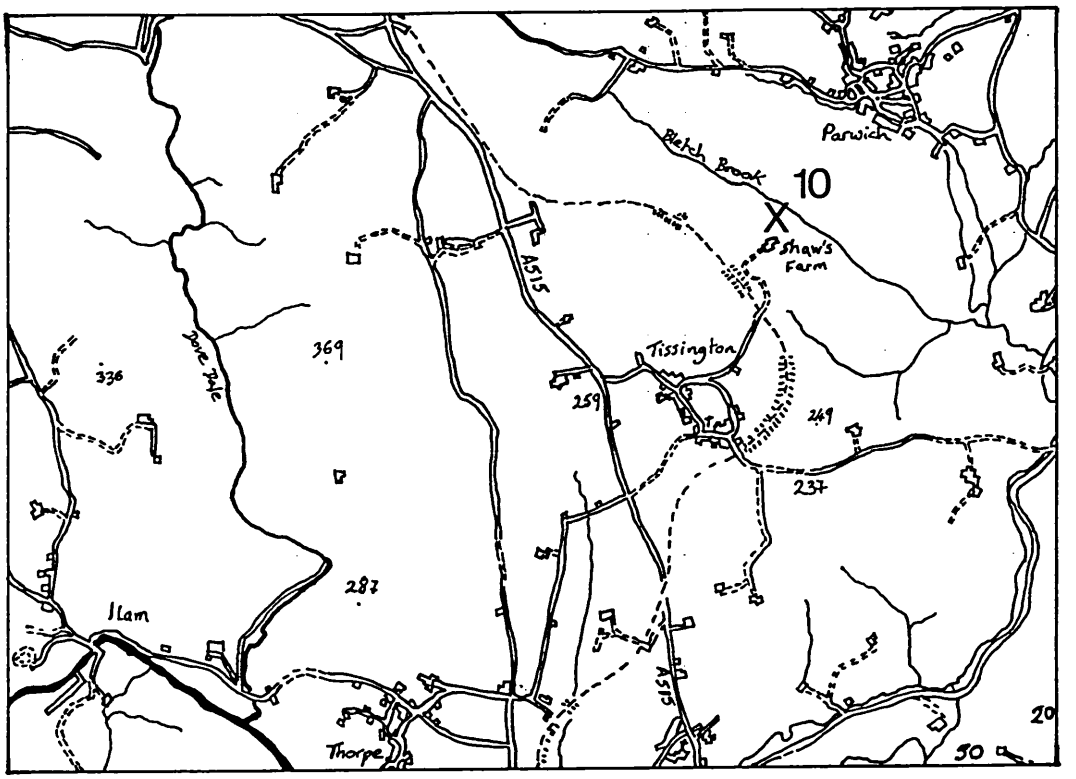
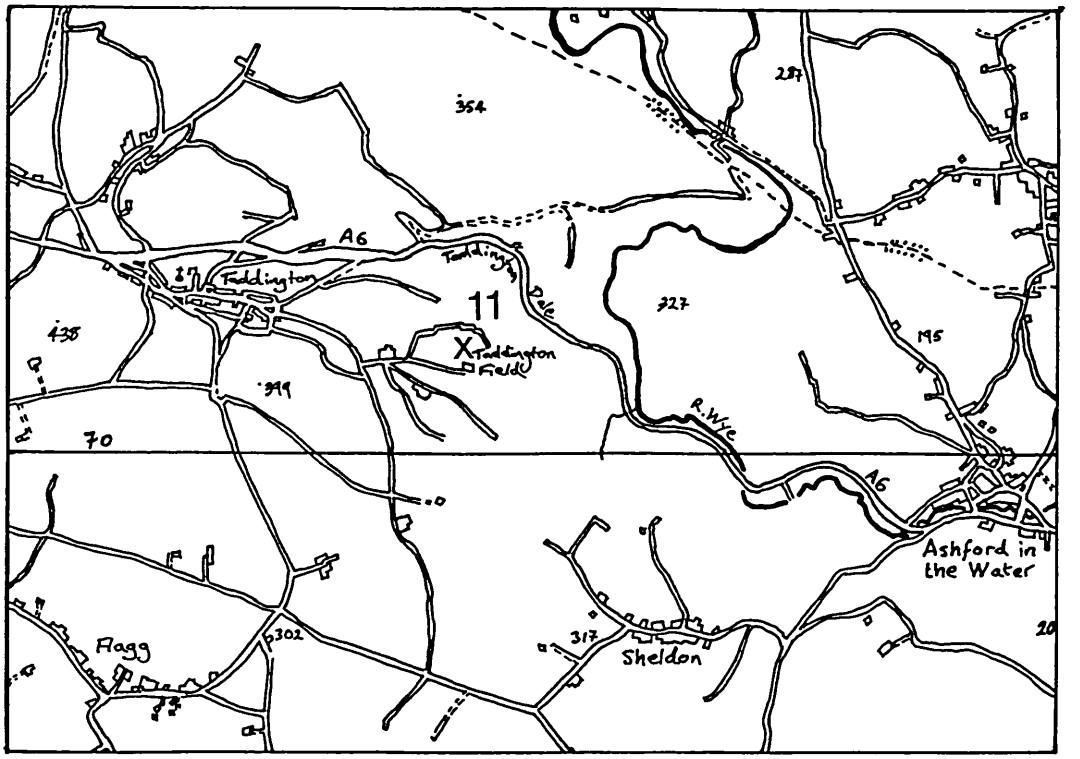


Figure 3.9 The Ordnance Survey map (1: 50,000 O. S. Landranger 119, Buxton) of the area around Taddington, Derbyshire (Site 11)



Key to Figure 3.7

A	Alluvium
S	Scree
Bc	Boulder clay
Cn	Carboniferous (Namurian) sand/mud/siltstones
Hc	Hyaloclastite (fragmented lava)
L	Limestone (Widmerpool formation)
Hp	Hopedale limestones
Wdf	Widmerpool formation (limestone-shales)
Mi	Milldale limestones-
Mi/dk	with dark facies-
K	and knoll-reefs
D	Dolomitised limestone

Key to Figure 3.8 (Soil Survey of England and Wales, Legend for the 1: 250,000 soil maps of England and Wales)

311c Wetton 1
313c Crwbin
421b Halstow
541n Trusham
541p Malham 2
581a Nordrach
711p Dunkeswick
712a Dale
811b Conway

Figure 3.7 The solid and drift geology map (1: 50,000 Geological Survey, sheet 124, Ashbourne) of the area around Tissington, Derbyshire (Site 10)

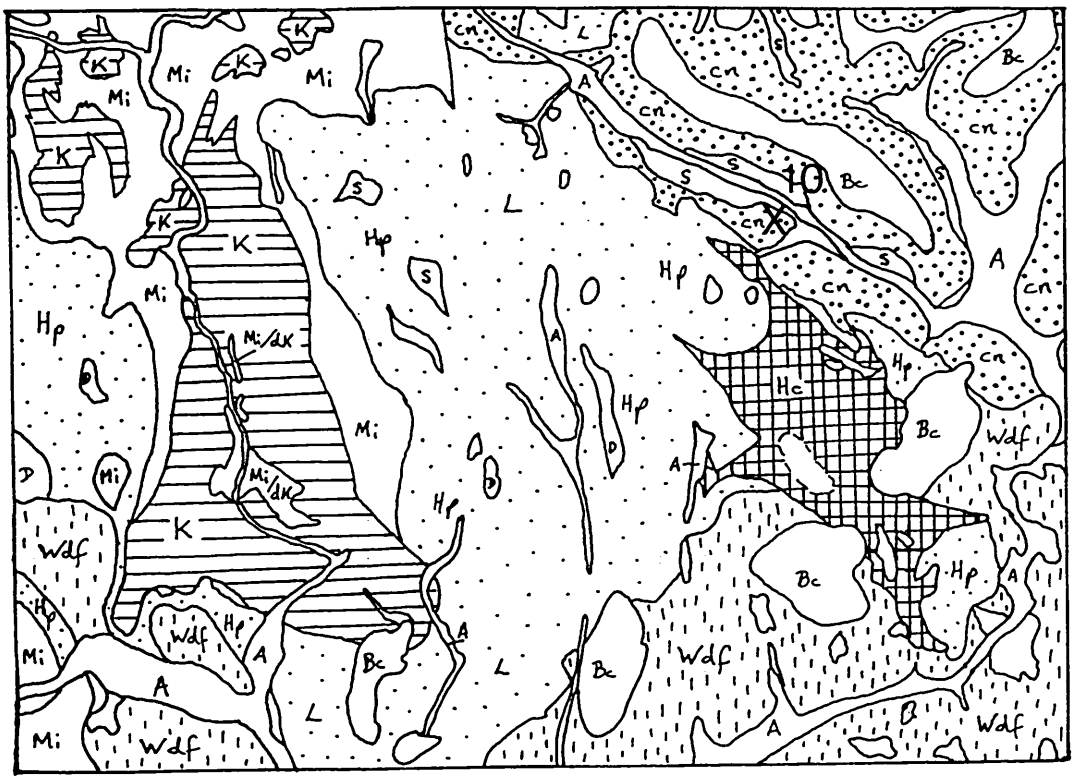
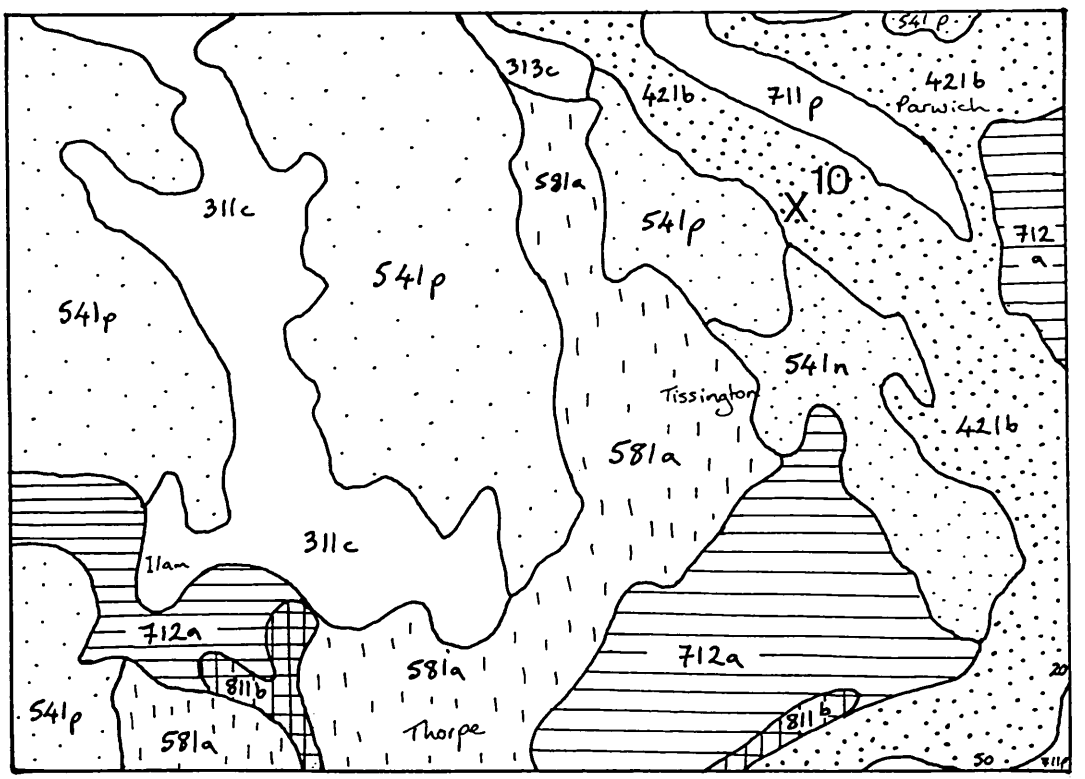


Figure 3.8 The soil map (1: 50,000 taken from the 1: 250,000 Soil Survey map of England and Wales, sheet 3, Midland and Western England) of the area around Tissington, Derbyshire (Site 10)



Key to Figure 3.10

A	Alluvium
S	Scree
Bc	Boulder clay
Cn	Carboniferous (namurian) sand/mud/siltstones
LsM	Longstone mudstones
EyL	Eyam limestones-
K	with knoll-reefs (K)
V	Lava
Mo	Monsal Dale limestones-
Mo/dk	with dark lithofacies
BLL	Bee Low limestones

Key to Figure 3.11 (Soil Survey of England and Wales, Legend for the 1: 250,000 soil maps of England and Wales)

311c	Wetton 1
541p	Malham 2
542	Nercwys
712a	Dale

Figure 3.10 The solid and drift geology map (1: 50,000 Geological Survey, sheet 111,) of the area around Taddington, Derbyshire (Site 11)

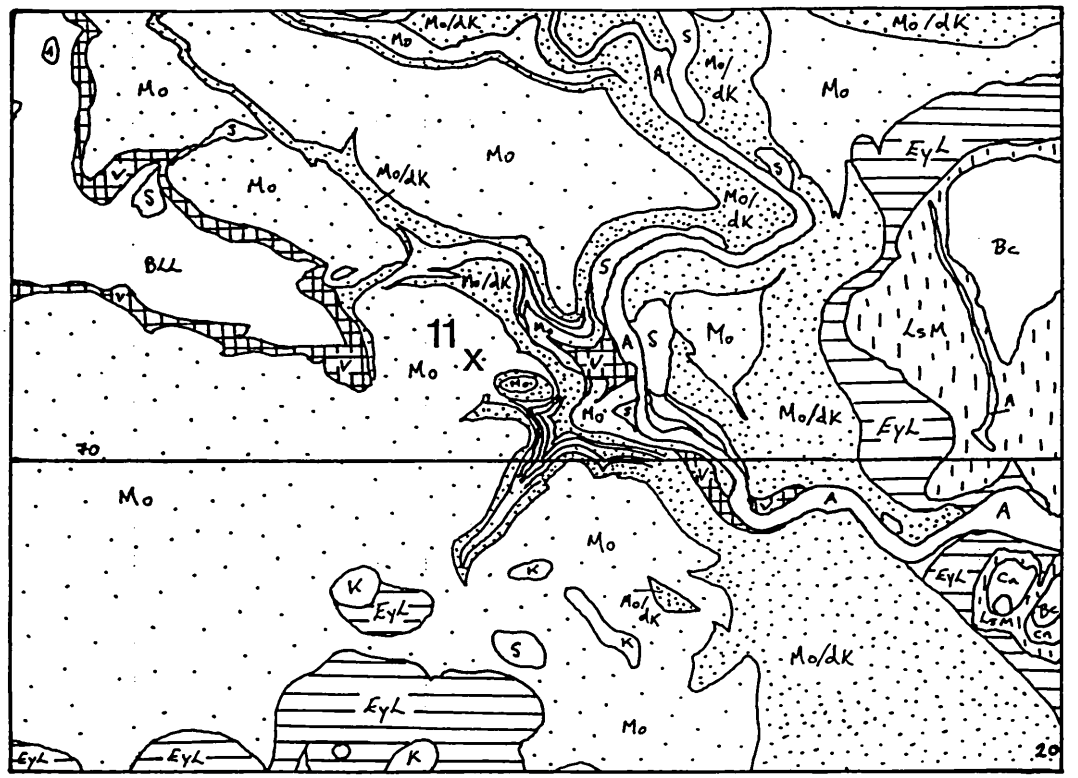
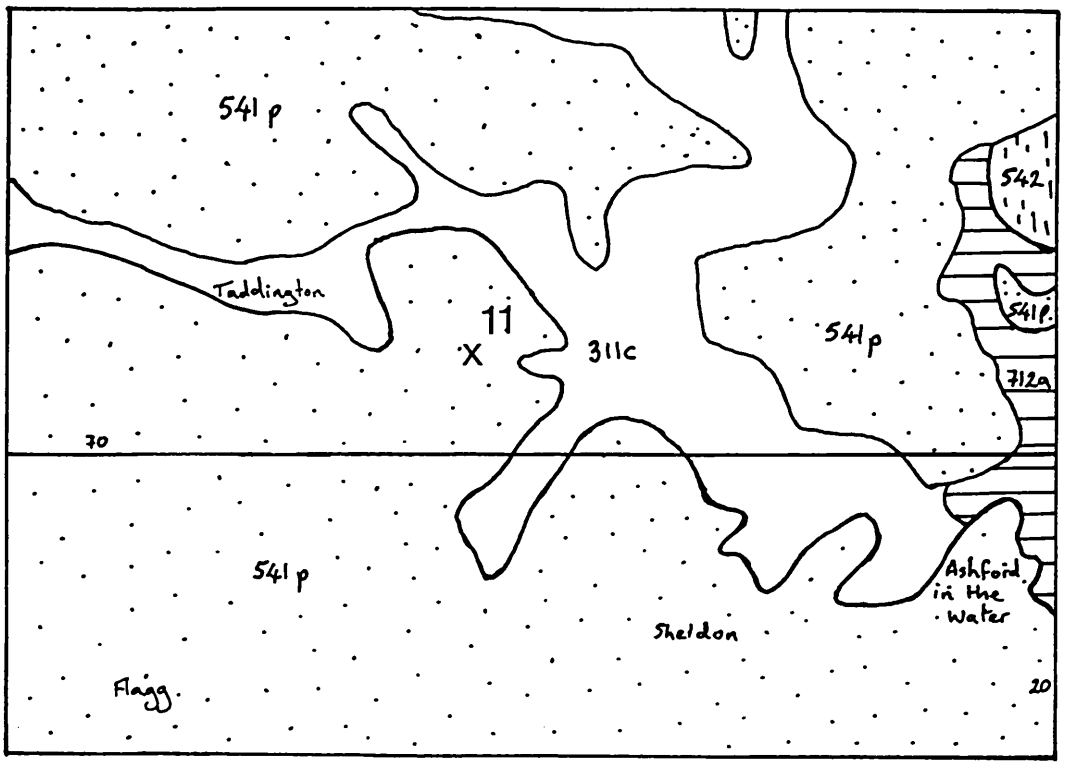


Figure 3.11 The soil map (1: 50,000 taken from the 1: 250,000 Soil Survey map of England and Wales, sheet 3, Midland and Western England) of the area around Taddington, Derbyshire (Site 11)



d) Romney Marsh

Romney Marsh is a 260 km^2 area of reclaimed coastal marshlands and beaches on the Kent-East Sussex border. The Marsh is bounded by the English Channel to the south and east and by old sea cliffs cut into the Wealden Beds in the north and west.

Romney Marsh is a collective name for several marshes, the largest being Romney Marsh proper and Walland Marsh. As well as marshes reclaimed from marine alluvium and partly overlying peat, there are extensive storm beaches of shingle and smaller areas of sand dunes, especially near the tip of the marsh at Dungeness. The highest dunes at Camber Sands reach 12 m a.m.s.l. but most of the land lies between 1-7 m a.m.s.l. with natural bank or ridge systems up to 2.5 m above the adjacent grounds. The marine alluvium lies at or below the high water level of spring tides and sea defences are therefore needed which are provided by artificial sea walls at Dymchurch and Camber and natural sand dunes and shingle beaches in other areas.

The marsh rests on a platform of Hastings Beds and Weald Clay in which rivers excavated a broad valley during the Pleistocene when the sea level was much lower. The old sea cliffs on the landward boundary of the marsh once fringed a bay between headlands which have gradually been eroded.

Marine sediments and upland materials carried from the Hastings Beds and Weald Clay by river have gradually accumulated in the Old Romney Bay in the lee of offshore spits and beaches formed by the sea. About 1000 B.C. forest vegetation developed widely and was the origin of the peat deposits benaeth many parts of the marsh. Later deposition was very complex with sand bank and shingle spits being formed and subsequently moved; river courses changed by tides and currents; lagoons and tidal flats developed as salt-marshes and established land was flooded and partly eroded or covered with fresh sediments. Enclosures or 'innings' have been formed by man during recent centuries to reclaim marshland, the youngest part of the marsh being enclosed about 100 years ago.

The flatness of the marsh and the fact that most parts are occasionally below sea level creates considerable drainage problems. The field are mostly bounded by watercouses ('sewers') taking water into main channels which discharge into the

sea or the river Rother at low tide. In some low lying areas pumps are used to convey water into high level channels such as the Royal Military Canal. The Royal Military Canal also serves as a reservoir, water trapped each spring is available later to replenish adjacent drains.

Ordnance Survey, geology and Soil Survey maps of this area are shown in Figures 3.12-3.14.

Figure 3.12 The Ordnance Survey map (1: 50,000 O. S. Landranger 189, Ashford and Romney Marsh) of Romney Marsh, near Rye (Site 12)

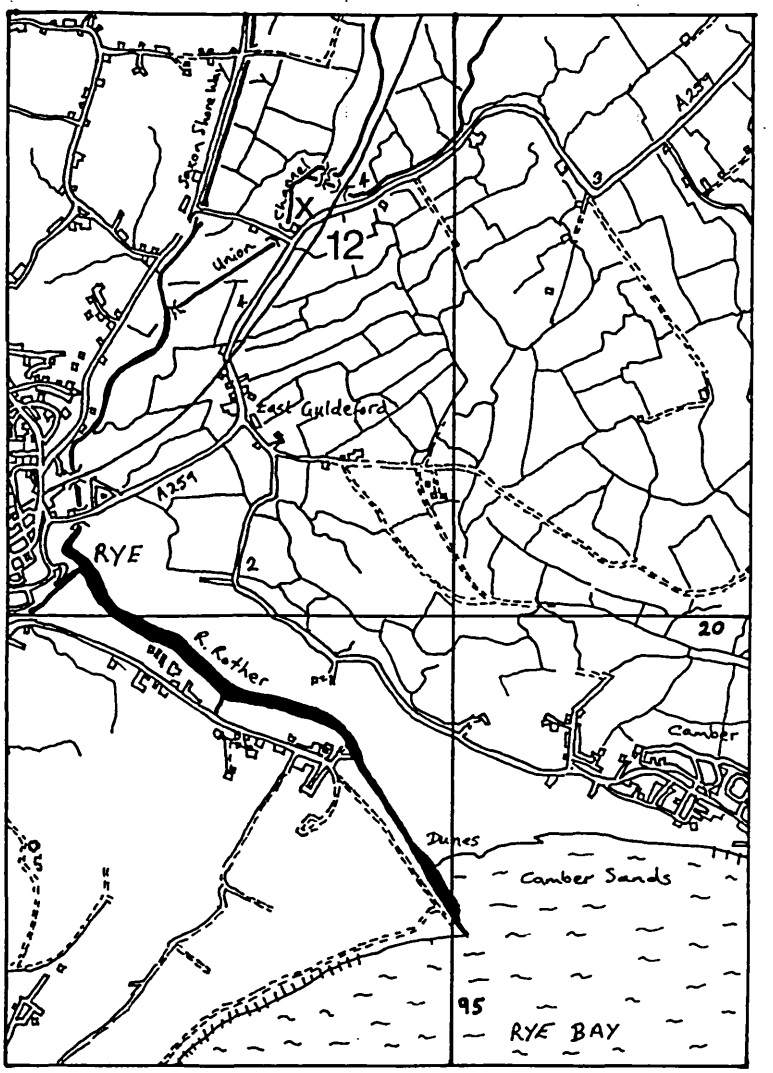
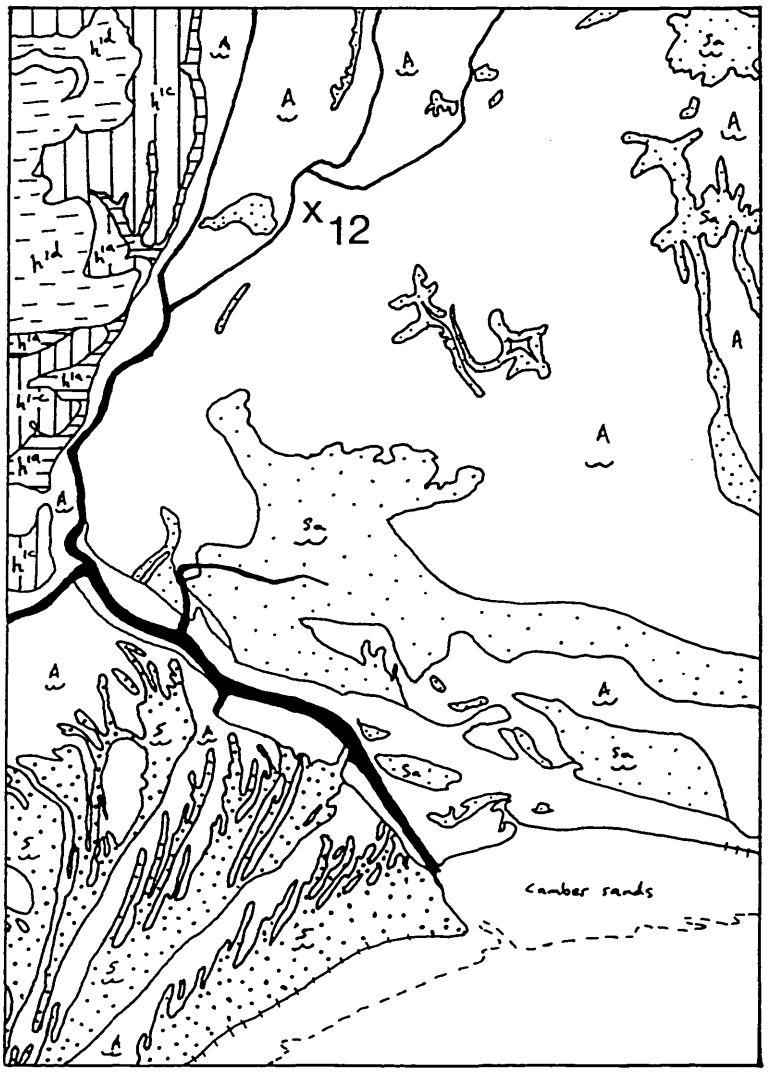


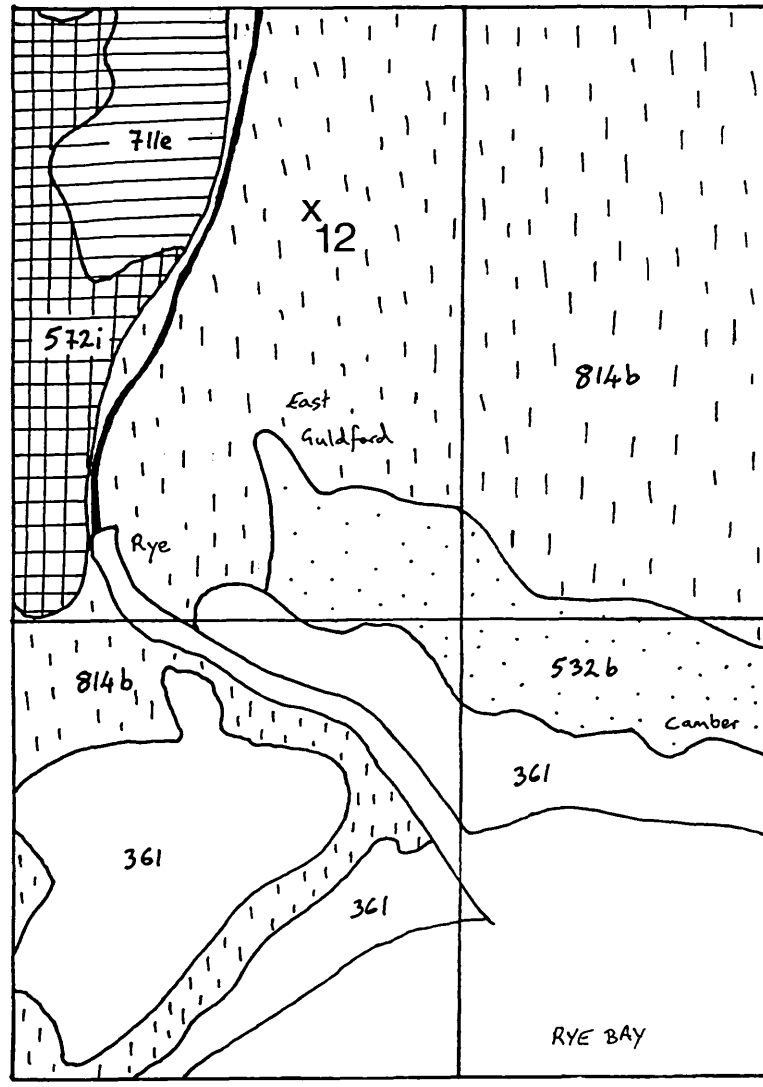
Figure 3.13 The solid and drift geology map (1: 50,000 Geological Survey, sheets 321/321 Hastings & Dungeness and 304 Tenterden) of Romney Marsh, near Rye (Site 12)



Key to Figure 3.13

A	Marine alluvium (clay/silt)
Sa	Marine alluvium (sand)
S	Storm gravel beaches
h^{1d}	Tunbridge Wells sand
h^{1c}	Wadhurst clay
h^{1a}	Ashdown beds

Figure 3.14 **The soil map (1: 50,000 taken from the 1: 250,000 Soil Survey map of England and Wales, sheet 6, South East England) of Romney Marsh, near Rye (Site 12)**



Key to Figure 3.14 (Soil Survey of England and Wales, Legend for the 1: 250,000 soil maps of England and Wales)

361 Sandwich

572i Curtisden

711e Wickham 1

814b Newchurch 1

e) **Woburn**

The Woburn site lies over sandstone rocks of the Woburn Sands formation. These sandstones are part of the Lower Greensands, and are ironsands which characteristically contain some fossils, quarz pebbles and ironstone.

Figure 3.15 **The Ordnance Survey map (1: 50,000 O. S. Landranger, 116) of the area near Woburn (Sites 13-16)**

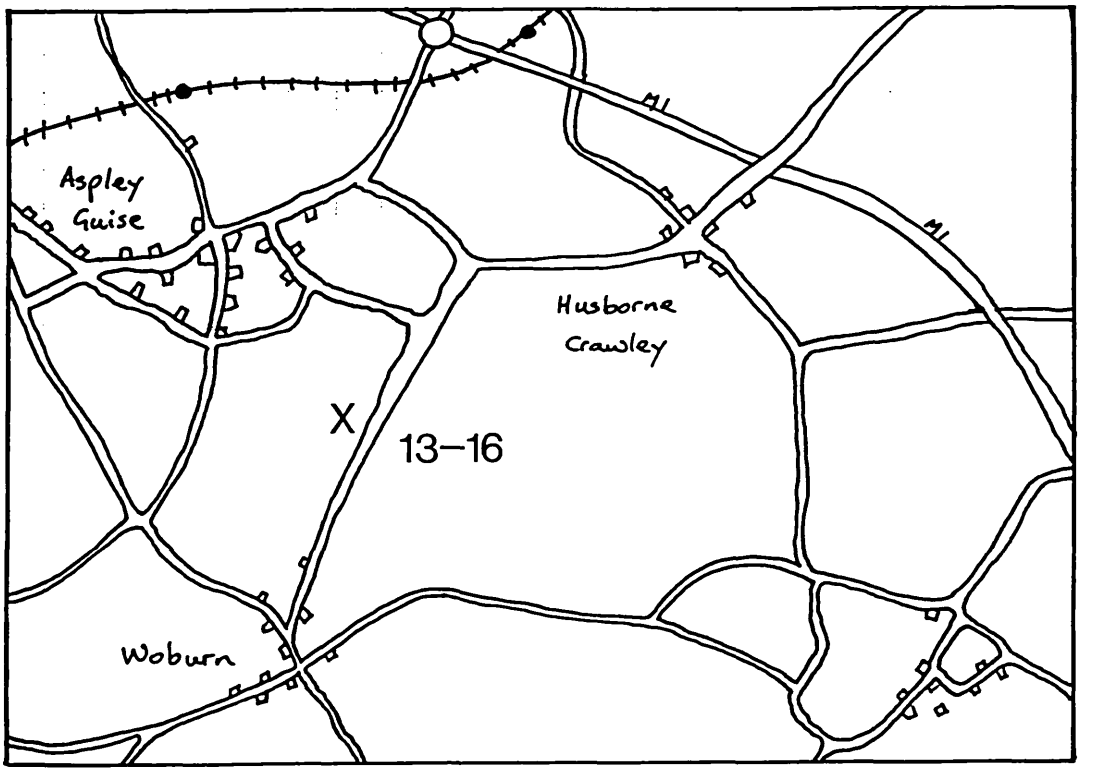
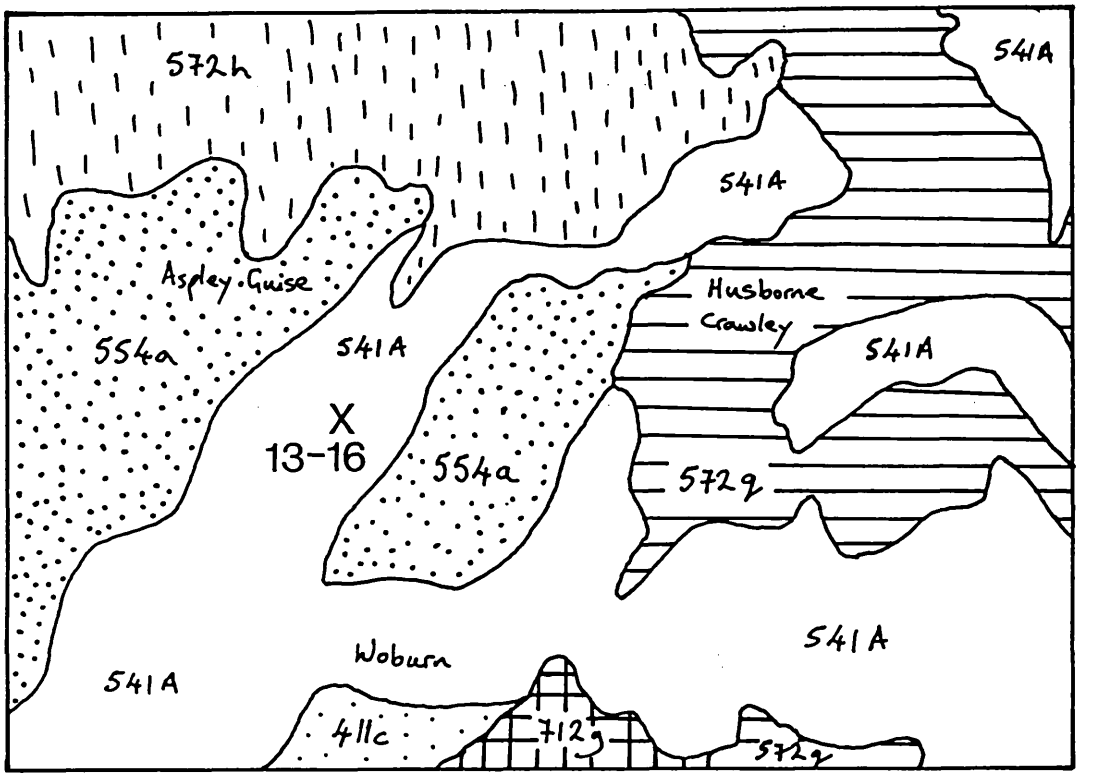


Figure 3.16 **The soil map (1: 50,000 taken from the 1: 250,000 Soil Survey map of England and Wales, sheet 4, Eastern England) of the area near Woburn, (Sites 13-16)**



Key to Figure 3.16 (Soil Survey of England and Wales, Legend for the 1: 250,000 soil maps of England and Wales)

411c Evesham 3

541A Bearsted 1

554a Frilford

572h Oxpasture

572q Ashley

712g Ragdale

3.4.2 Soil Survey Classifications (Soil Survey of England and Wales, 1983)

a) North Wales

1. Melai Farm on Moel Unben — Grid Reference: SH 909677

   Ht: 300 m Slope: 1 in 5 Aspect: S.W.

   Soil Association: 611c Manod (Brown podzolic soils)

   Geology: Palaeozoic slate, mudstone and siltstone.

   Soil and Site Characteristics: Well drained fine loamy or fine silty soils over rock. Shallow soil in places. Bare rock locally. Steep slopes common.

2. Ty Uchaf — Grid Reference: SH 904663

   Ht: 200 m Slope: 1 in 20 Aspect: N.E.

   Soil Association: 713d Cegin (Cambic stagnogley soils)

   Geology: Drift from Palaeozoic slatey mudstone and siltstone.

   Soil and Site Characteristics: Slowly permeable seasonally waterlogged fine silty and clayey soils. Some fine silty and fine loamy soils with slowly permeable subsoils and slight seasonal waterlogging on slopes.

3. Nant y Garreg — Grid Reference: SH 966619

   Ht: 370 m Slope: 1 in 10 Aspect: N.E.

   Soil Association: 654a Hafren (Ferric stagnopodzols)

   Geology: Palaeozoic slatey mudstone and siltstone.

   Soil and Site Characteristics: Loamy permeable upland soils over rock with a wet peaty surface horizon and bleached subsurface horizon often with a thin ironpan. Some peat on higher ground.

4. Pencraig Fawr — Grid Reference: SH 939653

   Ht: 190 m Slope: 1 in 10 Aspect: S.W.

   Soil Association: 611c Manod (Brown podzolic soils)

   Geology: As for site 1.

   Soil and Site Characteristics: As for site 1.

5. Hiraethog Grid Reference: SH 913603

Ht: 380 m Slope: 1 in 20 Aspect: S.E.

Soil Association: 654a Hafren (Ferric stagnopodzols)

Geology: As for site 3.

Soil and Site Charateristics: As for site 3.

6. Hiraethog Grid Reference: SH 911602

Ht: 370 m Slope: 1 in 20 Aspect: S.E.

Soil Association: 721d Wilcocks 2 (Cambic stagnohumic gleys)

Geology: Drift from Palaeozoic sandstone, mudstone and shale.

Soil and Site Characteristics: Slowly permeable seasonally waterlogged loamy upland soils with a peaty surface horizon. Some very acid peat soils.

7. Hiraethog Grid Reference: SH 923576

Ht: 390 m Slope: 1 in 20 Aspect: W

Soil Association: 721d Wilcocks 2 (Cambic stagnohumic gleys)

Geology: As for site 6.

Soil and Site Characteristics: As for site 6.

8. Plas Panton Grid Reference: SH 969625

Ht: 305 m Slope: flat Aspect: N

Soil Association: 611c Manod (Brown Podzolic Soils)

Geology: As for site 1.

Soil and Site Characteristics: As for site 1.

b) Brecon, South Wales

9. Bronydd Mawr, Experimental Farm Grid Reference: SN 886314

Ht: 310 m Slope: flat Aspect: ----

Soil Association: 541a Milford (Brown earth soils)

Geology: Devonian sandstone, siltstone, mudstone and slate.

Soil and Site Characteristics: Well drained fine loamy reddish soils over rock. Some steep slopes.

c) Derbyshire

10. Shaw Farm, Tissington Grid Reference: SK 183535

Ht: 180 m Slope: 1 in 20 Aspect: N.E.

Soil Association: 421b Halstow (Non-calcareous pelosols)

Geology: Carboniferous shale.

Soil and Site Characteristics: Slowly permeable clayey soils often over shale. Some well drained fine loamy soils.

11. Taddington Fields Farm Grid Reference: SK 163705

Ht: 300 m Slope: flat Aspect: ----

Soil Association: 541p Malham 2 (Brown earth soils)

Geology: Aeolian silty drift over Carboniferous and Jurassic limestone.

Soil and Site Characteristics: Well drained often stoneless silty soils over limestone, shallow in places, especially on crests and steep slopes.

d) Romney Marsh

12. Brookgates Farm Grid Reference: TQ 940227

Ht: 0 m Slope: flat Aspect: ----

Soil Association: 532b Romney (Gleyic brown calcareous alluvial soils)

Geology: Marine alluvium.

Soil and Site Characteristics: Deep stoneless permeable calcareous coarse and fine silty soils. Flat land. Groundwater controlled by ditches and pumps.

e) Woburn

13-16. Stackyards Field, Woburn Grid Reference: SP 494236

Ht: 90 m Slope: flat Aspect: ---

Soil Association: 541A Bearsted 1 (Typical brown earths)

Geology: Cretaceous sand and siltstone.

Soil and Site Characteristics: Well drained coarse loamy and sandy soils over sand or sandstone, in places ferrigunous. Some permeable coarse and fine loamy soils affected by groundwater. Risk of water erosion.

3.4.3 Soil Colour

The colours of the topsoil and subsoil collected from each site were described and recorded according to Munsell's classification scheme using a Munsell Colour Chart. The colour classification of the soils is shown in Table 3.2.

Table 3.2 The colour classification of collected soils using Munsell's colour chart

Site	Soil Type		Hue	Value/Chroma	Description
1	Topsoil	Wet	7.5 YR	3/2	Dark brown
		Dry	10 YR	3/3	Dark brown
	Subsoil	Wet	--	—	----
		Dry	--	—	----
2	Topsoil	Wet	2.5 Y	4/2	Dark greyish brown
			2.5 Y	4/1	Dark grey (Mottles)
		Dry	2.5 Y	7/2	Light grey
	Subsoil	Wet	2.5 Y	4/2	Dark greyish brown
			7.5 Y	5/8	Strong brown (Mottles)
		Dry	5 Y	8/2	White
3	Topsoil	Wet	10 YR	3/3	Dark brown
			10 YR	4/6	Dark yellowish brown
		Dry	10 YR	5/3	Brown
	Subsoil	Wet	10 YR	3/3	Dark brown
			10 YR	4/4	Dark yellowish brown
		Dry	10 YR	3/3	Pale brown
			10 YR	6/3	Very pale brown
4	Topsoil	Wet	10 YR	3/2	Very dark grey brown
		Dry	10 YR	6/3	Pale brown
	Subsoil	Wet	10 YR	3/3	Dark brown
		Dry	10 YR	6/3	Pale brown
5	Topsoil	Wet	7.5 YR	2/0	Black
			7.5 YR	5/2	Brown
			7.5 YR	5/6	Strong brown (Mottles)
		Dry	7.5 YR	2/0	Black
			7.5 YR	6/2	Pinkish brown
			7.5 YR	5/6	Strong brown (Mottles)
	Subsoil	Wet	10 YR	5/1	Grey
			10 YR	4/3	Brown
			7.5 YR	5/8	Strong brown
			10 YR	2/1	Black
		Dry	10 YR	7/1	Light grey
			10 YR	7/4	Very pale brown
			7.5 YR	7/8	Reddish yellow
			10 YR	3/1	Very dark grey

Table 3.2 Continued

Site	Soil Type		Hue	Value/Chroma	Description
6	Topsoil	Wet	7.5 YR	2/0	Black
		Dry	10 YR	2/1	Black
	Subsoil	Wet	10 YR	4/2	Dark greyish brown
			10 YR	7/3	Very pale brown
			10 YR	2/1	Black
		Dry	10 YR	6/2	Light brownish grey
			10 YR	8/2	White
			10 YR	2/1	Black
7	Topsoil	Wet	10 YR	2/1	Black
			10 YR	2/2	Very dark brown
		Dry	10 YR	2/1	Black
			10 YR	5/2	Greyish brown
	Subsoil	Wet	10 YR	8/2	White
			7.5 YR	2/0	Black
			10 YR	3/2	Very dark grey brown
			7.5 YR	5/8	Strong brown
		Dry	10 YR	8/1	White
			7.5 YR	2/0	Black
			10 YR	6/2	Light brownish grey
			7.5 YR	5/2	Brown
			7.5 YR	6/8	Reddish yellow
8	Topsoil	Wet	2.5 Y	4/2	Dark greyish brown
		Dry	2.5 Y	6/2	Light brownish grey
	Subsoil	Wet	2.5 Y	4/2	Dark greyish brown
		Dry	2.5 Y	6/2	Light brownish grey
9	Topsoil	Wet	2.5 YR	3/4	Dark reddish brown
		Dry	5 YR	5/3	Reddish brown
	Subsoil	Wet	2.5 YR	4/4	Reddish brown
		Dry	2.5 YR	6/4	Light reddish brown
10	Topsoil	Wet	10 YR	3/2	Very dark grey brown
		Dry	2.5 Y	5/2	Greyish brown
	Subsoil	Wet	10 YR	3/3	Dark brown
		Dry	10 YR	7/2	Light grey
			10 YR	5/8	Yellow brown (Mottles)

Table 3.2 Continued

Site	Soil Type		Hue	Value/Chroma	Description
11	Topsoil	Wet	10 YR	3/2	Very dark grey brown
		Dry	10 YR	5/3	Brown
	Subsoil	Wet	10 YR	3/2	Very dark grey brown
		Dry	10 YR	5/3	Brown
12	Topsoil	Wet	10 YR	4/3	Brown
		Dry	10 YR	6/3	Pale brown
	Subsoil	Wet	2.5 Y	4/2	Dark greyish brown
		Dry	2.5 Y	7/2	Light grey
13 14 15 16	Topsoil	Wet	10 YR	3/6	Dark yellowish brown
		Dry	10 YR	5/4	Yellowish brown
	Subsoil	Wet	10 YR	3/6	Dark yellowish brown
		Dry	10 YR	5/4	Yellowish brown

3.4.4 Sward Composition

At each sampling site a description of the dominant plant species was made and other species occuring occasionally were also listed. The books used for identification purposes included; Grasses, C. E. Hubbard (1954); Grasses, Ferns, Mosses and Lichens of Great Britain and Ireland, R Phillips (1980); The Wild Flowers of Britain and Northern Europe, R. Fitter, A. Fitter and M. Blamey (1974). A list of the plant species found at each site is given in Table 3.3.

Table 3.3 **The plant species found at each of the field sites**

Site	Plant Species	Abundance
1	Perennial Ryegrass *(Lolium perenne)* White Clover *(Trifolium repens)*	Dominant Very little
2	Perennial Ryegrass *(Lolium perenne)* Oval Sedge *(Carex ovalis)* Soft Rush *(Juncus effusus)* Meadow Buttercup *(Ranunculus acris)*	Dominant Small amount Small amount Very little
3	Perennial Ryegrass *(Lolium perenne)* Buttercup *(Ranunculus acris)* Common Mouse-eared Chickweed *(Cerastium fontanum)*	Dominant Small amount Very little
4	Perennial Ryegrass *(Lolium perenne)* Buttercup *(Ranunculus acris)* Ribwort Plantain *(Plantago lanceolata)* Dandelion *(Taraxacum vulgaria)*	Dominant Small amount Small amount Very little
5	**Heather:** Heather *(Calluna vulgaris)* Bell Heather *(Erica cinerea)* **Lichen:** *(Cladonia portentosa)* *(Cladonia arbuscula)* **Mosses:** *(Aulacomnium palustre)* *(Polytrichum formosum)* *(Hypnum jutlandicum)* *(Hypnum cupressiforme)* **Grasses:** Oval Sedge *(Carex ovalis)* Sheep's Fescue *(Festuca ovina)* **Other:** Bilberry *(Vaccinium myrtillis)* Wild Thyme *(Thymus serpyllum)*	Heather, lichens, mosses and grasses found in roughly equal proportions. Very little Very little

Table 3.3 **Continued**

Site	Plant Species		Abundance
6	Mosses:	*(Sphagnum capillifolium)*	Mosses found
		(Hypnum jutlandicum)	in greater
		(Polytrichum commune)	abundance
		(Hypnum cupressiforme)	than grasses.
	Grasses:		
	Oval Sedge	*(Carex ovalis)*	
	Sheep's Fescue	*(Festuca ovina)*	
	Other:		
	Bilberry	*(Vaccinium myrtillus)*	Very little
	Wild Thyme	*(Thymus serpyllum)*	Very little
7	Mosses:	*(Polytrichum commune)*	Mainly mosses
		(Hypnum cupressiforme)	and grasses
	Grasses:		
	Oval Sedge	*(Carex ovalis)*	
	Sheep's Fescue	*(Festuca ovina)*	
	Other:		
	Wild Thyme	*(Thymus serpyllum)*	Small amount
8	Perennial Ryegrass	*(Lolium perenne)*	Dominant
	White Clover	*(Trifolium repens)*	Small amount
	Meadow Buttercup	*(Ranunculus acris)*	Small amount
	Soft Rush	*(Juncus effusus)*	Small amount
	Dandelion	*(Taraxacum vulgaria)*	Very little
9	Perennial Ryegrass	*(Lolium perenne)*	Dominant
	Meadow Buttercup	*(Ranunculus acris)*	Small amount
		(Poa annua)	Small amount
	Common Mouse-eared Chickweed		Small amount
		(Cerastium fontanum)	
10	Lolium perenne	*(Lolium perenne)*	Dominant
	Meadow Buttercup	*(Ranunculus acris)*	Fair amount
	Poa annua	*(Poa annua)*	Small amount

Table 3.3 **Continued**

Site	Plant Species	Abundance
11	Perennial Ryegrass (*Lolium perenne*)	Dominant
	Meadow Buttercup (*Ranunculus acris*)	Small amount
	Common Mouse-eared Chickweed (*Cerastium fontanum*)	Small amount
12	Early Hair Grass (*Aira praecox*)	Dominant
	White Clover (*Trifolium pratense*)	Fair amount
	Dandelion (*Taraxacum vulgria*)	Small amount
	Daisy (*Bellis perennis*)	Small amount
	Moss (*Hypnum cupressiforme)*	Very little

3. 4. 5 Rainfall and Climate

Information on the climate and rainfall for each site during the two year sampling programme was obtained from the Meteorological Office Library in Bracknell. The mean monthly temperature and rainfall values were taken from the nearest recording stations to the field sites, or in some cases from the nearest two recording stations when the site lay between them.

Topsoil and subsoil temperatures were also available from some recording stations.

CHAPTER 4

SAMPLING METHODS, SAMPLE PREPARATION, ANALYTICAL TECHNIQUES AND QUALITY CONTROL PROCEDURES

4.1 SAMPLING METHODS

For each site chosen for study in this research, samples of topsoil, subsoil, herbage and rainwater were required.

Since the sites were to be studied seasonally and since there can be large differences in soil type across one field it was decided to sample the same area at each visit. In order to do this it was necessary to choose a position in the field which could be easily recognised on each return; however it was essential to sample well away from trees, hedges, overhead wires, gates and paths to avoid contamination. It was usually impossible to mark out sites due to the presence of grazing livestock and farm machinery so the sites had to be described using permanent markers such as gates or trees and simple measurements.

4.1.1 Soil Samples from Field Sites

For the collection of topsoils (0-15 cm) and subsoils (15-30 cm) a hand auger with a 2.5 cm diameter stainless steel screw was used to collect relatively small samples. Nine separate auger samples were taken from a 3 x 3 grid, with each point 3 m apart. These 9 samples were combined as they were collected in order to provide a composite sample which would more accurately represent the soil at that site.

The soil samples were placed in heavy-duty polythene bags and left loosely closed until returning to the laboratory in order to minimise water loss without allowing anaerobic conditions to develop.

At each seasonal sampling every attempt was made to return to the exact area of the original grid and in almost every case this was easy to achieve using careful descriptions of the position and simple pacing measurements from permanent markers.

4.1.2 Bulk Soil Samples

The topsoil and subsoil samples collected using the soil auger were intended for detailed chemical analysis. However, to ensure collection of sufficient soil for the analytical methods which required large volumes of soil, eg. particle size analysis and soil water extraction, bulk soil samples were also collected from an area close to but just outside the grid used for auger sampling of soil so as not to disturb the grid area for future sampling.

If the site was under permanent pasture the turf was removed using a stainless steel spade to just below the grass rooting depth (ca. 5 cm). A sample of soil was then taken with the spade and placed in polythene bags as before. Soil was not taken below 20 cm depth so the bulk soil samples were predominantly topsoil samples. After collection of the sample the turf was carefully replaced to minimise the damage to the fields.

4.1.3 Field Soils for Greenhouse Experiments

A large volume of soil was required from three sites for one of the greenhouse experiments. These were collected in the same manner as the bulk soil samples described above but the soil was taken from many small areas until sufficient soil had been obtained. These soils were collected in one or two very

large, heavy-duty polythene bags for transport back to the laboratory.

4.1.4 Herbage from Field Sites

Herbage samples were always collected from field sites before soil samples were taken to prevent contamination of the herbage.

Plant material was collected from an area inside and just outside the 3 x 3 (6 m^2) grid used for auger sampling. Samples of mixed pasture herbage were obtained by clipping the plants 2.5 cm above the soil surface using sharp stainless steel scissors. Care was taken to avoid extraneous soil material and dead plant material.

A representative mixture of herbage was collected at each site every season, however at sites where there was no individual dominant plant species, samples of the most commonly occurring plants were also collected separately in order to investigate the differences in elemental composition between the species.

The herbage was collected into polythene bags and left loosely sealed as for the soil samples. During the summer months the herbage and soil auger samples were placed in a cool box containing frozen cool-packs in order to keep the temperature relatively low until returning to the laboratory.

4.1.5 Herbage from Greenhouse Experiments

Herbage harvested from pots grown in the greenhouse was cut using stainless steel scissors and placed directly into paper sample bags for drying. The grasses and clovers were cut at the level of the top of the pots leaving sufficient plant material to allow growth to continue for several such harvests. The herbage was always collected prior to the addition of treatment solutions to prevent contamination.

4.1.6 Rainwater Samples

Samples of rainwater were collected over several seasons at one site in each of the 5 areas of the country under investigation. Plastic bottles (2 l) were thoroughly cleaned and then painted with black gloss paint to prevent excess light causing algal growth in the water samples. Plastic funnels (15 cm diameter) were covered with 1 mm nylon mesh using Araldite and attached to the necks of the bottles with insulation tape. These bottles were then left at the site, often attached to walls and fences, away from trees, houses, telegraph wires, etc and also out of range of livestock and children. On each sampling visit the bottles were removed, sealed and brought back to the laboratory and a fresh collection bottle left in its place. Approximately 1 l of rainwater was collected each time.

Site eight in North Wales bordered a small stream and a sample of the stream water was collected in a clean plastic bottle for comparison with the rainwater.

4.1.7 Duplicate Sampling

It has been shown (Markert, 1988) that the errors involved in sampling biological and environmental material can be very large and can easily outweigh the errors of the analytical methods being used. These sampling errors are presumably caused mainly by biological variation, but must also reflect the difficulty in accurately repeating a sample collection.

In order to obtain some idea of the magnitude of the sampling variation, duplicate samples were taken at several sites at different occasions and in different ways. In some cases the 9 separate soil auger samples were collected individually in order to detect the variation within the composite sample. Also duplicate composite soil samples were collected, treating the collections quite individually by relocating the site each time. Duplicate herbage samples were also collected at several sites.

4.2 SAMPLE PREPARATION

4.2.1 Field Soils

Soils collected from the field were dried on covered trays in a filtered-air drying cabinet at 30 °C for 48 hours. Losses of selenium can occur if samples are dried at temperatures above 50 °C (Hamdy and Gissel-Nielsen, 1976b; Zieve and Peterson, 1981). Once dry the soils were stored in paper sample bags prior to analysis.

The air dried soil was disaggregated using a mortar and pestle just sufficiently to pass through a 2 mm (10 mesh) sieve. Any stony material which did not pass through the sieve was retained and weighed to give an estimate of the stony fraction of the soil.

A 50 ml portion of the <2 mm soil fraction was mechanically ground in a tema mill (Siebtechnik TS 100 A) using an agate pot until the soil passed through a 200 μm (80 mesh) sieve. This finely milled soil was used for most chemical analyses using digestion methods, whereas the <2 mm fraction was used for all other analyses unless stated otherwise.

The milled soil was stored in resealable polythene bags in order to prevent excessive reabsorption of moisture from the atmosphere.

4.2.2 Field Herbage

Immediately on returning to the laboratory, herbage collected from the field was thoroughly washed with deionised water (DIW) using a nylon sieve until no soil particles could be seen on the herbage and none was being rinsed off with the water.

Although weak acids and detergents have been used to wash leaves (Little, 1973), it is possible that in addition to removing material on the outside of the

leaf, these solutions can leach metals from within the plant. Washing with DIW alone has been shown to remove a large percentage of metals present as surface deposits whether soluble or not.

After washing the herbage was dried on covered trays in a filtered-air drying cabinet at 30 °C for 48 hours as for the soils. The dry herbage was then stored in paper sample bags.

The dry herbage was milled to a fine powder using a Cyclotec 90 sample mill and the sample was collected directly into resealable polythene bags, and stored in a cool dark cupboard. This milled herbage was used for all subsequent analyses.

4.2.3 Herbage from Greenhouse Experiments

Plant material collected from the pot trials was not washed before drying since the greenhouse conditions produced very little, if any, soil contamination of the herbage. The plants were harvested directly into paper bags using stainless steel scissors and then dried at 30 °C in these paper bags before milling as above.

4.2.4 Water Samples

Rainwater and stream water samples collected from the field sites were stored in the dark in the laboratory and analysed for trace elements as soon as possible. For most purposes the water samples were filtered through 0.45 μm nucleopore filters prior to analysis to remove colloidal material and suspended particles.

Soil water samples were obtained from freshly collected soil using a centrifugation method described by Van Dorst (1984) and adapted from that of Davies and Davies (1963). The wet soils were placed in specially adapted centrifuge

tubes with a filter paper separating the lower chamber from the soil. The soil (50 g) was then centrifuged at 3000 rpm for 30 minutes and the soil water obtained was filtered through a 0.45 μm nucleopore filter prior to analysis.

An alternative method of obtaining the soil solution was also attempted using an inert, heavy organic liquid (trifluoroethane, 'Arklone') to displace the interstitial soil water when centrifuged at high speeds (Kinniburgh and Miles, 1983). This method was successful with mineral soils, however soils with a high organic matter content tended to float above the Arklone and so the soil water could not be separated.

4.3 ANALYTICAL TECHNIQUES

All chemicals used in this research were of AnalaR or AristaR grade and were obtained from the normal laboratory suppliers.

Deionised water (DIW) was used routinely throughout the laboratory work for all washing and preparation of equipment, preparation of standard solutions and any other general purposes. For the fluorimetric determination of selenium, double-distilled deionised water (DDDIW) was used for the preparation of the reagents and standard solutions in order to reduce the possibility of contamination still further.

The preparation of glassware is of utmost importance in trace element analysis in order to prevent low-level contamination. All glassware was cleaned with Decon 90, rinsed with tap water, left overnight in a 2% Decon 90 solution, rinsed again and left overnight in a 2% nitric acid solution. After soaking in acid the glassware was finally rinsed with DIW and dried in a filtered-air drying cabinet before use.

4.3.1 Moisture Content

The moisture content of the field soils was determined on samples immediately after returning from the field.

Approximately 10 g of field moist soil was accurately weighed into porcelain crucibles which had previously been dried to constant weight at 105 °C. The soils were then left at 30 °C in an oven for at least 24 hours and reweighed before heating to 105 °C for 24 hours. The crucibles containing the soils were allowed to cool in a desiccator before weighing once more. The moisture content is reported as a percentage of the wet weight of material.

The residual moisture content after drying at 30 °C was also calculated since samples for selenium analysis are dried at this temperature in order to prevent loss of volatile selenium compounds. Small corrections were made to

the analytical results to compensate for this residual moisture in the samples.

4.3.2 Loss on Ignition

Organic matter content of soils was determined by loss of weight on ignition in a muffle furnace. Many variations of ignition temperature and length of time ashing have been adopted in an attempt to distinguish between organic carbon, CO_2 from carbonates and interstitial water from clay minerals (Hesse 1971).

Ball (1964) showed that the greatest part of the weight loss due to the clay mineral water occurs in the temperature range of 450-600 ^oC and considered that the errors in the method could be minimised if the furnace temperature remained below 450 ^oC. This method was adopted in preference to igniting at 800 ^oC for 30 minutes. Smith (1983) calibrated the loss on ignition method against one for the determination of organic carbon using both clays and sandy soils and found excellent linear correlation between the two methods.

The crucibles containing the soil used for moisture content determination were dried to constant weight at 105 ^oC and then placed in a muffle furnace and ignited at 400 ^oC for 24 hours. The samples were cooled to 105 ^oC and then finally cooled in a desiccator before reweighing. The percentage loss on ignition was calculated from the dry weight (105 ^oC).

4.3.3 Soil pH Measurements

The pH of the soil may be determined either in the field or under laboratory conditions; the advantage of the latter is that it allows a standard procedure to be adopted and this method was used in the current research. However, measurements made on air dry soil may differ from those made on fresh soil in the field.

The pH of air dried soil (<2 mm fraction) was measured using a method described by Avery and Bascomb (1974), pH measurements were taken in a 1: 2.5 w/v suspension in both deionised water and in 0.01 M $CaCl_2$. The use of 0.01 M $CaCl_2$ has been suggested as more closely approximating the situation in the field (Schofield and Taylor, 1955).

Air dried soil (10 g, < 2 mm fraction) was placed in a 100 ml polythene bottle, 25 ml of deionised water was then added to the soil and stirred to form a slurry. The suspension was left to stand for 10 minutes, stirred again and the pH of the suspension was measured using a calomel electrode previously calibrated with buffer solutions at pH 4, 7 and 9.2. The pH reading was taken once the meter had stabilised. 2 ml of 0.125 M $CaCl_2$ solution was then added to the bottle and stirred to give an effective concentration of 0.01 M $CaCl_2$. The pH of this suspension was also recorded when stable.

4.3.4 Particle Size Analysis

The soil textural analysis was carried out using a method outlined by Smith and Atkinson (1975). After destruction of soil organic matter and the addition of a dispersant to completely separate the soil mineral particles, the density of the soil suspension was measured using a hydrometer. The hydrometer method was introduced by Bouyoucos in 1927, modified in 1953 (Bouyoucos) and is now widely used for the routine determination of the particle sizes in soils, replacing or as an alternative to the pipette method.

About 50 g of <2 mm air dried soil was accurately weighed into an 800 ml beaker and 60 ml of 9% w/v hydrogen peroxide was carefully added. The beaker was warmed until all frothing stopped and then gently boiled for a few minutes to destroy the excess hydrogen peroxide. When cool, 10 ml of Calgon (50 g sodium hexametaphosphate, 5.724 g Na_2CO_3 in 1 l DIW) was added and the suspension was stirred on a mechanical stirrer for 15 minutes.

The soil solution was then washed into a 1 l measuring cylinder and diluted to the litre mark with DIW. (Any remaining froth on the liquid was dampened with one drop of amyl alcohol.) The temperature of the solution was taken and then the cylinder was shaken end over end for 1 minute with the top sealed with Parafilm. The cylinder was then placed on the bench and a stopclock started immediately. The hydrometer was inserted gently and the readings were taken at 40 seconds and 4 minutes. The hydrometer was then removed, the cylinder was resealed and shaken again and then allowed to stand undisturbed for two hours. The hydrometer was reinserted just before the two hours and the final reading was taken.

The supernatant solution was discarded and the sediment transferred to an 800 ml beaker for analysis of the sand fraction. A mark was made 10 cm from the base of the beaker and the beaker was filled to this mark with DIW. The suspension was stirred and allowed to stand for the time required for all the sand to settle on the bottom of the beaker. The supernatant was poured off carefully without disturbing the sand sediment. This process of washing, stirring, sedimentation and decanting was repeated until the liquid remained clear at the appropriate time interval (4 minutes, 48 seconds at 20 ^{o}C). The sand residue was then dried at 105 ^{o}C, cooled in a desiccator and weighed.

After correction for temperature, hydrometer readings were used to calculate the particle size fractions of each soil. All the soils were classified according to the Avery (1973) size limits.

4.3.5 Cation Exchange Capacity

This method provides an estimate of the ability of a soil to bind cations. The soil complex is saturated with an index cation, the excess of this cation is washed out and the bound cations are removed using an extractant solution. The concentration of the index cation is then determined. Many procedures exist for estimating the cation exchange capacity using a range of indexing cations,

washing solutions and extractants. The method used in this research was based on that of Hesse (1971) with sodium used as the exchange ion, and adapted so that small quantities of soil could be analysed in large batches.

Air dried soil (0.5 g, <2 mm fraction) was weighed into polythene centrifuge tubes (10 ml). Sodium acetate solution (3 ml, 1 M, at pH 8.2) was added to the tubes which were shaken for 5 minutes in a box shaker. After shaking, the tubes were centrifuged at 2000 rpm for 5 minutes or until the supernatant solution was clear. The liquid was then decanted and discarded. This process of shaking, centrifuging and decanting was repeated four more times with fresh aliquots of sodium acetate solution. After saturation with sodium ions, the soil was washed by shaking with 3 ml of 95% ethanol for 5 minutes then centrifuged and decanted as before. The washing procedure was repeated three more times. The sodium ions bound to the soil exchange site were then extracted using ammonium acetate solution as follows.

Ammonium acetate solution (3 ml, 1 M, at pH 7.0) was shaken with the soil for 5 minutes prior to centrifugation as before. The clear supernatant solution was collected in clean polythene centrifuge tubes and then this process was repeated twice more with fresh 3 ml portions of 1 M ammonium acetate solution. The combined extracts collected from each soil was made up to 10 ml in the centrifuge tubes and retained for analysis of the sodium using atomic absorption spectroscopy.

The sodium concentration of the extractant solution (or dilutions of this if necessary) was measured using a Perkin Elmer 5000 atomic absorption spectrophotometer at 589.0 nm. The cation exchange capacity was calculated according to the equation given below:-

$$\frac{\text{Na conc } (\mu g/ml) \times \text{dilution factor} \times 2 \times 100}{23 \times 1000} = \text{Cation exchange capacity (me/100g)}$$

4.3.6 Pyrophosphate Extractable Iron Content

Selenium has been shown to be associated with the soluble iron content of some soils (Smith, 1983), and so the method of Avery and Bascomb (1974) was used to extract and measure a soluble iron fraction of the soils studied in this work.

Air dried soil (1 g, <2 mm fraction) was weighed into 125 ml polythene bottles. Potassium pyrophosphate $K_4P_2O_7.3H_2O$ (100 ml, 0.1 M) was added to the soil, the bottles were tightly stoppered and then shaken for 8 hours in a box shaker. After this time the suspension was centrifuged at 2000 rpm for 15 minutes. The supernatant solution was decanted into clean polythene bottles and retained for analysis of iron using atomic absorption spectroscopy. The iron concentration of the extracted solution was measured using a Perkin Elmer 5000 atomic absorption spectrophotometer at 373.7 nm.

4.3.7 Analysis of Selenium using Spectrofluorimetry

Spectrofluorimetry has proved to be a very reliable and sensitive method for the analysis of trace amounts of selenium. The method used in this research is essentially that first described by Hall and Gupta (1969), although modifications have been made by Van Dorst (1984), the MAFF analytical chemistry department (Chapman and Jane, 1985) and during this research. The method is based on the quantitative formation of a fluorescent piazselenol from selenium and 2,3-diaminonaphthalene.

The samples to be analysed are first digested by a wet oxidation method using nitric and perchloric acids to destroy organic matter which may otherwise interfere with the formation of the piazselenol. After digestion, boiling with hydrochloric acid reduces all the selenium present to the selenite ion (Se IV), the only form which reacts to produce the fluorescent compound. The digested solution is then buffered at pH 2 before the addition of a solution of

2,3-diaminonaphthalene (DAN). Selenite in acid solution reacts with DAN to form the fluorescent 4,5-benzopiazselenol which is subsequently extracted into cyclohexane (decahydronaphthalene is an alternative), a solvent in which the fluorescence yield is high. The fluorescence of this compound is then measured at 524 nm with an excitation wavelength of 366 nm.

i) Digestion of Samples prior to Fluorimetric Analysis

The same digestion procedure was followed for both soil and plant material since it produced satisfactory results with reference materials of both sample types. No loss of selenium was detected despite the differences in organic matter content. Since selenium can easily be lost at high temperatures, it is essential that the samples are never taken to dryness during the digestion.

The dry, finely milled sample (0.5 g) was weighed into large glass test tubes (140 mm x 25 mm) which have ground glass tops to accept 140 mm glass air condensers. Nitric acid (4 ml, 70%) was added to the sample and mixed, the air condensers were attached and the test tubes were left at 50 °C overnight in a thermostatically-controlled aluminium heating block. The tubes were then removed from the heating block and allowed to cool, and perchloric acid (2 ml, 60%) was added to the digest. This was mixed and replaced in the heating block with the air condensers still attached for 1 hour at 100 °C. The temperature of the block was then raised to 150 °C for two hours; the condensers were removed for the last 30 minutes at this temperature to allow some of the perchloric acid to evaporate. Finally the digest was heated to 170 °C until the sample had bleached due to the destruction of the organic material and most of the perchloric acid had evaporated. The samples were removed from the heating block when 0.5-1.0 ml of solution remained in the tubes. The time taken to reach this end point varied from sample to sample and had to be carefully monitored for successful results. Hydrochloric acid (2 ml, 5 M) was added to the digested samples, the condensers were replaced and the solution was boiled gently (130 °C) for 20

minutes to reduce all the selenium to the selenite ion after the oxidative digestion process.

This procedure proved successful in destroying the organic matter and removing the last traces of nitric acid, both of which interfere with the piazselenol formation, without any loss of selenium from volatilisation.

ii) Complexation and Fluorimetric Measurement of Samples

All the solutions mentioned in this section were made up using DDDIW. Formic acid (5 ml, 50%) and EDTA stabilising solution (10 ml; 9.3 g ethylenediaminetetraacetic acid, disodium salt and hydroxylammonium hydrochloride in 1 l) was added to the digest in the tubes and mixed. Ammonia solution (7 M) was added to the tubes until the solutions were at pH 2, using Whatman narrow range paper pH 1-4, which is the optimum pH for the piazselenol formation (Nye, 1975). DAN solution (2 ml, section 4.3.7iv) was added to the solutions and mixed by shaking, and the tubes were left in a water bath at 50 °C for 30 minutes to allow the complex to develop. The tubes were then removed from the water bath, allowed to cool to room temperature and then 7 ml of cyclohexane was added. The tubes were stoppered with ground glass tops and shaken vigorously by hand for 1 minute. The cyclohexane layer was then removed into a glass centrifuge tube containing 4 ml of 0.1 M HCl using a Pasteur pipette. The centrifuge tube was stoppered with a silicone bung and shaken vigorously for 30 seconds to wash the extract. The lower water layer was removed and discarded using a Pasteur pipette and the cyclohexane layer was clarified by centrifugation at 3000 rpm for 1 minute.

The fluorescence of the piazselenol complex was measured immediately using a Baird Nova 2 spectrofluorimeter reading the emission at 524 nm with an excitation wavelength of 366 nm. The response of the fluorimeter was calibrated at regular intervals using standard solutions of selenium.

iii) Purification of 2,3-Diaminonaphthalene (DAN)

2,3-Diaminonaphthalene (DAN) was obtained as 95% pure from Aldrich Chemical Company. This compound is a suspected carcinogen and somewhat light sensitive, so appropriate precautions had to be taken during its handling. During the purification of DAN all stages were carried out in diffuse light.

A slurry of 5 g of DAN in 20 ml of chloroform was prepared and transferred with additional chloroform to a 1 l flask fitted with a reflux condenser. The total volume was made up to 360 ml with chloroform and this was refluxed for 10-15 minutes until the DAN had dissolved. After removing from the heat until the boiling just ceased, a slurry of 6 g of decolourising charcoal in 20 ml of chloroform was added and the mixture was reheated and refluxed for a further 2 minutes.

The boiling DAN solution was filtered rapidly under reduced pressure through a pre-prepared filter (sintered glass funnel of No. 3 porosity, with a 2 cm bed of sodium sulphate under a Whatman No. 4 filter paper all heated to 80 ^{o}C). The filtrate was kept at -20 ^{o}C for 2-3 hours to crystallise out the DAN. After this time the crystals were filtered through a pre-cooled sintered funnel (porosity No. 1, covered with a filter paper) and washed with 2 x 20 ml portions of chloroform at -20 ^{o}C. The remaining solvent was removed under slightly reduced pressure and the DAN crystals were dried in a desiccator over calcium chloride (anhydrous) in the dark. The crystals were stored in an airtight amber bottle in the fridge.

iv) DAN Working Solution

Immediately before it was required 200 mg of purified DAN was dissolved in 100 ml of hydroxylamine hydrochloride solution (5 g hydroxylamine hydrochloride, 10 ml HCl (70%) in 1 l DDDIW), warming to 50 ^{o}C for 15 minutes in a water bath to aid dissolution.

v) Speciation and Analysis of Selenium in Water Samples

The selenium content of natural waters, both rainwater and fresh soil solution, was also analysed using spectrofluorimetry. It was considered important to filter the water samples using 0.45 μm millipore membranes, in order to remove all the suspended particles and much of the organic material which can interfere with the complexation process in selenium analysis, (0.45 μm is assumed to be the limiting size for colloidal material). Analysis was attempted with unfiltered samples but a digestion process is probably needed to destroy the organic material in soil solutions.

Speciation studies can be attempted on filtered natural waters. Since no oxidative process is required the inorganic selenium in the sample should be present in its original form.

The spectrofluorimeter method is specific for the selenite ion and so if the untreated sample was complexed with DAN (section 4.3.7ii) a value for the selenite ion concentration in the sample was obtained. However if the filtered sample was boiled gently with 5 M HCl to reduce selenate to selenite prior to complexation, a value of (selenite + selenate) ion concentration in the sample was obtained. The relative proportions of selenite and selenate ions in the waters and soil solutions could thus be measured.

4.3.8 Elemental Analysis using ICPAES

This method of analysis provides data for some 25 elements simultaneously and has the advantage that interference problems can be largely eliminated. Using the hydride generation mode, selenium can also be determined.

Soils and herbage collected from the field were analysed for selenium, sulphur and many other elements by this method using an ARL 3400 ICP Atomic Emission Spectrometer. This instrument measures the concentration of

many elements with low detection limits, good precision and linear calibration over several orders of magnitude. As it is a solution method, matrix and interference effects can be minimised. Perhaps the most important aspect of this method is that many elements can be measured together in a few seconds so that analysis is very rapid.

The elements to be analysed are presented as an aqueous solution which is pumped by peristaltic pump into the spray chamber where a flow of pure argon gas converts a fraction of the solution to an aerosol. This aerosol is then sprayed into the centre of the argon plasma.The inductively coupled plasma is a stream of argon atoms which is heated by inductive heating using a radio frequency coil run from a generator. The inductive heating of argon as it flows through the radio frequency field effectively strips electrons from the argon atoms and produces a plasma of argon ions with an operating temperature of 6,000-10,000 K at its centre. The plasma is ignited by a high frequency spark.

Thus the solution to be analysed is sprayed into a very high temperature flame which is stable and of sufficient temperature to dissociate the chemical bonds and excite a large number of spectral lines. The light emitted is focused into a conventional air path 1.5 m spectrometer in which the spectral lines are detected by fixed photomultipliers mounted along the Rowland circle of the spectrophotometer. The signal from the photomultiplier may be sent directly to a printer to give intensity measurements or alternatively calibration lines can be prepared from the intensity readings obtained from solutions containing known concentrations of the elements. These calibrations may then be stored on the computer linked to the system for later reference.

i) Multi-Element Analysis

Soils:

A nitric/perchloric acid oxidative digestion was used on 0.25 g of finely milled soil in Pyrex test tubes (180 mm x 18 mm). Nitric acid (4 ml, 70%) and

perchloric acid (2 ml, 60%) was added to the soils and mixed thoroughly. A sequential heating process was then carried out with the tubes placed in a programmable aluminium heating block. The tubes were sequentially heated for 3 hours at 50 °C, 3 hours at 150 °C, 18 hours at 190 °C and 5 minutes at 195 °C to leave the tubes completely dry and the samples digested. Once cool, hydrochloric acid (2 ml, 5 M) was added to each tube and they were heated to 60 °C for 1 hour to leach the dried sample. After removing from the heat, 8 ml of DIW was added to the tubes and thoroughly mixed using a vortex mixer. This solution was then transferred to disposable polystyrene centrifuge tubes, stoppered and centrifuged at 2000 rpm for 2 minutes. The solutions were then analysed using the GEN5 calibration on the ICP Atomic Emission Spectrometer for a suite of 25 elements.

Herbage:

A similar principle of digestion was employed for the multi-element analysis of herbage to that for soils, however a larger weight of sample was used to increase the detection limit of elements, many of which are present in low concentrations in plant material. Consequently a larger volume of acids was used in larger test tubes and a longer digestion period was used to prevent ignition of the organic material.

2 g of the finely milled herbage was weighed into large test tubes (140 mm x 25 mm). The herbage was dampened with 2 ml of 0.1% Decon solution and left for 2-3 hours. Air condensers were then attached to the tubes and 5 x 1 ml portions of fuming nitric acid (95%) was added to the samples mixing carefully each time and allowing the frothing to subside between additions. A further 3 x 5 ml of fuming nitric acid was carefully added to the tubes. The tubes were then placed in the aluminium heating block and sequentially heated for 3 hours at 50 °C, 3 hours at 100 °C, 10 hours at 150 °C and 5 minutes at 160 °C. When cool, the condensers were removed and perchloric acid (3 ml, 60%) was added to the tubes which were then taken slowly to 150 °C and heated for 18 hours at this temperature. The dry tubes were then leached using 2 ml of 5 M hydrochloric acid

and heated at 60 °C for half an hour and 70 °C for a further half hour with the condensers attached. After leaching, 8 mls of DIW was added to the tubes, mixed using a vortex mixer and decanted into disposable polystyrene centrifuge tubes prior to ICPAES analysis. The solutions were analysed using the GEN5 calibration on the ICPAtomic Emission Spectrometer for a suite of 25 elements.

ii) Sulphur Analysis in Soil and Herbage

Sulphur in Herbage:

Sulphur in herbage was measured by ICPAES using aliquots of the solution prepared as above. However a different calibration is required for the analysis of sulphur.

Sulphur in Soil:

Sulphur is precipitated from soils during the digestion described above and so a different digestion method was employed prior to sulphur analysis by ICPAES.

0.25 g of finely milled soil was weighed into a 50 ml beaker, 1 ml of saturated magnesium nitrate was added to the beaker and thoroughly mixed with the sample. The sample was then placed in a muffle furnace and heated to 450 °C for 6 hours and then allowed to cool. Hydrochloric acid (5 ml, 36%) was added to the beakers which were covered with clingfilm and left on a rocking machine overnight. 0.5 ml of this solution was carefully pipetted into a disposable polystyrene centrifuge tube without taking up any of the residue, 4.5 ml of DIW was then added to each of the tubes which were capped and shaken to mix the solutions thoroughly prior to analysis by ICPAES.

iii) Selenium Analysis using Hydride Generation ICPAES

Analysis of both soil and herbage selenium was carried out using the hydride generation method described by Pahlavanpour et al. (1980).

The digestion method is the same for both soil and herbage except that, as a safety precaution, the herbage is allowed to remain at room temperature overnight before heating after the first addition of nitric acid.

0.5 g of dried, milled sample was weighed into Pyrex test tubes (180 mm x 18 mm). Nitric acid (2 ml, 70%) was added to the samples and thoroughly mixed. The herbage samples were then left to stand overnight. The tubes were placed in an aluminium heating block and heated at 50 °C overnight. The tubes were then allowed to cool and perchloric acid (1ml, 60%) was added to the samples and mixed. The samples were heated sequentially for 1 hour at 100 °C, 2 hours at 150 °C and then up to 170 °C until the samples were bleached but not dry. At this point the tubes were removed from the block and allowed to cool. Hydrochloric acid (4 drops, 36%) was added to the tubes and mixed, followed by 5 ml of DIW and further mixing. The solution was transferred into disposable polystyrene centrifuge tubes with washing and made up to 10 ml with DIW. The samples were centrifuged at 2000 rpm for two minutes and decanted into clean disposable centrifuge tubes.

To remove many other metal ions which may interfere in the hydride generation a lanthanum precipitation method was employed. Lanthanum nitrate (0.5 ml, 5% w/w) solution was added to the solution in the tubes and mixed thoroughly, ammonia solution (2 ml, 35%) was also added and the mixture shaken by hand prior to centrifugation at 2000 rpm for 2 minutes. The supernatant solution was discarded and the precipitate was redissolved by shaking with 5 ml of warm (50 °C) potassium bromide (4%) in 5 M HCl. Once dissolved the sample solution was made up to 10 ml with more 4% KBr in 5 M HCl. The tubes were left in a water bath at 50 °C for 1 hour. Once cool the volume was checked and made up to exactly 10 ml prior to hydride generation for selenium analysis the following day.

The hydride generation method employs a peristaltic pump to mix the sample solution with $LiAlH_4$ in a closed chamber. The selenium hydride which is generated is then swept into the ICP Atomic Emission Spectrometer and the selenium concentration is detected.

iv) Analysis of Water Samples

Water samples, whether rainwater or soil solutions, were analysed for a suite of elements on the ICPAES. The only preparation required was to filter the samples through a 0.45 μm millipore filter and to bring the solutions to 1 M HCl by addition of concentrated (36%) HCl.

4.4 ANALYTICAL QUALITY CONTROL PROCEDURES

Analytical quality control is an integral part of geochemical analysis. The two parameters used to assess analytical quality are accuracy and precision. The term accuracy is used to denote the extent to which the mean approaches the true concentration of the analyte. As the true value can never be ascertained, a consensus of estimates, made by a variety of analytical methods, is used. This is known as the referred or accepted value (Thompson, 1983). Accuracy therefore describes the extent to which the mean approaches the accepted value. Random errors are assumed to follow a normal Gaussian distribution about the mean concentration. Precision (P) refers to the spread of results (s) about the mean (x). It is a function of the coefficient of variation and is usually defined in geochemistry as:

$$P = (2s - x). 100\%$$

indicating, relative to the concentration of the analyte, the range in which

approximately 95% of the observations fall.

Quality control methods may be used to monitor within batch variations, between batch variations and the overall accuracy of the analytical method. The following methods of analytical quality control were carried out in every type of analysis used in this research but were of especial importance in monitoring the performance of the ICPAES method.

4.4.1 Duplicate Samples

Duplicate samples are used as an estimate of the precision within each batch of analysis.

In each batch, 10% of the samples were analysed in duplicate and included randomly in each batch with each duplicate being taken through the analytical procedure individually.

Precision can be rapidly tested against an empirical standard of precision using the special control chart (section 5.3.1). The use of these charts is based on the methodology of Thompson and Howarth (1976) and involves plotting the absolute difference between pairs of duplicate analyses against the mean value of the pairs.

Difficulties in the use of duplicates to estimate precision arise when differences between them do not follow a Gaussian distribution. This happens if :-

i) the sample is heterogeneous and sampling errors are skewed;

ii) the concentrations are close to the resolution of the analytical method and results are reported as discrete values and therefore give a discontinuous distribution;

iii) the concentrations are close to their detection limit. Values less than the detection limit are either set to zero or to the detection limit;

iv) systematic bias arises within a batch.

4.4.2 International and Departmental Reference Materials

Reference materials are essential for monitoring the accuracy of a method, especially the internationally recognised reference materials. Departmental reference materials are very important for assessing the between batch variation of an analytical method.

Reference materials were included at random to make up 10% of the batch. It is important that the reference materials are of a similar compositional and mineralogical make-up to the samples and that the particle sizes are similar.

Departmental reference materials (5%) were used during every batch of analyses, and international reference materials were analysed occasionally to ensure the accuracy of the method. Bowen's Kale (donated by MAFF) was used regularly as the main reference material for herbage selenium analysis as internal reference materials were not entirely suitable. Where available, two reference materials were always chosen with concentrations near both the detection limit and the threshold level in order to monitor the accuracy across the whole range of concentrations analysed.

4.4.3 Reagent Blanks

Reagent blanks were included as 10% of the samples in each batch and were used to ascertain the background level of the analyte and hence the detection limit of the analytical method. Reagent blanks can also monitor contamination either from extraneous material added to the sample during analysis or contamination due to carry over when anomalous and background samples are analysed together.

CHAPTER 5

A COMPARISON OF SELENIUM ANALYTICAL TECHNIQUES AND RESULTS OF THE QUALITY CONTROL PROCEDURES

5.1 INTRODUCTION

Many methods for the analysis of selenium in environmental and biological samples exist, however only a few are sensitive and accurate enough to detect selenium at the low levels which are currently of interest in this study of selenium deficiency.

A review of the methodology for selenium analysis is presented in this chapter followed by a detailed description of the precision and accuracy obtained for the two methods of selenium analysis used in this work, spectrofluorimetry and ICPAES. A discussion of the advantages and disadvantages of both these methods for selenium analysis is also included here.

The results of the quality control practices outlined in Chapter 4 are also presented in this chapter.

5.2 ANALYTICAL TECHNIQUES FOR THE DETERMINATION OF TRACE AMOUNTS OF SELENIUM

The identification of selenium toxicity problems in livestock in the 1930's led to the development of gravimetric, titrimetric and turbidimetric methods of selenium analysis in environmental samples. The method most commonly used was specific for selenium and quite sensitive for its time, capable of detecting 0.01

mg selenium (Robinson, 1933). The titrimetric method of Klein (1943) later superseded this, being somewhat more reproducible and sensitive.

With the realisation that selenium was also an essential trace element and that there were associated livestock deficiency problems in some areas, more sensitive methods of selenium analysis were needed to detect selenium in the μg and ng region.

Since the 1960's very sensitive methods have been developed for the analysis of environmental samples of low selenium concentration. Some of these methods capable of determining trace (μg/g) amounts of selenium include: spectrophotometry (Cheng, 1956), polarography (Faulkner et al., 1961), neutron activation analysis (Bowen and Cawse, 1963; Allaway and Cary, 1964), atomic absorption spectrometry, (Rann and Hambly, 1965), fluorescence spectrometry (Watkinson, 1960), gas chromatography (Tanaka and Kawashima, 1965), X-ray fluorescence analysis (Strausz et al., 1975), and inductively coupled plasma atomic emission spectrometry, ICPAES (Thompson et al., 1978).

A description of the development of those methods most widely in use at present is given in the following sections.

5.2.1 Decomposition Techniques

All environmental materials, with the possible exception of water samples, require a decomposition procedure prior to any method of analysis. A wide range of decomposition methods have been reported in the literature (Olson, 1976; Shendrikar, 1974), including wet oxidation using a variety of oxidants, dry ashing and oxygen flask combustion.

Wet oxidation of samples has been used most frequently employing various mixtures of HNO_3, $HClO_4$, H_2SO_4 and H_2O_2. Concentrated HCl is avoided since this would lead to the formation of volatile selenium compounds such as $SeOCl_2$. Volatilisation of selenium during wet oxidation procedures is the main obstacle to this method of sample destruction. Mixtures of HNO_3 and $HClO_4$ have been widely and successfully used for digestion prior to atomic absorption spectrometry

and fluorimetry, and these acids were used for sample decomposition throughout this research.

5.2.2 Neutron Activation Analysis

This method was first described for selenium analysis in biological samples by Bowen and Cawse (1963) and still provides one of the most sensitive methods of selenium analysis with detection limits in the range of 1-10 ng selenium (Bem, 1981). Thermal neutron activation is the most commonly used procedure for irradiating samples containing selenium. Of the radio-nuclides of selenium that this produces, the isotopes ^{75}Se ($t_{1/2}$ =120 days), ^{81}Se ($t_{1/2}$ = 18.6 mins) and ^{77m}Se ($t_{1/2}$ =17.5 secs) are useful in analysis. The material to be examined is usually irradiated in a nuclear reactor with a flux of 10^{13} -10^{15} n/cm^2 sec for 7-14 days (for ^{75}Se) or for several seconds (for ^{77m}Se). The activity of the irradiated samples is measured with a γ-ray multichannel analyser and high resolution Ge/Li detectors.

Neutron activation analysis can be used with and without sample destruction, non-destructive methods have been used for multi-element analysis but they are subject to more errors, because of interference, than destructive methods followed by chemical separation.

Neutron activation analysis can be very accurate, sensitive and specific, especially when used with sample destruction and chemical separation of the selenium. However it requires sophisticated equipment, including a nuclear reactor, to which most laboratories do not have access, and hence its most important use to date has been as a reference method against which other methods can be evaluated, or as a means of establishing the selenium content of reference materials (Nadkarni and Morrison, 1978).

5.2.3 Atomic Absorption Spectrometry

Methods for selenium analysis based on atomic absorption spectrometry (AAS) have been widely reported, and most require some type of wet digestion to decompose the sample material. Flame atomisation methods can only detect selenium in relatively high concentrations but other more sensitive methods have been developed, most based on hydrogen selenide generation or flameless atomic absorption spectrometry (FAAS). Hydride generation has the advantage of separating the selenium from many other elements and removing most of the possible interference. Ihnat (1976a) compared the performance of hydride generation AAS with that of a graphite furnace atomisation FAAS method and found the hydrogen selenide generation method to be superior.

Flameless atomic absorption spectrometry techniques offer a high sensitivity (0.1 ng Se) but are neither simple nor free from interference due to the high volatility of selenium (Bem, 1981). FAAS is particularly suitable for direct analysis of samples (Fry and Denton, 1977). The addition of nickel has been shown to enhance the sensitivity of graphite furnace FAAS by about 30% (Ihnat, 1976b) and to remove interference from other metals capable of forming selenides such as Ba, Cu, Fe, Mg and Zn. Montaser and Mehrabzadeh (1978) reported a detection limit of 0.001 ng Se using electrothermal graphite furnace FAAS and background correction with a deuterium lamp. However, the graphite furnace method is not always reliable due to the strong and variable matrix effects and signal splitting, and the technique of hydride generation is now widely used as an improvement on graphite furnace FAAS.

The hydride generation method involves the measurement of atomic absorption or emission of the selenium hydride formed by reduction of selenium in the sample solution, usually with $NaBH_4$ (Thompson, 1975). Hydride generation techniques are far more sensitive for the detection of selenium than ordinary atomic absorption spectrometry and are generally preferable to graphite furnace atomic absorption spectrometry since the selenium is separated from the matrix before atomisation thus avoiding the interference effects inherent in the other methods.

For ICPAES the technique of hydride generation is identical but the emission spectrum is used for the quantitative identification of selenium after atomisation in a high temperature plasma (Thompson et al., 1978). Pahlavanpour et al. (1980) reported a detection limit of 1 ng/ml Se using hydride generation ICPAES. Although hydride generation is not subject to interference from most constituents of soils or other biological samples, small traces of copper inhibit the release of hydrogen selenide, so the selenium is separated by co-precipitation with lanthanum hydroxide (Bedard and Kerbyson, 1976). In the final solution, the selenium must be present as Se IV as the efficiency of the sodium tetraborohydride reduction depends on the oxidation state of the selenium. This is achieved by adding 4% of potassium bromide to the final solution and heating at 50 °C (Pahlavanpour et al., 1980).

5.2.4 Spectrofluorimetric Analysis

This is currently one of the most widely used methods for the determination of selenium at low levels, and is based on the measurement of a fluorescent piazselenol formed in the reaction of Se IV with 1-diamines.

Hoste (1948) first reported the use of diaminobenzidine (DAB) as an analytical reagent for selenium, then in 1955, Hoste and Gillis used this same reagent for the spectrophotometric analysis of trace amounts of selenium. Cheng (1956) was among the first to employ this reagent (DAB) in a spectrophotometric method and then Watkinson (1960) in a fluorimetric method. However a more sensitive reagent was required and in 1960, Ariyoshi et al. described a spectrophotometric method using o-phenylenediamine (o-PDA), and Parker and Harvey (1962) described a sensitive fluorimetric method using 2,3-diaminonaphthalene (DAN). The spectrophotometric method was developed separately for gas chromatography analysis (section 5.2.5).

Fluorimetric analysis of selenium was found to be ten times more sensitive than the atomic absorption spectrometry method (Lott et al., 1963), since the wavelength of 1960 nm used in atomic absorption spectrometry is in the u.v.

region where extraneous absorption occurs (Shendrikar, 1974).

The fluorimetric method first described by Parker and Harvey (1962) was substantially modified by Hall and Gupta (1969) to provide a sensitivity of 5 ng Se in 5 g samples. This method has been proved sufficiently accurate and reliable to be adopted, with modifications, as the official method for selenium determination at low levels in plants by the American Association of Official Analytical Chemists (AOAC) (Olson et al., 1975) and is recommended for general samples by the Environmental Protection Agency (Shendrikar, 1974) and the Ministry of Agriculture, Fisheries and Food (HMSO, 1986).

This method was adopted for use in this research with some modifications in both the digestion process and the complexing procedure using recommendations from Chapman and Jane (1985) and Van Dorst (pers. comm.), and is described in detail in Chapter 4.

5.2.5 Gas Liquid Chromatography

Present methods for determining selenium by gas chromatography are based on the analysis of a piazselenol formed by the reaction of Se IV with an o-phenylenediamine (o-PDA) in an acidic solution. This analysis evolved alongside the fluorimetric analysis for piazselenols and has since been developed by Takana and Kawashima (1965), Goto and Toei (1965) and Nakashima and Toei (1968). The present gas chromatography method is used in conjunction with an electron capture detector to provide detection limits of 1-10 ng Se (Dilli and Sutikno, 1984).

5.2.6 X-ray Fluorescence Analysis

There has been a growing interest in determination of many elements, including selenium, by X-ray induced fluorescence analysis (XFA). This method allows simultaneous estimation of a number of elements, often without the

necessity for previous sample preparation. Selenium is determined by measurement of its Kα line (11.2 KeV) with a semiconductor detector (eg. Si/Li) and a computer coupled multi-channel analyser (Bem, 1981). Selenium has been measured in geological samples and coal fly ash by this method (Giauque, et al., 1977) and in mineralised biological samples with a detection limit of 0.2 μg Se in a 5 g sample (Strausz, et al., 1975).

5.2.7 Selenium Speciation Techniques

The identification and quantification of chemical forms of selenium in the soil is important to the understanding of plant selenium uptake and the behaviour of selenium in soils. However some fundamental problems exist in the study of selenium species in the soil. Almost all analytical techniques for selenium require a digestion to obtain the sample in a homogeneous solution and the selenium content of an untreated sample cannot therefore be determined. With any form of chemical treatment prior to analysis there is the probability of redox reactions occurring in the sample, or destruction and/or volatilisation of organic material, and the natural speciation of the sample is presumed to be changed.

One method of producing a sample for speciation studies with the minimum of interference is to obtain the soil solution from a sample of wet soil, either fresh soil or one which has been allowed to equilibrate with added water in laboratory conditions. The soil solution is obtained either by centrifugation (Davies and Davies, 1963) or by displacement with a heavy organic liquid (Kinniburgh and Miles, 1983).

A variety of methods have been used in the past for identifying and measuring a number of forms of selenium in different materials; paper chromatography (Hamilton, 1975) and ion-exchange chromatography (Martin and Gerlach, 1969) for selenium compounds in plant extracts; gas chromatography for volatile selenium compounds (Doran and Alexander, 1976); and ion-exchange chromatography (Shrift and Virupaksha, 1965) or spectrofluorimetric

determination for measuring selenite and selenate in solutions (Van Dorst and Peterson, 1983).

The separation of organo-selenium compounds in plants by chromatographic and ion-exchange techniques is well documented (Martin et al., 1971, Peterson and Robinson, 1972 and Brown and Shrift, 1980). Separation of inorganic selenium compounds has been attempted using ion-exchange chromatography followed by analysis using hydride generation/graphite furnace atomic absorption spectrometry; however this is not very sensitive (Roden and Tallman, 1982).

Van Dorst and Peterson (1983) carried out a comprehensive study of selenium speciation in soil solutions using four different methods: paper chromatography, anion exchange column chromatography, fluorimetric analysis and high voltage paper electrophoresis. They found that paper chromatography was unsuccessful for separating a mixture of selenium compounds in soil solution due to streaking of colloidal selenium, although selenate, selenite and selenoamino acids could be identified. Anion exchange chromatography was also only useful for estimating selenate and selenite, as organic forms of selenium were assumed to be destroyed during the column elution. The fluorimetric technique relied on the fact that selenite only is complexed with DAN at pH 2.5 to form a fluorescent piazselenol. Selenite could therefore be measured directly with good sensitivity and selenate could be measured indirectly after a reduction step with hydrochloric acid to selenite. High voltage paper electrophoresis was found to give a comprehensive fingerprint of the selenium compounds present in the soil solution, although high blank values limited the sensitivity. All of these methods were also attempted with ^{75}Se labelled soil solution which increased the sensitivity of high voltage paper electrophoresis and anion exchange chromatography for inorganic species of selenium.

The use of the fluorimetric method for speciation of inorganic selenium compounds in soil solution was adopted with some success for a limited number of samples in this research (section 6.6.2).

5.3 A COMPARISON OF SPECTROFLUORIMETRY AND ICPAES FOR ANALYSIS OF TRACE LEVELS OF SELENIUM

Two methods were available within the department for the analysis of selenium, spectrofluorimetry and hydride generation ICPAES, and a comparative study was undertaken in order to check the performance of each method and to detect differences between them.

The ICPAES method allows for much larger batches of samples to be processed (around 250 in 4 days) than the fluorimetric method (38 in 2 days) and so would be the preferred method of analysis if the accuracy and precision were found to be sufficient for the samples of low selenium content which were collected in this research.

Some improvements were made to the spectrofluorimetric analysis during the course of this research (section 5.3.2). No method development was, however, attempted for the ICPAES method since the maintainance and calibration of the ICPAE Spectrometer was carried out by the analytical services department.

It was suspected that the ICPAES method, having a stated system detection limit of 0.024 μg/g Se, would not be sufficiently sensitive for some herbage and water analyses although all of the collected soils would be within the calibration range.

Internal and certified reference materials for both soil and herbage were analysed using both methods to check the accuracy of each. Results of duplicate analyses were used to estimate the precision of each method and blank measurements were used to obtain the machine and method detection limits.

5.3.1 Results of the Comparative Study for Spectrofluorimetry and ICPAES

The analysis of soil and herbage samples for this comparative study was carried out using the methods described in detail in Chapter 4. Reference materials for soil and herbage were analysed using both spectrofluorimetry and hydride generation ICPAES and the measured values are given in Table 5.1.

Key to Table 5.1

The values given are mean selenium concentrations ± 95% confidence limits.

The selenium concentrations for NBS Citrus Leaves are not certified.

SO 1-4 are soil reference materials from the Canadian Reference Material Project. (S. Abbey, 1983. Studies in standard samples of silicate rocks and minerals, 1969-1982. Geological Survey of Canada, Paper 83-15, Ottowa.)

The accepted selenium concentration given for Bowen's Kale was provided by the MAFF analytical laboratory in Newcastle. The certified value for the selenium concentration in Bowen's Kale is 0.148 ± 0.0137 μg/g (H. G. M. Bowen, 1967. Analyst (Lond.), 92:124-131.).

Table 5.1 **The selenium content obtained for some reference materials using spectrofluorimetry and ICPAES**

	Reference Material	Accepted Value µg/g Se	Fluorimetry µg/g Se	ICPAES µg/g Se
International				
Herbage	Bowen's Kale	0.134 ± 0.02	0.147 ± 0.04 n=29	0.069 ± 0.02 n=10
	NBS Citrus Leaves	0.025	0.05 ± 0.01 n=4	0.015 ± 0.04 n=2
	NBS Rice Flour	0.4 ± 0.1	0.30 ± 0.03 n=4	0.20 ± 0.10 n=2
Soil	SO 1	0.1	0.12 ± 0.06 n=2	<0.02 ± 0.04 n=2
	SO 2	0.3	0.33 ± 0.02 n=2	0.21 ± 0.08 n=2
	SO 3	0.05	0.01 ± 0.01 n=2	<0.02 ± 0.01 n=2
	SO 4	0.4	0.29 ± 0.12 n=2	0.30 ± 0.06 n=2
House (Internal)				
Herbage	HRM 12	0.33	0.41 ± 0.10 n=21	0.29 ± 0.08 n=24
	HRM 13	0.03	0.05 ± 0.04 n=17	0.021 ± 0.03 n=32
	HRM 14	0.08	0.13 ± 0.06 n=18	0.08 ± 0.04 n=22
Soil	HRM 1	0.07	0.07 ± 0.09 n=6	0.05 ± 0.09 n=15
	HRM 2	2.23	3.06 ± 0.28 n=6	1.81 ± 0.30 n=14

The values obtained for the international certified reference materials using spectrofluorimetry all agree well with the accepted values apart from NBS Citrus Leaves and SO3, where the accepted values are very low and greater uncertainties would be expected. However, the values obtained for these reference materials using the ICPAES method are all lower than the accepted values, especially for the herbage samples which are around 50% low. The values obtained for the soil reference materials are probably acceptable, especially for those of higher selenium content, however it is apparent that the ICPAES method is underestimating the selenium content of herbage. The selenium may be lost during digestion or during the lanthanum nitrate precipitation stage, but no experiments were carried out to determine this and the herbage selenium content was analysed by spectrofluorimetry throughout the rest of the research.

The internal or 'house' reference materials analysed by both methods all show good agreement with the accepted values. However here it must be noted that the accepted values were obtained by ICPAES analysis only and so the usefulness of these reference materials to the assessment of the fluorimetric method is limited. For these internal reference materials the fluorimetric determination again produces larger values than the ICPAES method in both soil and herbage, suggesting that some selenium is lost during the ICPAES analysis.

Using the internal reference materials alone, both methods would seem to be acceptable for the analysis of soil and herbage samples, however the use of international reference materials has shown that the ICPAES method does not have sufficient accuracy for the analysis of herbage samples of low selenium content. Unfortunately due to their cost, only a few samples of international reference materials other than Bowen's Kale could be analysed, and on such a small data set the results cannot be conclusive, but they do suggest that the hydride generation ICPAES method for selenium analysis in herbage needs some improvement.

The detection limit of an analytical method is taken to be twice the standard deviation on the blank sample. Two methods of describing the detection limit were used in this research, the instrumental detection limit and the system detection limit. The instrumental detection limit is found by measuring repeated

machine readings on one blank solution giving an estimate of the machine variation, and producing a value which is often very low. The system detection limit is more realistic and is found by measuring many blank solutions all prepared in the same way as the samples. This detection limit value therefore gives a reasonable estimate of the selenium content of a sample which can be detected above the background variation of blank sample solutions.

The fluorimeter was found to have a much better instrument detection limit (0.13 ng/g Se) than the ICPAE spectrometer (10 ng/g Se) which was expected since the fluorimeter produces less background interference. The system detection limit was also lower for the fluorimetric method (4.5 ng/g Se) than the ICPAES method (24 ng/g Se) due to the greater sensitivity of the fluorimetric method.

The precision of an analytical method can be estimated using the means and differences of duplicate samples analysed by that method. The use of precision charts is a simple way to estimate the precision of a method (section 4.4.1), and the precision charts for the selenium analysis of soil and herbage using spectrofluorimetry and ICPAES are given in Figures 5.1-5.4. In these charts, 90% of the points should lie below the diagonal lines shown for any particular percentage precision. For the analysis of selenium in soil, the precision obtained from both methods is the same, around 25%, however the fluorimetric method does show improved precision (10%) on samples with a selenium content above 0.1 μg/g. For the analysis of herbage the spectrofluorimetric method shows a much better precision (15%) than the ICPAES method (28%) overall, however for samples with a selenium content above 0.2 μg/g both methods show a precision of around 10%. The ICPAES method has far worse precision for herbage samples of low selenium content than the fluorimetric method due to the higher detection limit of the ICPAES method.

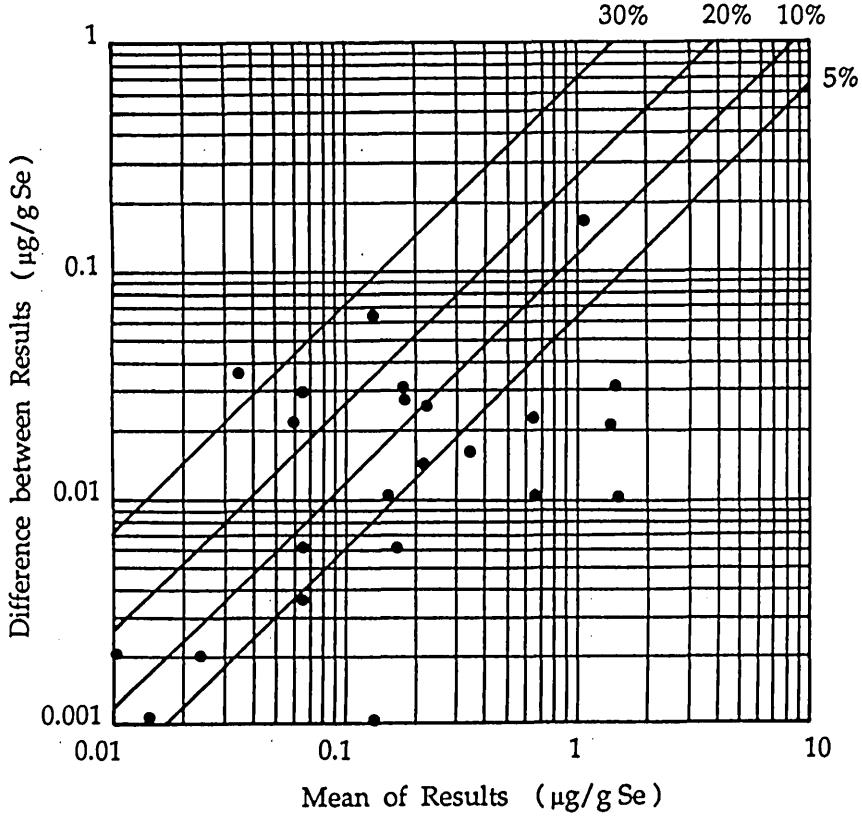


Figure 5.1 The precision chart for the analysis of selenium in soil by spectrofluorimetry showing a precision of 25% (10% at >0.1 µg/g Se) at the 95% confidence limit

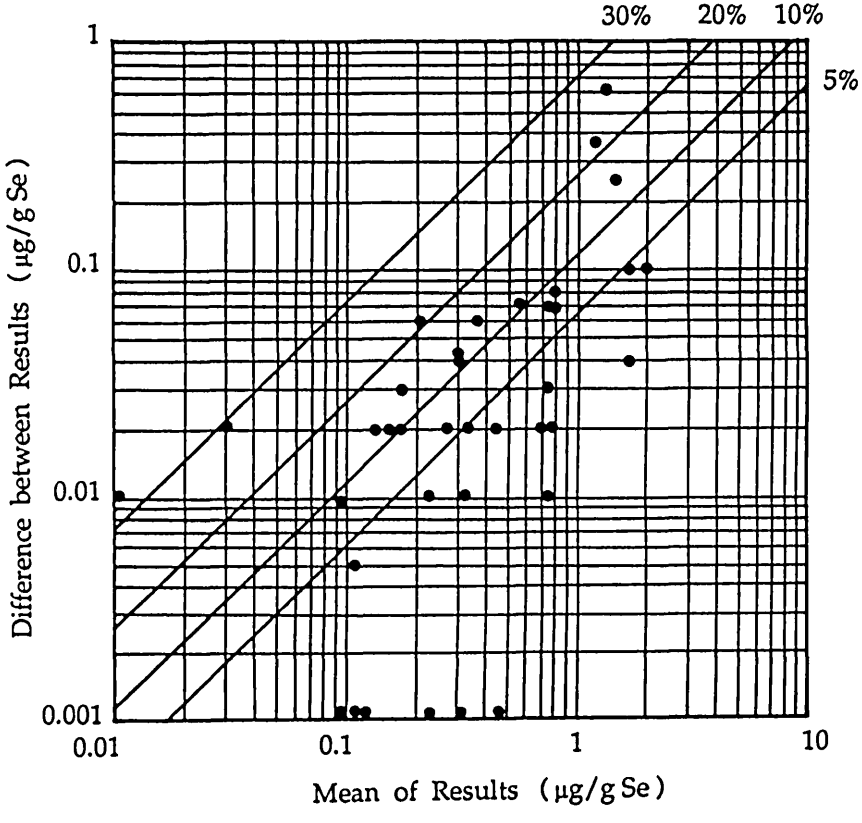


Figure 5.2 The precision chart for the analysis of selenium in soil by ICPAES showing a precision of 25% at the 95% confidence limit

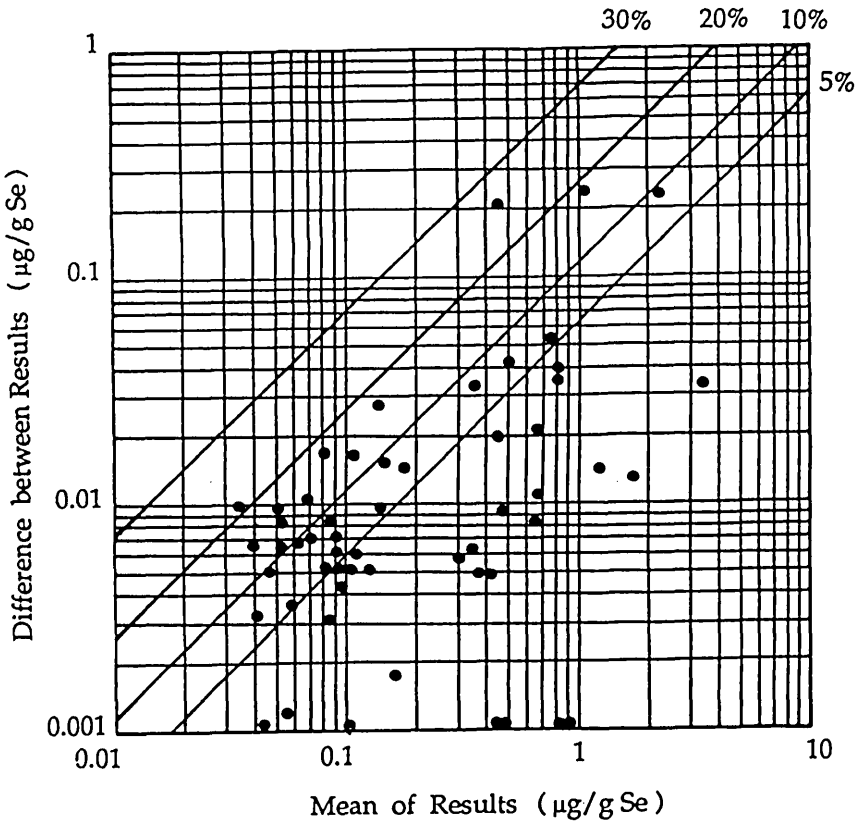


Figure 5.3 The precision chart for the analysis of selenium in herbage by spectrofluorimetry showing a precision of 15% (10% at >0.2 µg/g Se) at the 95% confidence limit

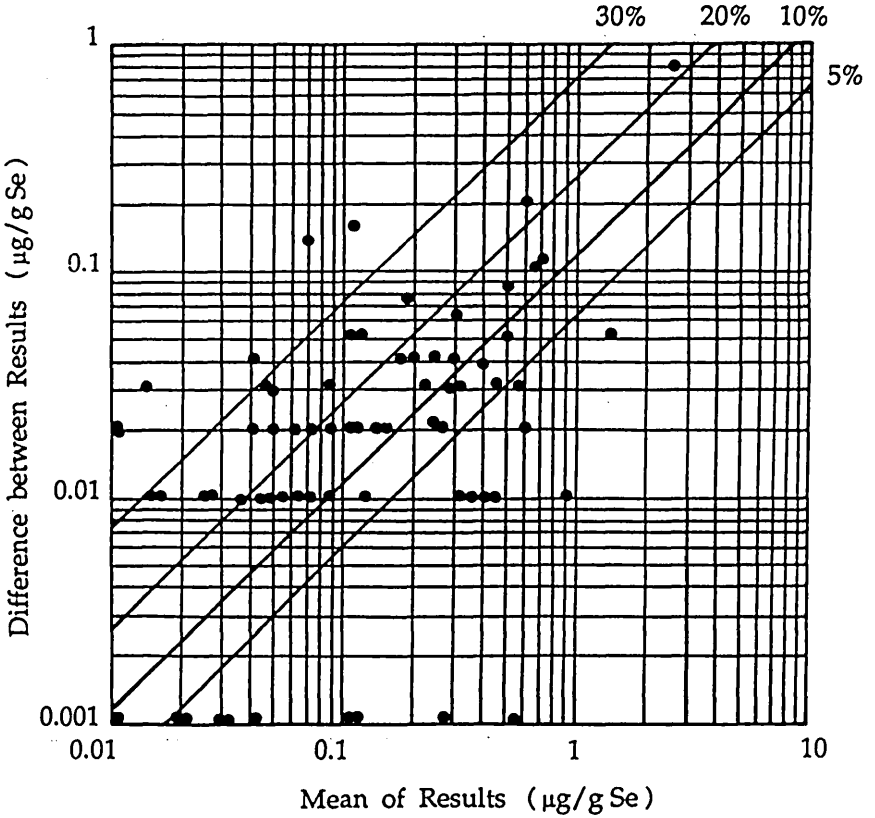


Figure 5.4 The precision chart for the analysis of selenium in herrbage by ICPAES showing a precision of 28% (10% at >0.2 µg/g) at the 95% confidence limit

5.3.2 Improvements made to the Spectrofluorimetric Analysis during the Research

The spectrofluorimetric method adopted during this research was essentially that recommended by the Ministry of Agriculture, Fisheries and Food (MAFF, 1981), based on the method of Hall and Gupta (1969) and further revised in 1985 (Chapman and Jane, 1985). This method, especially the digestion process, has been adapted so that it uses smaller weights of sample and is compatible with the equipment available in this department. Several stages of the method have been checked during this research and the detection limit of the method was lowered by the use of DDDIW in all reagents.

The digestion procedure used by Hall and Gupta was lengthy, involved the use of nitric acid, hydrogen peroxide and perchloric acid and was liable to produce charring in the samples. This method was replaced (Chapman and Jane, 1985) by a simpler nitric/perchloric acid digestion using 2 g of sample material and 50 ml Kjeldahl digestion flasks.

This nitric/perchloric acid digestion process was modified for this research in order to use boiling tubes (140 mm x 25 mm), with ground glass tops and a thermostatically controlled aluminium heating block with holes to accept these tubes. A smaller sample weight and acid volume was used since these tubes are smaller than the Kjeldahl flasks. The tubes accept air condensers which were used to prevent the nitric acid evaporating until the majority of the digestion was complete. Nitric and perchloric acids are added at the start of the digestion in the MAFF method (Chapman and Jane, 1985) and heating is continued until 15 minutes after white perchloric acid fumes are first evolved. The digestion method used in this research is given in detail in Chapter 4, but nitric acid alone was used on the samples overnight at 50 °C, then perchloric acid was added, and the samples heated slowly to 150 °C over three hours with the air condensers only removed for the last half hour at 150 °C to allow the nitric acid to evaporate. The temperature was finally raised to 170 °C for about 20 minutes until the samples bleached and the liquid volume in the tubes was reduced (0.5 - 1 ml remaining), but not dry.

Following this method, charring of the samples was never encountered and

all the nitric acid was removed, which may otherwise have caused interference in the complexing procedure. During the development of this method, it was found that removal of the air condensers earlier in the digestion led to occasional charring and often loss of selenium when the volume of liquid in the tubes became too low. If the condensers were removed as the temperature was raised to 170 °C then some nitric acid remained in the sample, providing interference in the complexing process and producing a peak in the spectrum corresponding to nitrate in the sample and generally raising the measured fluorescence.

Bowen's Kale was used consistently as a reference material to check that no loss of selenium occurred during the digestion.

In order to ensure that all the selenium present in the sample was in the Se IV oxidation state prior to complexation, the digest was boiled with hydrochloric acid for 15 minutes. Using standard solutions of sodium selenite and sodium selenate this reduction step was checked to ensure that the reduction was complete. Figure 5.5 shows the fluorescence emission spectra of standard solutions of sodium selenite and sodium selenate reduced by this method, and the spectrum of the same sodium selenite solution without the reduction step. It can be seen from these spectra that the sodium selenate solution has been completely reduced to selenite prior to the complexation.

The complex formation method used was essentially that recommended by MAFF (Chapman and Jane, 1985) although the use of DDDIW instead of DIW in this research for making up all reagents, standards and as a final rinse for glassware reduced the method detection limit from 9.3 ng/g Se to 4.5 ng/g Se.

The purification of DAN was carried out as described in the MAFF method, and the purified DAN was then stored in an amber bottle in the freezer until required. Fresh DAN solution was made up for each batch of analyses.

The addition of formic acid as buffer, EDTA stabilising solution and ammonia to the digest in the boiling tubes prior to the addition of DAN maintained the mixture at pH 2 which is essential for the formation of the fluorescent piazselenol. The contents of the tubes were mixed by shaking before and after the addition of the DAN solution.

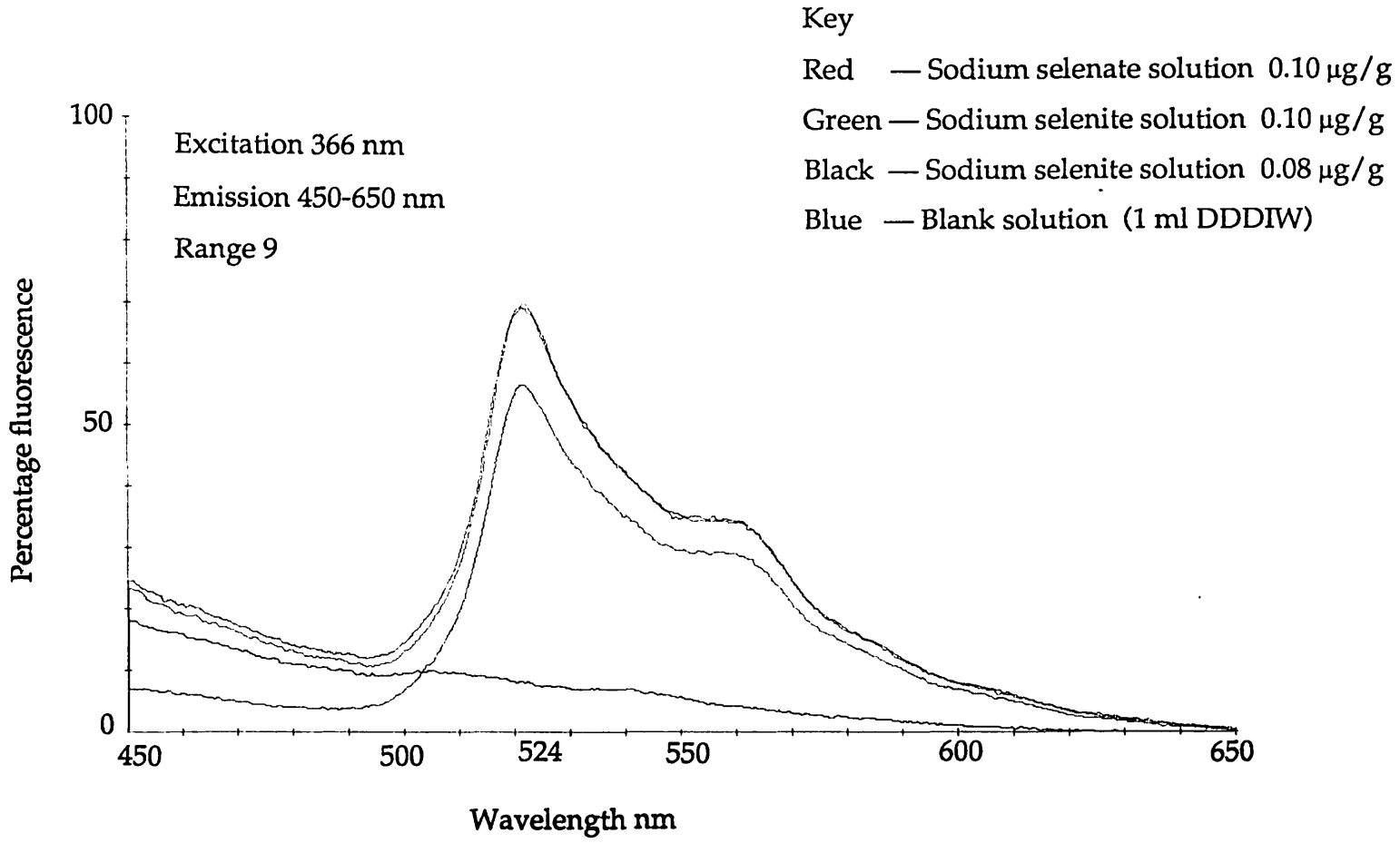


Figure 5.5 The fluorescence emission spectra of standard solutions of sodium selenite and sodium selenate following reduction using hydrochloric acid

Once formed the piazselenol complex was extracted into cyclohexane. MAFF used dekalin (decahydronaphthalene) since this is less volatile and therefore safer, however cyclohexane has been used successfully instead of dekalin by other workers.

The fluorescence emission spectrum of the cyclohexane was examined (see Figure 5.6) and shown to be very low in the region of 524 nm where the piazselenol peak occurs. This also showed that there were no impurities in the cyclohexane which interfere with the fluorescence spectrum of the piazselenol.

MAFF (Chapman and Jane, 1985) reduced the extraction procedure from 3 individual pooled extractions to just one extraction using 7 ml of cyclohexane or dekalin. They tested this method to ensure that all the piazselenol was extracted in the single step and found no difference in the results of the two methods. As a separate check during this research, a further extraction was carried out with a second 7 ml volume of cyclohexane to see if the first extraction left any measurable amount of piazselenol in the aqueous phase. No selenium was detected in these second extractions except for one sample of very high selenium content, however the amount of selenium present in the second extract was less than 2% of the original selenium content of the sample and was therefore considered negligible.

Silicone bungs were used successfully for the washing procedure in the 10 ml glass centrifuge tubes, and were also helpful throughout the measurement stage in preventing the evaporation of cyclohexane which could otherwise produce elevated values of selenium due to concentration.

The quartz spectrometer cells were rinsed with clean cyclohexane between each fluorescence measurement to prevent contamination between samples.

The fluorescence of the samples was read at 524 nm with an excitation wavelength of 366 nm. The spectra were also examined to check that the samples produced the characteristic spectra for selenium, and especially that there was no nitrate interference.

Standard solutions of sodium selenite were used to calibrate all ranges of the spectrofluorimeter (Figure 5.7), and sodium selenite solutions, sodium selenate solutions and standard reference materials were included in each batch of samples to monitor the performance of the method.

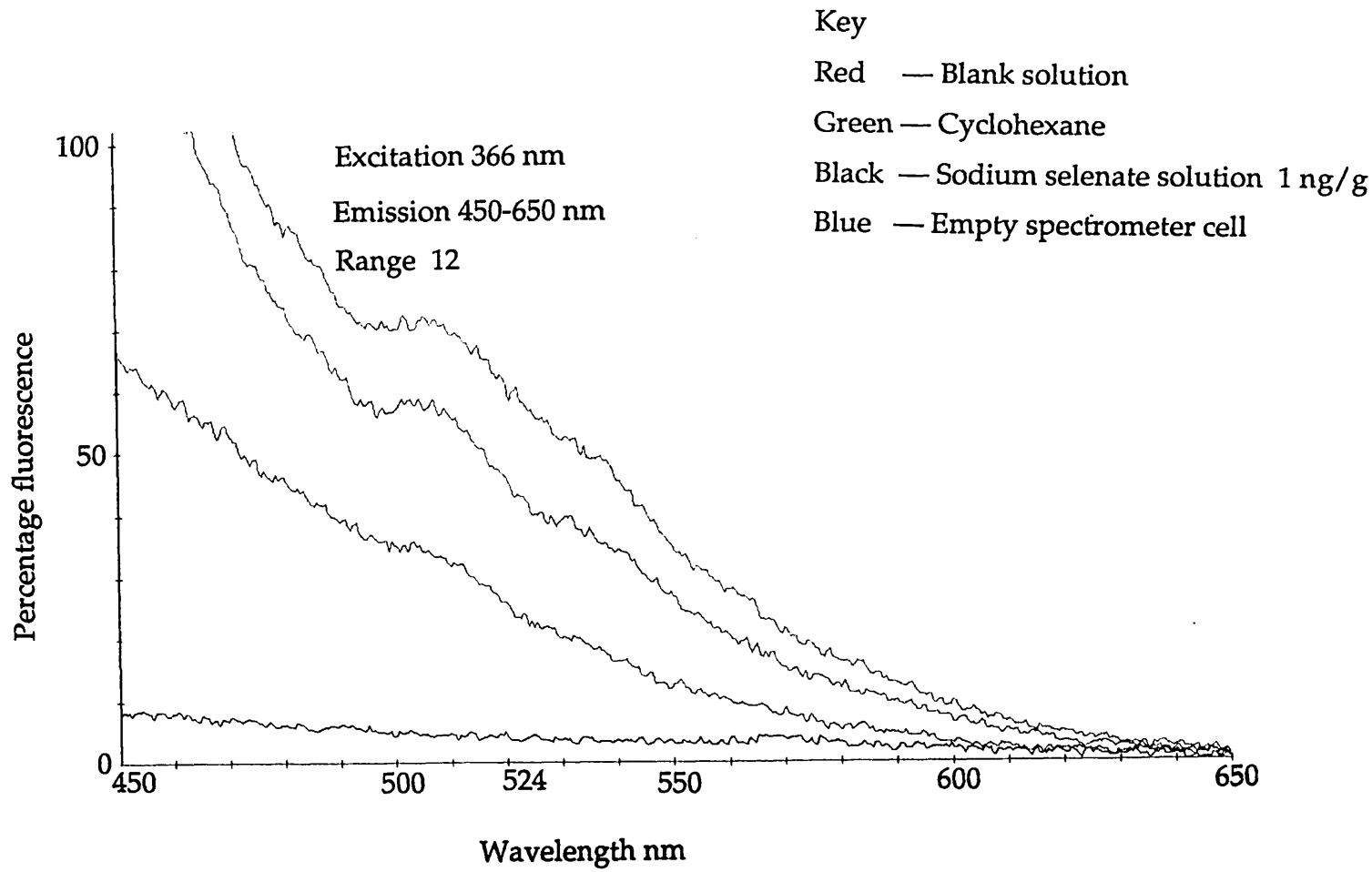


Figure 5.6 The fluorescence emission spectra of cyclohexane and blank solutions

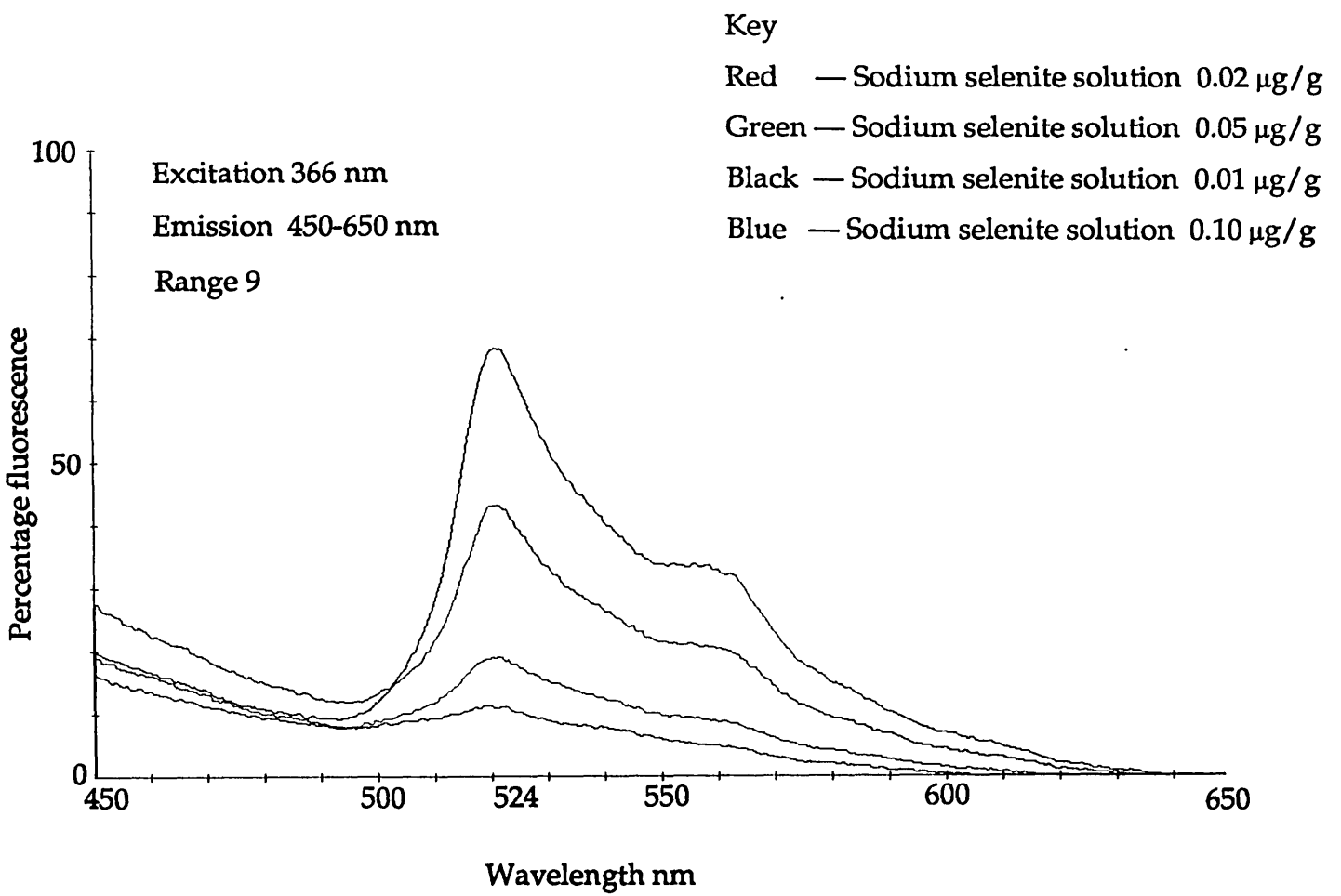


Figure 5.7 The fluorescence emission spectra of standard solutions of sodium selenite used to calibrate the spectrofluorimeter

The emission spectra of samples and reference materials were plotted where necessary to ensure that the characteristic selenium spectrum was obtained. The spectra obtained from standard selenium solutions and a herbage sample is given in Figure 5.8 and shows that the measured fluorescence is due to the selenium complex and not some other source. Figure 5.9 shows the emission spectra of standard sodium selenite solutions and blank solutions analysed with and without going through the digestion process. The results show that a small amount of blank contamination occurs during the digestion, possibly due to the addition of more reagents, and consequently the selenium concentration for the standard solution is slightly higher if the sample has gone through the digestion process. Figure 5.10 shows the emission spectra from several standard reference materials, illustrating the spectra obtained from samples with a range of selenium concentrations.

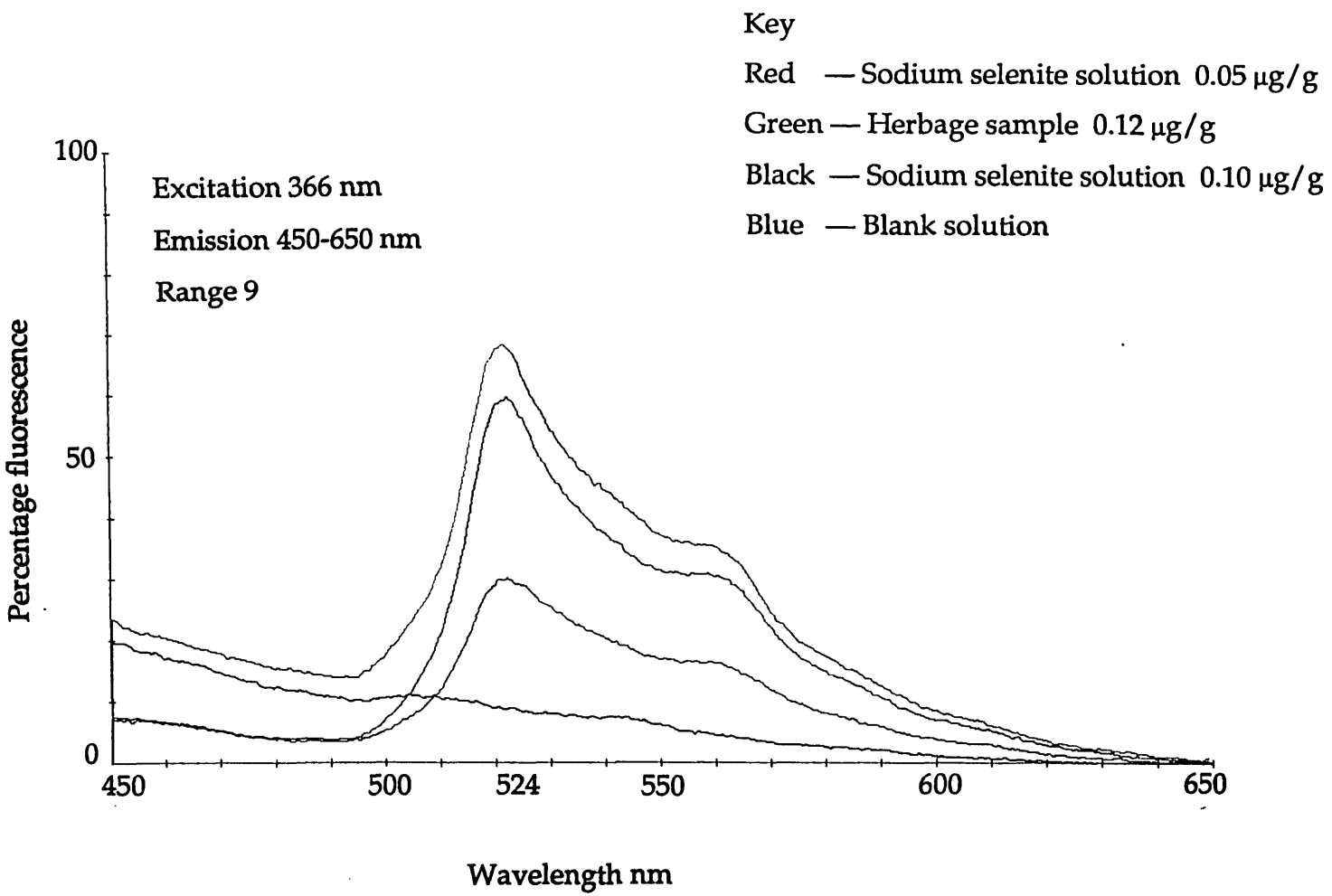


Figure 5.8 The fluorescence emission spectra of herbage samples and standard sodium selenite solutions showing the characteristic spectrum for selenium

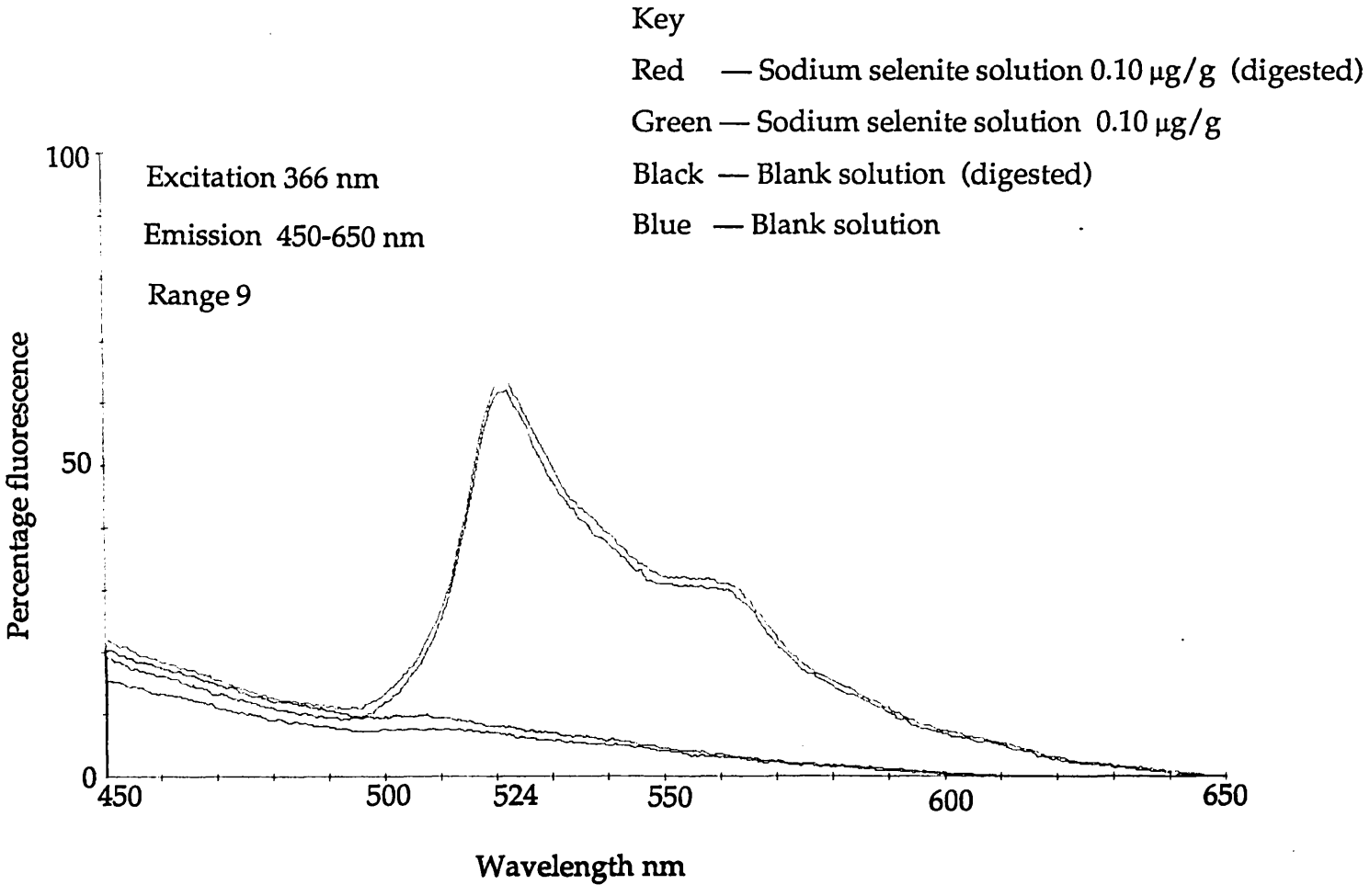


Figure 5.9 The fluorescence emission spectra of blanks and standard solutions analysed with and without a digestion process

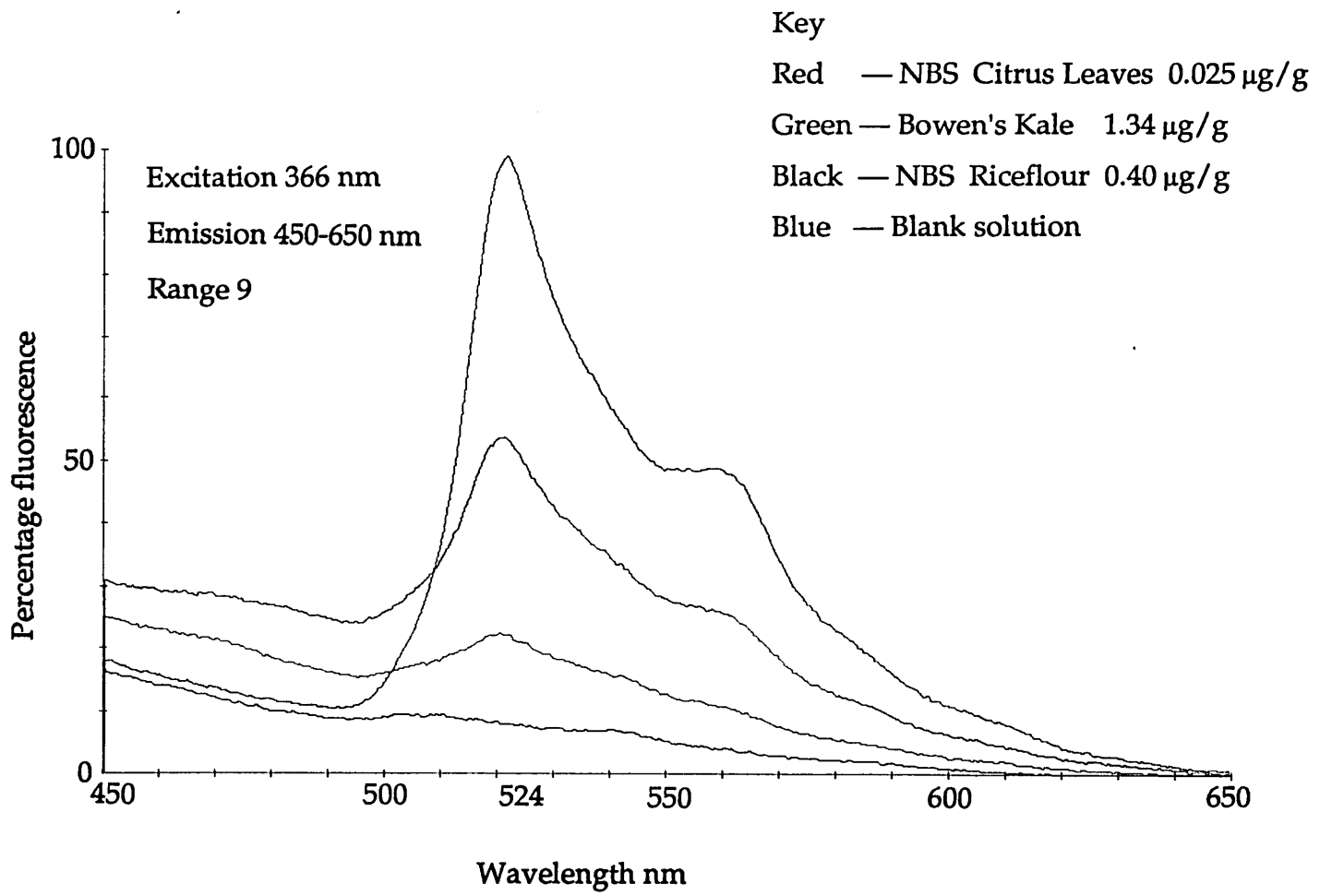


Figure 5.10 The fluorescence emission spectra of several reference materials with a range of selenium concentrations

5.3.3 Advantages and Limitations of ICPAES and Spectrofluorimetry for Trace Analysis of Selenium

Table 5.2 compares the main factors which were considered in this comparison of the two analytical techniques. During this research the spectrofluorimetric method has been shown to have lower detection limits, slightly better precision and better agreement with the accepted international reference materials especially for herbage selenium analysis than the hydride generation ICPAES method. The spectrofluorimetric method also offers greater scope for selenium speciation studies since DAN complexes with only the selenite (Se IV) ion in aqueous solution. Selenium speciation in water samples using the hydride generation ICPAES method is theoretically possible but the higher detection limits of the method and the current use of 1M HCl as a solvent would make it impractical.

ICPAES is, however, much better suited to the routine analysis of selenium in large numbers of samples due to the greater number of samples that can be analysed in one batch. This method would be more practical for large scale reconnaissance surveys for example, but in more detailed studies the loss in sensitivity may not be acceptable despite the time saving. Spectrofluorimetry is considerably slower than ICPAES but this may often be justified by the greater analytical flexibility provided by spectrofluorimetry.

Table 5.2 **A comparison of ICPAES and spectrofluorimetry for the determination of selenium**

	ICPAES	Spectrofluorimetry
Instrument	ARL 34000C	Baird Nova 2
Detection limit		
i) Instrumental	10 ng/g	0.13 ng/g
ii) System	24 ng/g	4.5 ng/g
Precision	soil: 25% plant: 28% (10% >0.2 μg/g)	soil: 25% (10% >0.1 μg/g) plant: 15%(10% >0.2 μg/g)
Calibration range	10^5 concentration	10^1 concentration (x 16 ranges)
Interference from other elements	Cu, Ni, Pb ->$La(OH_2)$ ppt As, Sb, Bi	NO_3^- removed in digestion
Speciation	Theoretically possible but detection limit too high	Se IV complex formed Speciation possible in water samples
Sample type	Soil, plant, water	Soil, plant, water
Productivity	High, 200 per batch	Low, 30 per batch
Capital cost	£150-180 thousand	£15-20 thousand
Safety	$HClO_4$ -explosive	$HClO_4$ -explosive DAN -carcinogen?

5.4 QUALITY CONTROL RESULTS FOR OTHER ANALYTICAL METHODS

5.4.1 Quality Control for the ICPAES Method

The collected samples were analysed for many elements using the ICPAES method. One method of analysis allowed a suite of 25 elements to be measured simultaneously, and sulphur was also determined separately. The quality control procedures outlined in Chapter 4 were followed for all analyses and Table 5.3 shows the results of this monitoring for some of the elements which have proved to be of greatest interest in this research.

Table 5.3 The results of the quality control for the ICPAES analyses for selected elements

Analysis type	Detection limit µg/g	Precision
Sulphur in herbage	12.6	5 %
Sulphur in soil	0.14	10 %
Iron in herbage	0.57	10 %
Iron in soil	6.20	5 %
Titanium in herbage	0.07	20 %
Titanium in soil	0.84	20 %

The analysis of reference materials gave measurements which were all within the accepted range of values for each element.

5.4.2 Quality Control for other Soil Analyses

Duplicate samples were also measured for pH (DIW and $CaCl_2$), cation exchange capacity and pyrophosphate extractable iron analyses. The pH measurements were checked using fresh standard buffer solutions, however no reference material was available for the cation exchange capacity or pyrophosphate extractable iron measurements. The pH measurements were found to have a precision below 5% in both DIW and $CaCl_2$ solution. The precision for the cation exchange capacity and the pyrophosphate extractable iron was 10% for both measurements. The detection limit for the cation exchange capacity was 0.27 me/100 g soil and for the pyrophosphate extractable iron was 1.09 μg/g Fe.

A few duplicate measurements were made during the soil texture analysis and these showed satisfactory similarity, although there were insufficient duplicate samples to make an estimate of the precision of the method.

5.4.3 Analysis of Sampling Variation

The sampling programme was designed to monitor the seasonal variations in the selenium content of herbage and consequently the samples were taken from the same part of the field at each visit. In order to establish the amount of variation inherent in this repeat sampling method, duplicate samples were taken at several sites on various occasions. If the sampling variation in selenium concentration was found to be exceptionally large then the seasonal differences in selenium concentration which were observed might be merely due to sampling errors.

The percentage difference in selenium content between the duplicate herbage samples was around 13% on average, ranging from 6% to 16%, and a t-test of the duplicate measurements showed that there was no significant difference between the two sets of results ($t=3.18$, $p=0.021$).

Similar results were obtained for the selenium content of the duplicate soil samples, both topsoil and subsoil. The percentage difference between samples was

around 10% on average, ranging from 2% to 21%. A t-test on the duplicate measurements showed no significant difference between the two sets of results ($t=1.94$, $p=0.041$).

The soil samples obtained for analysis were the bulked samples of 9 subsamples taken from a 3 x 3 (6 m^2) grid (section 4.1.1). This method of sampling was chosen in order to provide a composite sample that was representative of the overall area and to remove the possibility of large variations in soil samples which may occur in a point sampling system.

On several occasions the 9 subsamples of soil were collected and analysed individually to gain an estimate of the variation present within this subsampling system. The results of the 9 analyses were averaged to obtain the elemental concentration for the overall sample.

The selenium concentrations of the 6 samples collected in this way, the average selenium content of the 9 subsamples, 95% confidence limits (1.96 σ) and percentage difference of these confidence limits from the average value are shown in Table 5.4. It can be seen from this table that the percentage differences are all in the range of 10% to 22%.

The analysis of both the duplicate sampling for soil and herbage and the subsampling for soil show that the maximum variation in the selenium content due to sampling is around 20%. This value is acceptable since sampling errors are invariably quite large in field surveys.

Table 5.4 **The variation in selenium content of the subsamples of soil taken for individual analysis**

Sample	Subsample Selenium μg/g	$\bar{x}$ μg/g	1.96 σ μg/g	Difference %
701 T	0.360 0.312 0.378 0.288 0.312 0.330 0.312 0.330 0.312	0.326	0.051	15.7
711 T	0.282 0.318 0.336 0.306 0.342 0.348 0.360 0.282 0.270	0.316	0.060	19.0
712 T	0.108 0.090 0.096 0.090 0.090 0.066 0.096 0.096 0.096	0.092	0.021	22.5
712 S	0.108 0.084 0.096 0.096 0.108 0.108 0.078 0.102 0.108	0.099	0.021	21.0
811 T	0.282 0.306 0.258 0.270 0.300 0.294 0.282 0.324 0.33	0.294	0.044	15.0
811 S	0.300 0.300 0.282 0.252 0.288 0.288 0.276 0.264 0.288	0.282	0.029	10.4

CHAPTER 6

THE RESULTS OF THE FIELD SAMPLING PROGRAMME

6.1 INTRODUCTION

A two year study of specific field sites in various areas of England and Wales was carried out during this research to investigate the effect of specific soil, plant and climatic factors on the uptake of selenium by pasture plants. Table 6.1 summarises the geology, soil type and total selenium content of the topsoil at each site studied.

The 16 sites which were chosen for sampling and the reasons for choosing them have been described in detail in Chapter 3. The geology, soil classification, land use, sward composition and drainage characteristics are all listed with the site descriptions. All the sites were visited every three months for two years so that a seasonal sampling programme could be carried out. The dates of each seasonal collection and the samples which were collected from each site are given in Table 6.2. Occasionally samples could not be collected, especially herbage samples, due to deep snow cover, reseeding of fields or, in the case of the Woburn sites (13-16), where other experimental work sometimes meant that the plots could not be disturbed. As Site 2 in North Wales was completely altered during drainage improvements at the farm halfway through the sampling programme, this site was replaced at the next visit by site 8, another poorly drained soil, and so consequently there are some samples missing for both site 2 and site 8.

The sampling and analytical methods used in this field investigation have been described in Chapter 4, and the analytical quality control procedures used for all methods of analysis have been discussed in detail in Chapter 5.

Where not reproduced in this chapter, the data obtained from the analysis of the field samples have been given in Appendix A.

Table 6.1 The soil type, geology and total soil selenium concentration of the sites sampled

Site No.	Area	Soil Type	Parent material	Total Soil Se* µg/g
1	N. Wales	Brown earth	Silurian Shale	0.329 ± 0.057
2	N. Wales	Stagnogleyic brown earth	Silurian Shale	0.183 ± 0.036
3	N. Wales	Brown podzolic soil	Silurian Shale	0.434 ± 0.069
4	N. Wales	Brown earth	Silurian Shale	0.200 ± 0.030
5	N. Wales	Ferric stagnopodzol	Silurian Shale	0.323 ± 0.056
6	N. Wales	Stagnohumic gley soil	Silurian Shale	0.717 ± 0.203
7	N.Wales	Stagnohumic gley soil	Silurian Shale	0.755 ± 0.149
8	N. Wales	Stagnogleyic brown earth	Silurian Shale	0.125 ± 0.011
9	Brecon	Brown earth	Old Red Sandstone	0.134 ± 0.081
10	Derbyshire	Non-calcareous pelosol	Marine Black Shale	1.363 ± 0.084
11	Derbyshire	Brown earth	Limestone	0.330 ± 0.042
12	Romney Marsh	Calcareous alluvial soil	Silt Alluvium	0.125 ± 0.085
13	Woburn	Brown earth	Devonian Sandstone	0.195 ± 0.054
14	Woburn	Brown earth	Devonian Sandstone	0.179 ± 0.062
15	Woburn	Brown earth	Devonian Sandstone	0.169 ± 0.037
16	Woburn	Brown earth	Devonian Sandstone	0.157 ± 0.052

* The mean selenium concentration of 8 seasonal samples +/- 95% confidence limits

Table 6.2 The samples collected during the two year sampling programme

Site No.	1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	T H	T H	T H	T H	T H	T H	T H	T H
2	T H	TSH	T H					
3	T	TSH	T H	T H	TS	TSH	TSH	TSH
4	T H	TSH	T HR	T HR	TSHR	TSHR	TSHR	TSH
5	T	TSH	T H	T H	TSH	TSH	TSH	TSH
6	T	TSH	T H	T H	TSH	TSHR	TSH	TSH
7	T	TSH	T H	T H	TSH	TSH	TSH	TSH
8					TSH	TSH	TSH	TSH
9	T	TSH	T HR	T HR	TSHR	TSH	TSH	TSH
10	T H	TSH	T H	T H	TSH	TSH	TSH	TSH
11	T H	TSH	T HR	T HR	TSHR	TSHR	TSHR	TSH
12	T H	TSH	T HR	T HR	TSHR	TSHR	TSHR	TSH
13	T		T	T R	TSHR	TSHR	TSHW	TS R
14	T		T	T	TSH	TSH	TSHW	TS
15			T	T	TSH	TSH	TSHW	TS
16			T	T	TSH	TSH	TSHW	TS

KEY: T = Topsoil H = Herbage
S = Subsoil R = Rainwater
W = Wheat

6.2 SELENIUM CONTENT OF THE SOILS AND HERBAGE

Table 6.3 shows the mean selenium concentrations of the soils and herbage sampled seasonally from all sites. The values are therefore seasonal averages and the seasonal variation is dicussed in section 6.3. The large values for the 95% confidence limits in the herbage samples are due to considerable seasonal variation in the selenium content of the herbage (see section 6.3). The original data for selenium in the field samples is given in the Appendix (Tables A1-A4).

The selenium content of the topsoil (0-15 cm) and the subsoil (15-30 cm) at each site is clearly correlated (r=0.926); Figure 6.1 shows the plot of selenium in the topsoil against selenium in the subsoil using the original data rather than the seasonal averages. From Figure 6.1 it can be seen that two groups of data lie away from the main set of results, these have been labelled on the plot. One group corresponds to Site 10 with high selenium levels in both topsoil and subsoil, and the other group to the peat soils of the North Wales moorland sites. The linear relationship between all the sites is shown by line 1 on the graph. However if the group of peat soils are removed from the data, the correlation becomes r=0.987 and this is shown by line 2 which lies closer to the points for Site 10 with high selenium concentrations. The peat soils show a marked difference between the selenium content of the topsoil and that in the subsoil, the topsoil containing higher concentrations of selenium. This difference is greater than for the brown earth soils at the other sites and consequently the moorland soils lie in a separate group in this plot.

The overall average from all the sites for selenium in the topsoil (0.387 μg/g Se) is slightly higher than that for the subsoil (0.349 μg/g Se). This has been found to be the case in other studies (eg. Thornton et al., 1983), and is presumably due to the association of selenium with organic matter, topsoil generally having the higher organic matter content. The selenium content of the underlying rock in some of the areas studied in this research has been measured previously by other workers in the department and these values are given in Table 6.4 (S. Van Dorst, pers. comm.).

Table 6.3 **The total selenium concentration of herbage, topsoil and subsoil at the field sites**

Site No.	Selenium in herbage (μg/g) *	Selenium in topsoil (μg/g) *	Selenium in subsoil (μg/g) *
1	0.094 ± 0.127	0.329 ± 0.057	--- ---
2	0.107 ± 0.072	0.183 ± 0.036	0.180 ---
3	0.059 ± 0.044	0.434 ± 0.069	0.413 ± 0.070
4	0.082 ± 0.091	0.200 ± 0.030	0.190 ± 0.037
5	0.212 ± 0.133	0.323 ± 0.056	0.403 ± 0.044
6	0.166 ± 0.167	0.717 ± 0.203	0.430 ± 0.073
7	0.130 ± 0.156	0.755 ± 0.149	0.428 ± 0.062
8	0.106 ± 0.153	0.125 ± 0.011	0.198 ± 0.276
9	0.093 ± 0.148	0.134 ± 0.081	0.113 ± 0.095
10	0.258 ± 0.390	1.363 ± 0.084	1.383 ± 0.143
11	0.135 ± 0.181	0.330 ± 0.042	0.306 ± 0.069
12	0.085 ± 0.072	0.125 ± 0.085	0.110 ± 0.011
13	0.114 ± 0.096	0.195 ± 0.054	0.206 ± 0.041
14	0.122 ± 0.032	0.179 ± 0.062	0.216 ± 0.040
15	0.093 ± 0.058	0.169 ± 0.037	0.191 ± 0.050
16	0.207 ± 0.104	0.157 ± 0.052	0.167 ± 0.026
Range	0.059 - 0.258	0.125 - 1.363	0.110 - 1.383

* The mean selenium concentration of 8 seasonal samples +/- 95% confidence limits

Figure 6.1 **The relationship between selenium concentration in the topsoil and subsoil**

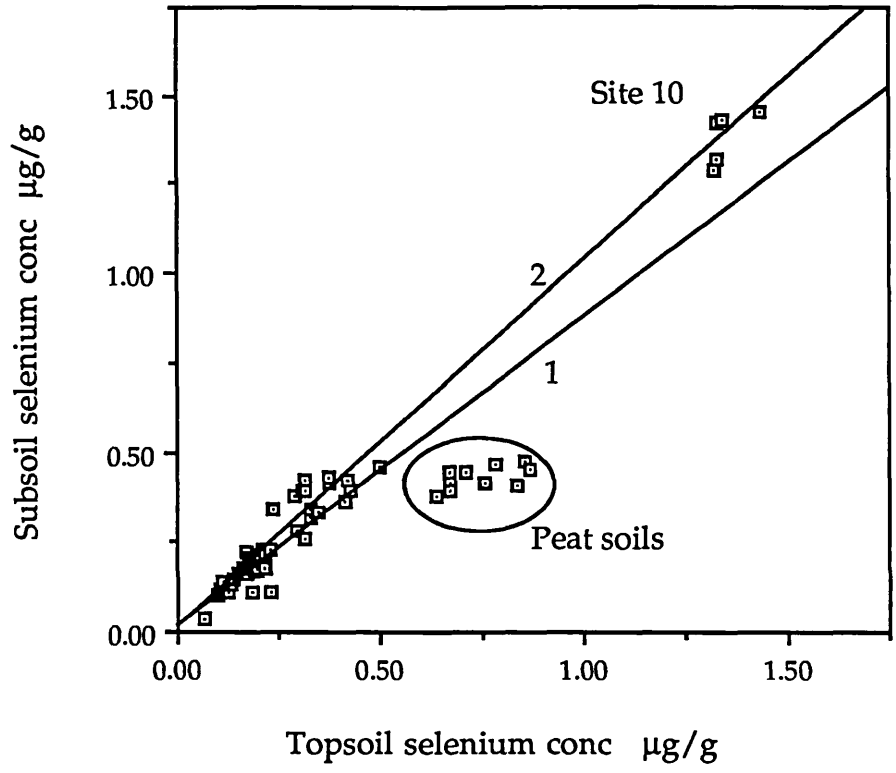


Figure 6.2 **The relationship between selenium concentration in the herbage analysed by spectrofluorimetry and ICPAES**

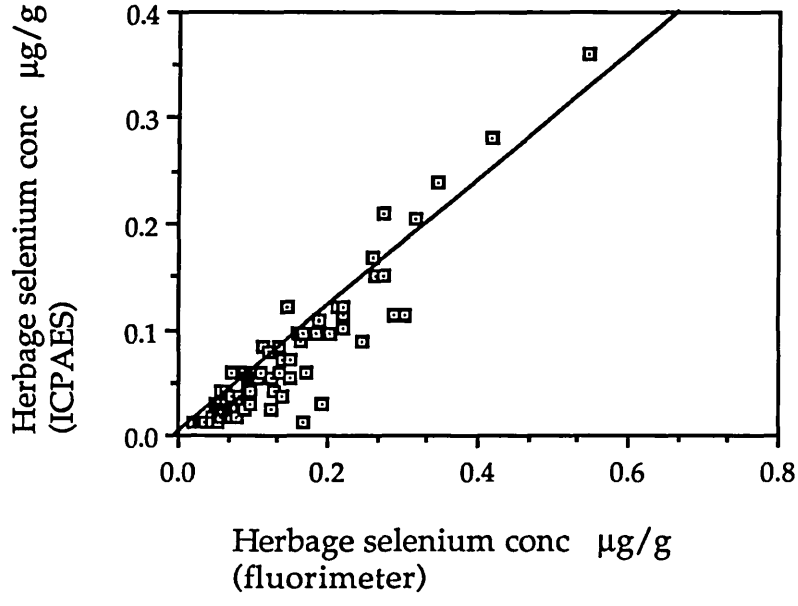


Table 6.4 The selenium concentration of the parent material at some field sites

Site	Se (μg/g)
Site 1-8	0.078 ± 0.042
Site 9	0.028 ± 0.011
Site 10	1.318 ± 0.213

For sites 1-9, the selenium levels in the parent materials are much lower than those in the corresponding soils, and it has been found that selenium is generally enriched in soils compared to their parent material (Smith, 1983). The accumulation of organic material in soils during their formation serves to increase the total soil selenium levels since organic matter tends to contain more selenium than the majority of parent materials.

However, the soil selenium content at site 10 (1.363 μg/g Se, topsoil) is very similar to that of the parent material (1.318 μg/g Se). The parent material at this site is a marine black shale, which has a high selenium concentration due to the accumulation of organic material in the shale during its formation. Consequently, the soil formed on this parent material has a higher selenium content derived from the parent material than the other soils, and the incorporation of organic matter in the soil will not further increase the selenium levels.

The enrichment of selenium associated with organic matter tends to continue up the profile, with the topsoil having the greatest selenium content unless leaching or podzolisation conditions are prevalent. Sites 6 and 7 on the peat soils of the Hireathog in North Wales have a considerably higher selenium content in the topsoil than the subsoil due to the exceptionally high organic matter content of the topsoil. This highlights the selenium accumulation in the surface layers associated with organic matter accumulation which is the general case in these field sites.

However some exceptions to this trend are found in site 5, 8 and 13-16. Site

5 on the North Welsh moorland has a higher selenium content in the subsoil (0.403 μg/g Se) than the topsoil (0.323 μg/g Se) due to the association of selenium with the iron pan just below the subsoil (see section 2.3). Site 8 also has a slightly higher selenium content in the subsoil (0.198 μg/g Se) than the topsoil (0.125 μg/g Se). This may be due to the waterlogged conditions at this site, which could cause the selenium to be transported to the lower layers of the soil, where it becomes associated with iron oxides and clay minerals in the subsoil. Or the selenium may be present as insoluble, and therefore persistent, selenides or elemental selenium in the more reducing conditions of the waterlogged subsoil. The subsoil at sites 13-16 is also a little higher in selenium than the topsoil which is probably due to slight leaching from this very sandy soil causing some depletion of selenium from the topsoil.

The herbage selenium content at the field sites was analysed using both spectrofluorimetry and ICPAES and although the correlation between the two methods was good (r=0.877 Figure 6.2), the values obtained from the spectrofluorimetric analysis were used throughout the data analysis because this method was shown to be more accurate, precise and to have better detection limits, and was therefore more suitable for low level selenium analysis than ICPAES (Chapter 5).

Selenium in herbage did show a positive correlation with the selenium content of the soils, although this is not a particularly strong correlation and the variation of selenium in herbage is not fully described by the differences in soil selenium levels. The correlations of selenium in herbage (using the results from both analytical methods), topsoil and subsoil are given in Table 6.5 and Figures 6.3 - 6.6 show the corresponding graphs.

Table 6.5 **The correlation coefficients (r) between herbage and soil selenium concentrations**

	Se topsoil	Se subsoil
Se herbage (fluorimetry)	0.405 p<0.001	0.396 p<0.005
Se herbage (ICPAES)	0.485 p<0.001	0.479 p<0.005

In 1983, MAFF obtained a correlation of r = 0.12 between soil and herbage total selenium concentrations and concluded that there was little relationship between the soil selenium concentrations and the selenium concentration of the herbage growing on the soil. However the results in this research do provide evidence of a positive significant correlation (r=0.405 p<0.001) between soil and herbage total selenium levels.

Figure 6.3 **The relationship between selenium concentration in the topsoil and selenium concentration in the herbage analysed by spectrofluorimetry**

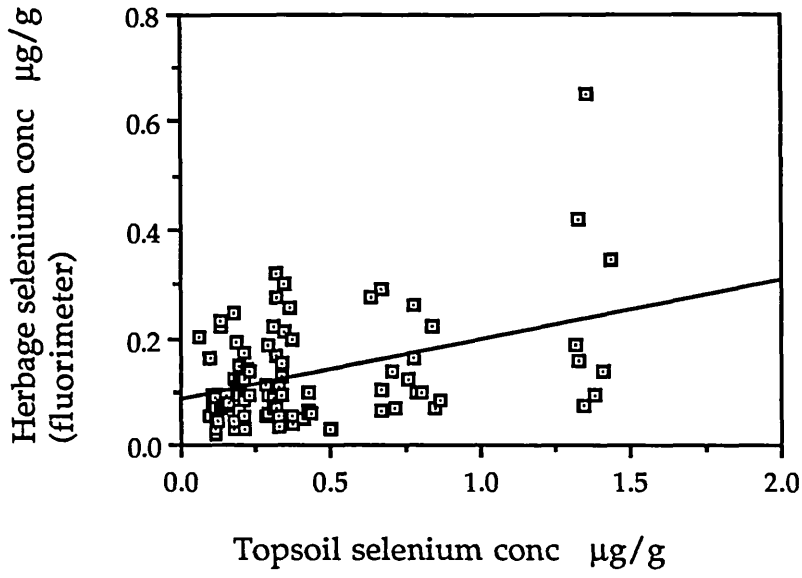


Figure 6.4 **The relationship between selenium concentration in the topsoil and selenium concentration in the herbage analysed by ICPAES**

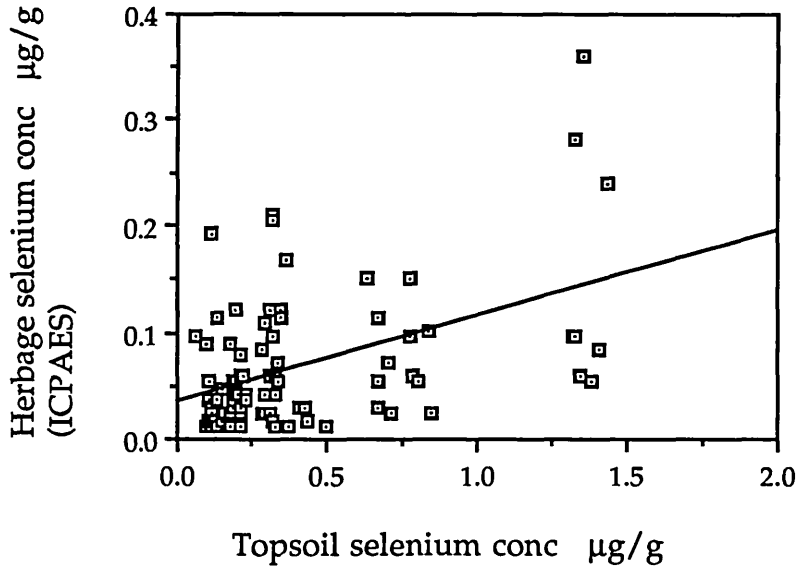


Figure 6.5 **The relationship between selenium concentration in the subsoil and selenium concentration in the herbage analysed by spectrofluorimetry**

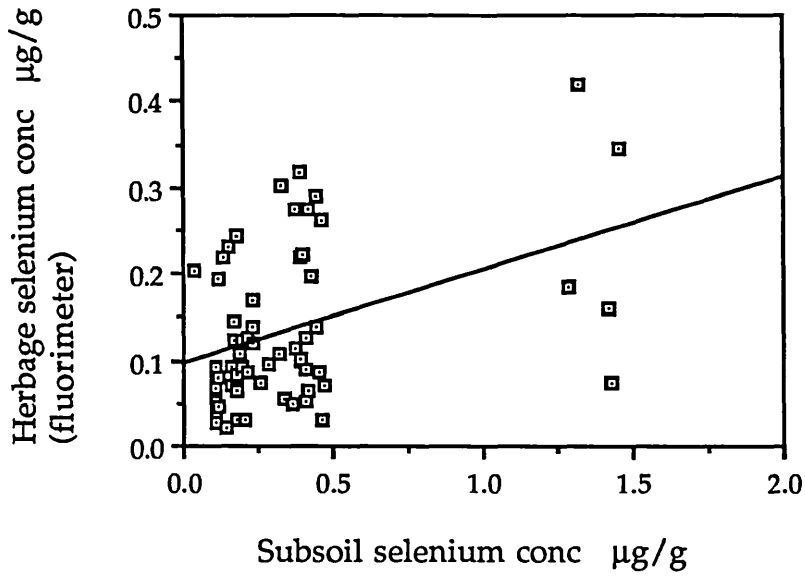
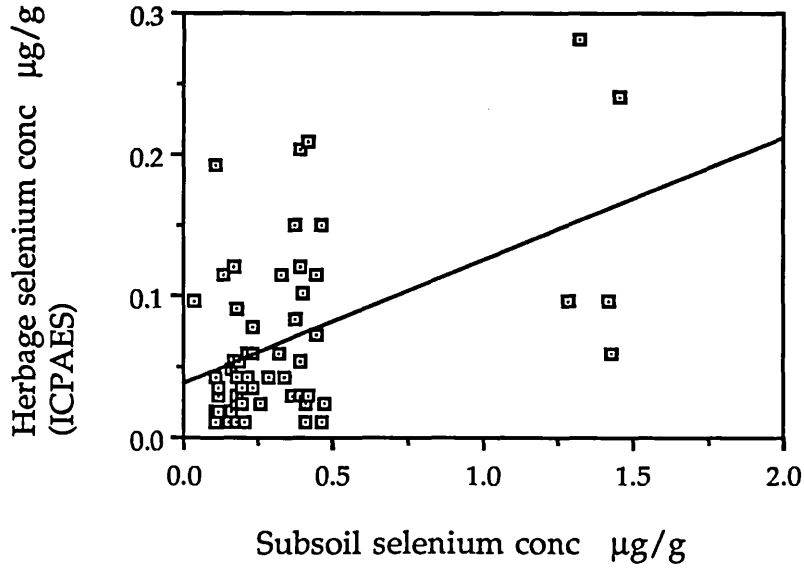


Figure 6.6 **The relationship between selenium concentration in the subsoil and selenium concentration in the herbage analysed by ICPAES**



It can be seen from Table 6.3 that the levels of selenium in herbage are invariably lower than levels of selenium in the soil, although the proportion in these field herbages varies from about 20% to over 80% of that of the topsoil at different sites. At site 10 in Derbyshire for example, the soil selenium concentration is high (1.363 μg/g Se), the soil being derived from marine black shale and although the herbage selenium concentration is the highest of the sites studied (0.257 μg/g Se), it is very close to the herbage concentration of 0.212 μg/g Se found at site 5 in North Wales which had a soil selenium concentration of 0.323 μg/g Se, about 20% of that at site 10. This suggests that herbage growing on soils of low total selenium content may accumulate a greater percentage of the total soil selenium than herbage growing on a soil with higher selenium levels. To illustrate this point further, Table 6.6 shows the herbage selenium concentration expressed as a percentage of both the topsoil and the subsoil selenium content at each site. This is not a measure of uptake since it takes no account of growth rate but gives an indication of the variation in 'availability' of the soil selenium to the herbage under different soil conditions.

The other two columns in Table 6.6 show that, in general, the lower the selenium concentration of the soil the greater the percentage of soil selenium in the herbage. The values of selenium in topsoil have been coded from 1 to 16 in decreasing order and the selenium content of the herbage as a percentage of the topsoil selenium concentration has also been coded from 1 to 16 but in ascending order. It can be seen from the table that the order of the two sets of numbers is generally similar. This leads to the suggestion that plants growing on a soil low in selenium absorb proportionally more selenium from the soil than plants growing on a soil containing higher selenium levels.

Table 6.6 **The selenium concentration of herbage expressed as a percentage of the selenium concentration in the soil at each site**

Site No.	% topsoil Selenium in herbage	% subsoil Selenium in herbage	Se in topsoil (coded)	% Se in herbage (coded)
1	28.4	—	6	5
2	58.5	59.4	10	9
3	13.6	14.3	4	1
4	41.0	43.2	8	7
5	65.6	52.6	7	11
6	23.2	38.6	3	4
7	17.2	30.4	2	2
8	84.8	53.5	15	15
9	69.4	82.3	14	14
10	18.9	18.6	1	3
11	40.1	44.1	5	6
12	68.0	77.3	15	12
13	58.5	55.3	9	9
14	68.2	56.5	11	13
15	55.0	48.7	12	8
16	132	124	13	16

Figure 6.7 The uptake of soil selenium by herbage as a function of soil selenium concentration

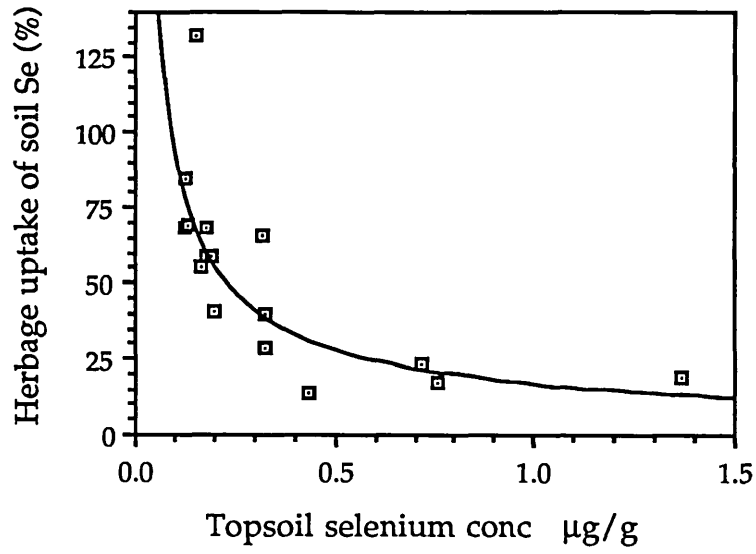


Figure 6.7 shows the exponential relationship between the uptake of soil selenium by plants and soil selenium concentration, from soils of varying selenium concentrations.

Active uptake of selenium by plants to fulfil their nutritional requirements would explain this difference in absorption of selenium from different soils; however neither active uptake, nor a nutritional requirement for selenium by plants has ever been proved. Differing soil factors, chemical and/or physical, are the more obvious explanation for the difference in selenium availability and this research has attempted to delineate the soil factors which affect the uptake of soil selenium into herbage. These factors are discussed in the following sections.

6.3 SEASONAL VARIATION IN TRACE ELEMENT CONCENTRATIONS

It was considered that the seasonal changes in trace element concentrations and other soil and climatic factors could play an important part in the variation of selenium uptake into herbage. Little is known about the amount of variation in selenium concentration which can occur with the seasons, and for many field sampling surveys the collections are only carried out during the summer months which could produce rather biased results if seasonal variations are shown to be large.

The sampling programme undertaken for this research involved collecting soil and herbage samples seasonally, four times a year, over two years.

For each element concentration, or other measurement, that was made on the samples, the results of the first years sampling have been compared with those of the second year using paired t-tests and one-way analysis of variance (ANOVA). Where there was no significant variation ($p<0.05$) between the two years samplings the results for the two years have been combined and the data analysed using one-way ANOVA in order to detect any factors which have statistically significant differences across the seasons.

The data was studied to ensure that it was normally distributed by plotting histograms and the plots of N-scores against the original values. For much of the herbage elemental data the distribution was skewed and this was corrected by a logarithmic (base 10) data conversion. Both the original data and the logged data were used for statistical analysis and any differences have been reported. The soil data often fell into 2 or 3 groups or populations, each approximately normally distributed. This was perhaps to be expected from the sampling regime of a detailed survey of a few areas, the groups are merely the populations from within each area of the country that was sampled.

6.3.1 Variation between years

a) Soil

Of the 25 elements analysed in the soil together with the other properties determined (pH etc.), the only soil factors which showed any statistical differences between the two years were lithium and phosphorus in the topsoil and aluminium in the subsoil (see Appendix A). For all three sets of data the variation was only just significant (at $p<0.05$) and for lithium and phosphorus, one-way ANOVA did not detect any significant difference between the years but the paired t-test did; for aluminium the t-test did not detect any differences but the one-way ANOVA did. From this it was concluded that there was large overall variation in these measurements leading to some statistical tests finding significant differences, and that no strong annual or long-term changes were occurring. No seasonal variation was detected for these factors, which were anyway of peripheral importance in the overall research.

b) Herbage

The trace element concentrations in herbage which showed a significant difference ($p<0.05$) between the two years samplings were Se, Ti, Li, Al, V, Cr, Pb, Fe, Ca, Sr, Ni and Cu (see Appendix). The results of the paired t-tests for the element variation in herbage between the years, for each element showing significant variation, are given in Table 6.7. All of these trace elements, except Ca, Sr, Ni, and Cu, also show seasonal variation in the herbage concentrations. For those factors which show seasonal variation, this variation between the years reflects the variation of the seasons in these two years; the spring in 1987 was extremely late and was followed by a rather better summer than in 1986. It is clear that factors which are related to the climatic conditions, whether due to soil contamination or plant growth rate, will vary between two years of rather different weather patterns.

The significant variation between the years shown for the other four elements Ca, Sr, Ni and Cu cannot easily be explained. For Sr and Ni, the concentrations measured are close to the detection limit of the analytical method

and so large random errors in the measurements would be expected and may account for the significant differences between the years. However for Ca and Cu, the reason for the differences between the years is not known. This study was too limited to detect long term changes in trace element concentrations.

Table 6.7 The results of the paired t-tests for the element variation in herbage between years

Element	t-value	Probability (p)
Se	3.60	0.001
Ti	2.30	0.029
Li	2.55	0.016
Al	2.67	0.012
V	3.11	0.004
Cr	2.60	0.015
Pb	2.28	0.030
Fe	2.42	0.022
Ca	2.81	0.009
Sr	4.92	0.000
Ni	2.06	0.049
Cu	3.36	0.002

6.3.2 Variation between seasons

a) Soil

None of the soil factors measured in this research showed any significant ($p<0.05$) seasonal variation using either one-way ANOVA or t-tests. Even the moisture content of the soils showed no significant variation with season which is surprising considering the large variation in rainfall through the year. Moisture content of the soil is, of course, very dependent on the weather conditions immediately before the collection times and therefore a very changeable variable, however the stability of the measured soil moisture content suggests that the soils studied have a strong capacity to maintain the moisture status of the soil.

b) Herbage

The use of ANOVA and paired t-tests revealed significant ($p<0.05$) seasonal variation in the following trace element concentrations in herbage; Se, Li, Na, K, Al, V, Ti, Fe, Pb, Mg, Mo and Cr (see Appendix). Of these, Se, Li, Al, V, Fe, Pb and Cr were found to correlate strongly with Ti in herbage (see Table 6.8). Titanium is not taken up by plants and so any titanium measured in the herbage will be due to soil contamination (Cherney et al., 1983). For this reason titanium can be used as an indicator for soil contamination in the herbage. The herbage trace elements listed above which are correlated with titanium in herbage are therefore assumed to be affected by soil contamination. The elements showing seasonal variation which have no correlation with herbage titanium levels are Na, K, Mg and Mo, all essential nutrients for plants. The titanium correlated elements all show some increase in concentration during the autumn and winter samplings due to extra soil contamination in these conditions. The other elements (Na, K, Mg and Mo) are seen to increase slightly during the summer months and are presumably linked to plant growth rate. The correlation matrix of Table 6.8 clearly shows these two groups of elements.

An estimation of soil contamination can be given by expressing the herbage titanium concentration as a percentage of the soil titanium concentration (Mitchell, 1960). These percentages are given in Table 6.9 and the seasonal

variations in soil contamination are shown in Figures 6.7-6.9. The herbage collected from the field was washed extremely thoroughly with DIW and the estimate of soil contamination given here is for the residual soil which could not be removed by the washing procedure.

Table 6.8 The correlation matrix of those trace elements in herbage which show seasonal variation

	Se	Ti	Li	Al	V	Fe	Pb	Cr	Na	K	Mg	Mo
Se	1.00											
Ti	0.59*	1.00										
Li	0.61*	0.86*	1.00									
Al	0.57*	0.88*	0.97*	1.00								
V	0.78*	0.88*	0.92*	0.93*	1.00							
Fe	0.47*	0.85*	0.88*	0.94*	0.82*	1.00						
Pb	0.68*	0.74*	0.56*	0.54*	0.66*	0.53*	1.00					
Cr	0.61*	0.90*	0.93*	0.95*	0.92*	0.87*	0.61*	1.00				
Na	-0.34*	0.02	-0.24*	0.01	-0.09	0.01	-0.21	-0.08	1.00			
K	-0.57*	-0.24*	-0.26*	-0.28*	-0.43*	-0.25*	-0.44*	-0.32*	0.42*	1.00		
Mg	-0.37*	0.07	0.13	0.15	-0.06	0.15	-0.25*	0.05	0.61*	0.50*	1.00	
Mo	-0.21	-0.19	-0.16	-0.22	-0.29*	-0.27*	-0.30*	-0.25*	0.15	0.37*	0.45*	1.00

* Significant correlation ($p<0.05$)

Table 6.9 **The estimated percentage soil contamination on herbage (herbage Ti concentration expressed as a percentage of soil Ti concentration)**

	Sampling Date							
Site No.	1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	5.55	---	1.33	1.23	4.73	0.79	0.71	1.33
2	1.51	1.21	1.74	---	---	---	---	---
3	---	1.45	0.87	1.25	---	0.39	0.75	1.36
4	2.54	2.68	1.04	0.98	4.80	0.53	0.79	4.60
5	---	1.10	0.57	0.95	2.97	0.91	0.55	---
6	---	1.73	1.03	1.70	1.96	0.59	1.55	---
7	---	1.67	0.71	1.30	4.06	1.05	1.24	---
8	---	---	---	---	6.40	2.00	1.32	2.58
9	---	1.33	---	0.39	6.82	0.35	0.31	0.86
10	8.16	7.03	0.64	1.42	7.82	0.53	0.53	1.11
11	6.22	2.72	0.91	1.41	9.18	0.43	0.44	1.91
12	2.66	4.66	1.23	3.87	2.75	0.81	0.83	2.80
13	---	---	---	---	1.81	0.60	0.78	---
14	---	---	---	---	1.70	0.53	0.55	---
15	---	---	---	---	1.52	0.65	0.61	---
16	---	---	---	---	2.28	0.8	0.67	---

In general the level of soil contamination is low (0 - 10%), with the lowest values occurring in summer and autumn when the grass is growing freely and rainfall is lower, and the higher values during winter and spring when the grass is short and easily contaminated by rain-splash and livestock trampling. The spring in 1986 was wetter prior to sampling than in 1987 which accounts for the higher level of soil contamination in spring 1986 at some sites.

Figure 6.8 Seasonal variation in soil contamination of herbage at Sites 1 & 4

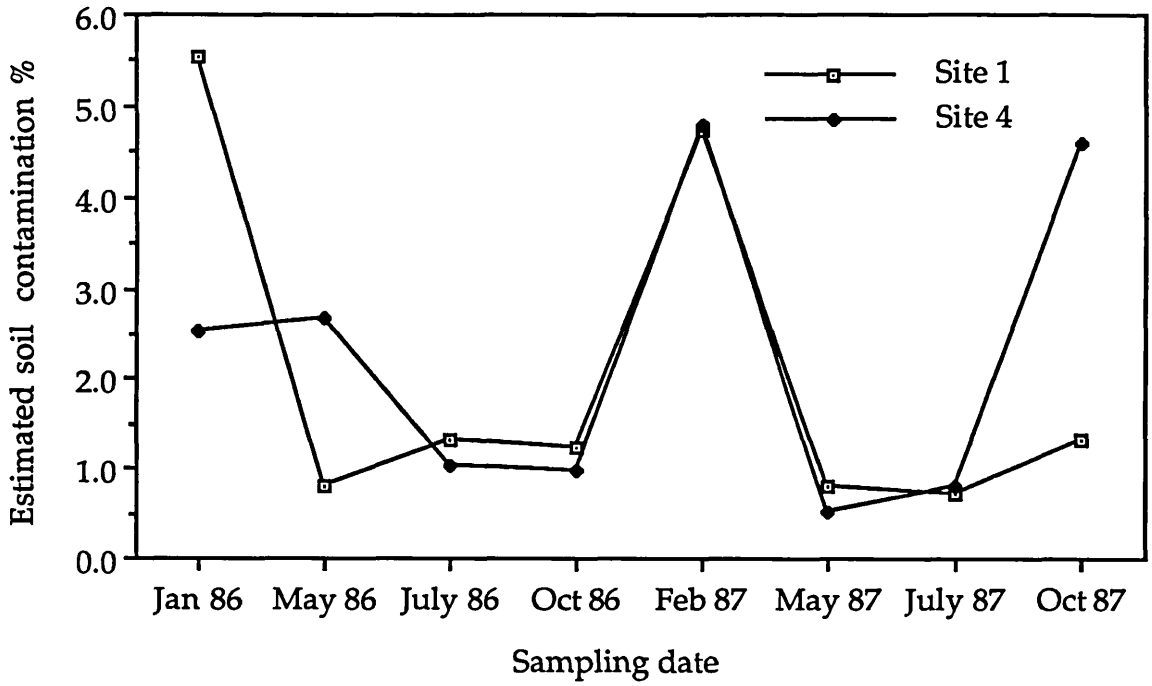


Figure 6.9 Seasonal variation in soil contamination of herbage at Sites 9 & 10

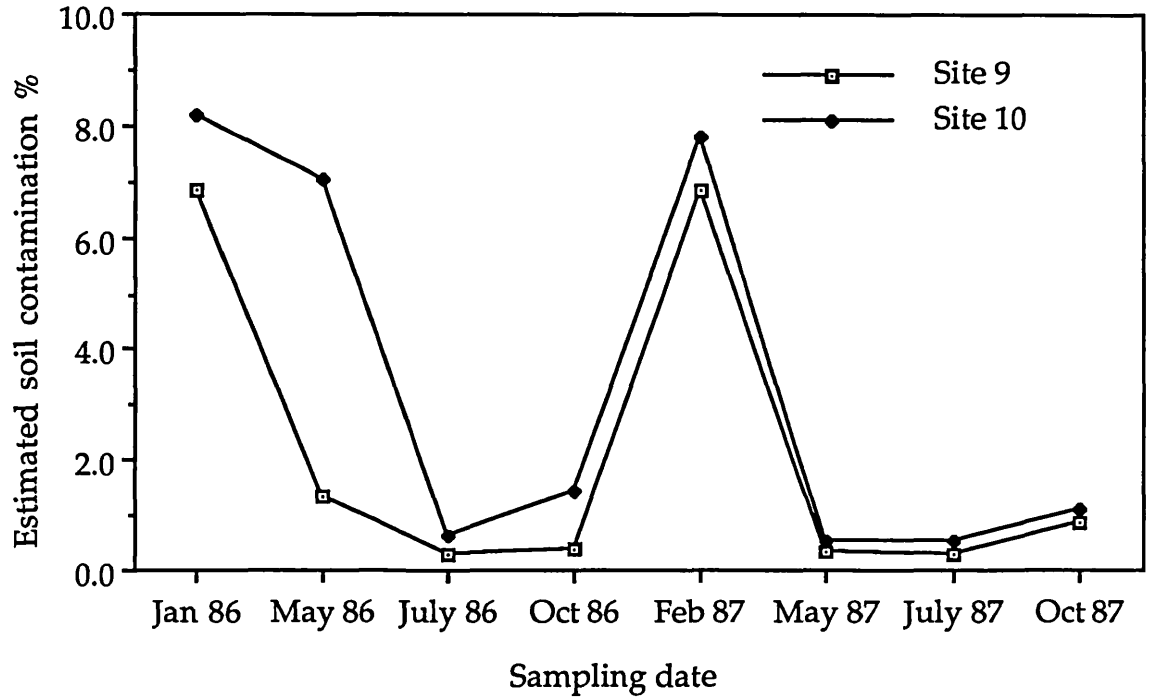
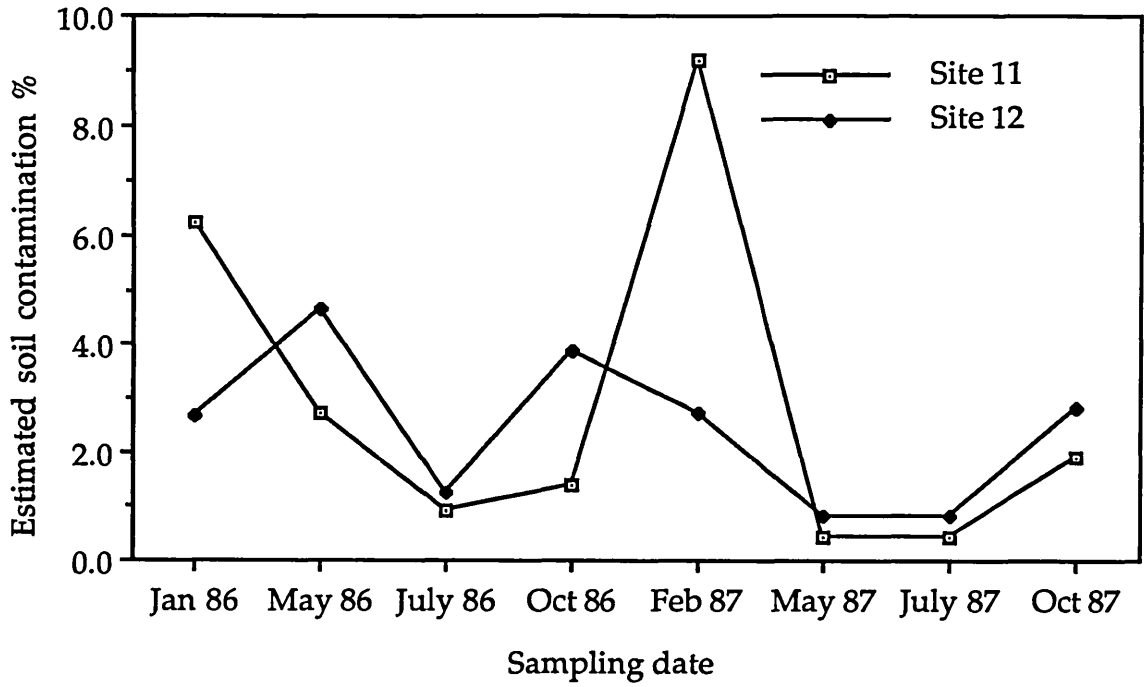


Figure 6.10 Seasonal variation in soil contamination of herbage at Sites 11 & 12



Although the percentage soil contamination of herbage (by weight) may be small, the elemental concentrations in soil are generally greater than those in herbage (especially where the soil selenium concentration is high, eg. Site 10), and so a small amount of soil contamination could give rise to a proportionally larger increase in the measured elemental content of the herbage. By using the estimate of soil contamination (Table 6.9) , the amount of selenium in the herbage attributed to soil contamination has been calculated (Table 6.10) and this has also been given as a percentage of the measured herbage selenium concentration (Table 6.11). Site 10 on the marine black shale in Derbyshire has the highest percentage of herbage selenium concentration due to soil contamination (29% max.) although the level of soil contamination is not particularly high (8% max.). This is because this site has high soil selenium levels and so the soil contaminates the herbage with a proportionally greater amount of selenium.

The selenium content of the herbage has been corrected to remove the amount estimated to be present due to soil contamination and these corrected values are given in Table 6.12. From this table it can be seen that seasonal differences are still obvious in the selenium content of herbage, with lower levels in summer than winter and spring. This seasonal difference is still significant (ANOVA, $F=10.0$, 3 d.f., $p<0.05$) and therefore the seasonal variation is not just explained by soil contamination. During the summer when the herbage is growing strongly, the selenium concentration drops considerably, probably due to a dilution effect where the selenium uptake does not increase with increase in plant growth rate. Whether any other seasonal changes in temperature or soil microbial activity affect the seasonal variation in selenium uptake by herbage is not known. Figures 6.10-6.15 show some examples of the seasonal variation in herbage selenium levels, with and without correction for soil contamination.

It can be seen from these graphs that the residual soil contamination does not appreciably alter the selenium content of the herbage throughout the seasons. The only example where a large proportion of the measured herbage selenium is derived from the soil contamination is at Site 10 which has a high soil selenium content. This proportion of selenium from soil contamination increases slightly in winter due to the higher rates of soil contamination at this time of year.

Table 6.10 **The selenium concentration (ng/g) of herbage attributed to soil contamination**

	Sampling Date							
Site No.	1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	19	---	4.3	3.5	16.2	3.0	2.3	3.9
2	2.9	2.0	3.4	---	---	---	---	---
3	---	6.2	3.8	5.4	---	19	3.1	5.1
4	5.6	5.5	2.2	1.8	9.5	0.9	1.7	8.7
5	---	3.5	1.8	2.8	9.4	2.8	1.6	---
6	---	11	6.6	13	13	4.2	13	---
7	---	14	5.0	10	32	7.1	8.3	---
8	---	---	---	---	8.4	2.4	1.7	3.1
9	---	2.5	---	0.4	4.5	0.4	0.3	1.3
10	110	101	8.8	20	104	7.1	7.0	15
11	23	9.0	2.9	4.8	32	1.4	1.4	3.2
12	2.6	11	1.7	4.3	3.0	0.9	0.8	3.0
13	---	---	---	---	3.9	1.3	1.7	---
14	---	---	---	---	3.9	1.1	1.0	---
15	---	---	---	---	2.7	1.3	0.9	---
16	---	---	---	---	4.1	1.5	0.9	---

Table 6.11 The percentage of herbage selenium content attributed to soil contamination

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	8.8	---	6.3	6.3	10.7	8.1	6.9	6.6
2	2.0	2.5	3.7	---	---	---	---	---
3	---	6.4	6.5	8.2	---	6.1	6.2	9.5
4	3.8	4.3	4.0	3.8	7.7	2.9	5.4	9.4
5	---	1.3	1.1	1.5	3.0	1.3	1.4	---
6	---	4.0	6.6	8.0	4.5	2.0	2.3	---
7	---	6.3	7.2	10.3	12.1	6.9	12.8	---
8	---	---	---	---	3.8	3.6	3.7	3.5
9	---	1.3	---	0.7	2.2	1.9	1.1	1.8
10	16.9	2.92	9.2	14.7	24.8	9.7	3.8	9.4
11	8.9	8.2	3.3	5.2	10.5	2.5	1.9	3.3
12	1.6	11.8	2.4	4.5	3.7	2.2	1.5	3.8
13	---	---	---	---	2.3	1.5	2.0	---
14	---	---	---	---	2.8	0.9	0.9	---
15	---	---	---	---	2.2	2.0	1.0	---
16	---	---	---	---	1.7	1.0	0.4	---

Table 6.12 The selenium concentration (μg/g) of herbage corrected to remove the contribution from soil contamination

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	0.196	---	0.064	0.052	0.135	0.034	0.031	0.055
2	0.146	0.077	0.089	---	---	---	---	---
3	---	0.091	0.055	0.060	---	0.029	0.047	0.049
4	0.140	0.122	0.053	0.045	0.115	0.030	0.030	0.084
5	---	0.271	0.158	0.185	0.309	0.217	0.113	---
6	---	0.265	0.093	0.149	0.227	0.136	0.058	---
7	---	0.209	0.065	0.087	0.232	0.096	0.056	---
8	---	---	---	---	0.212	0.065	0.045	0.086
9	---	0.192	---	0.057	0.200	0.021	0.276	0.071
10	0.540	0.245	0.087	0.116	0.315	0.066	0.178	0.145
11	0.236	0.100	0.086	0.088	0.272	0.055	0.074	0.093
12	0.016	0.083	0.070	0.092	0.079	0.040	0.052	0.076
13	---	---	---	---	0.167	0.085	0.083	---
14	---	---	---	---	0.135	0.122	0.106	---
15	---	---	---	---	0.122	0.064	0.091	---
16	---	---	---	---	0.241	0.145	0.230	---

Figure 6.11 **Seasonal variation in herbage Se concentration at Site 1 before and after correction to remove the contribution from soil contamination**

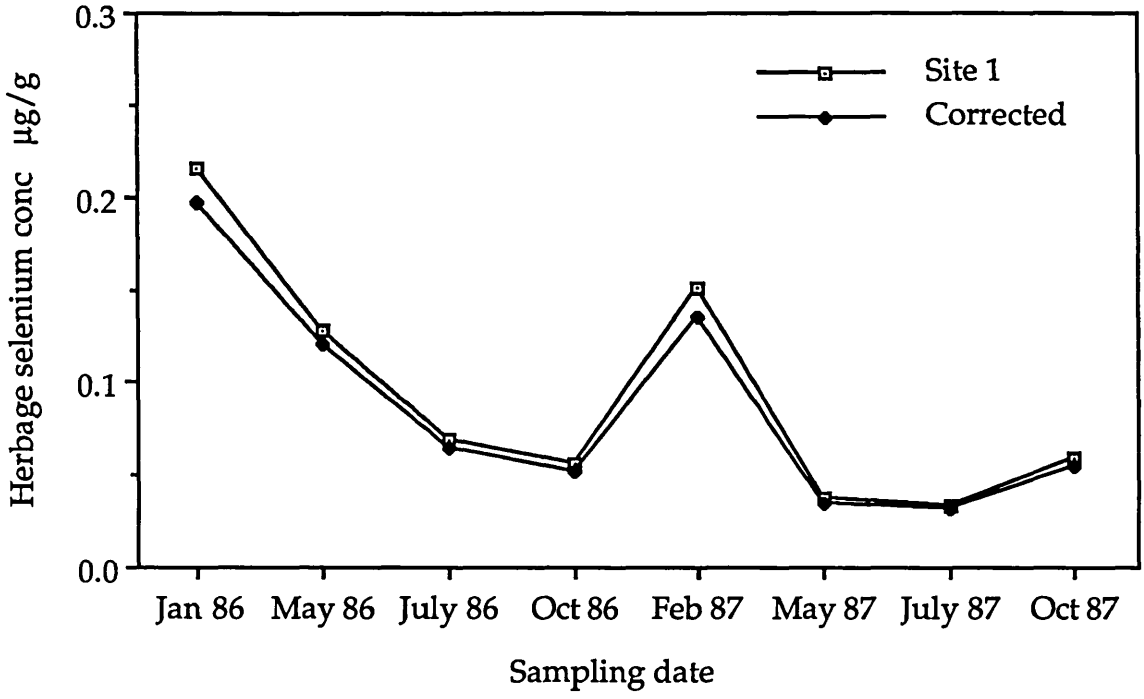


Figure 6.12 **Seasonal variation in herbage Se concentration at Site 4 before and after correction to remove the contribution from soil contamination**

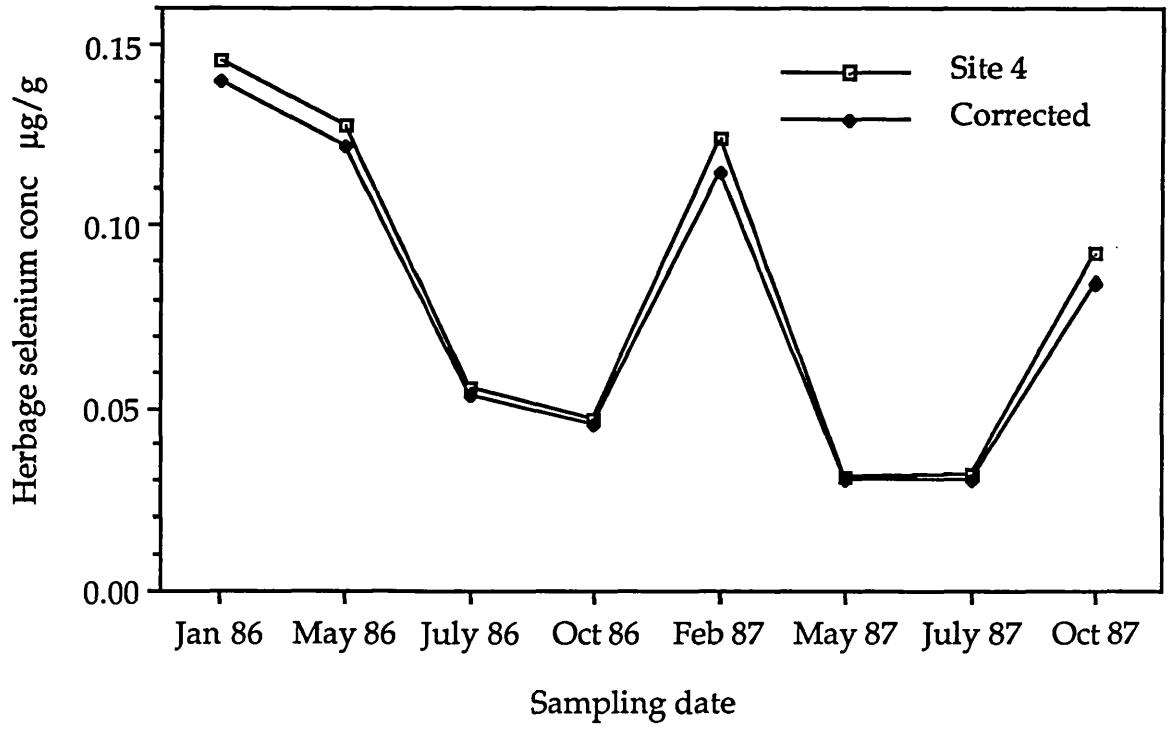


Figure 6.13 Seasonal variation in herbage Se concentration at Site 9 before and after correction to remove the contribution from soil contamination

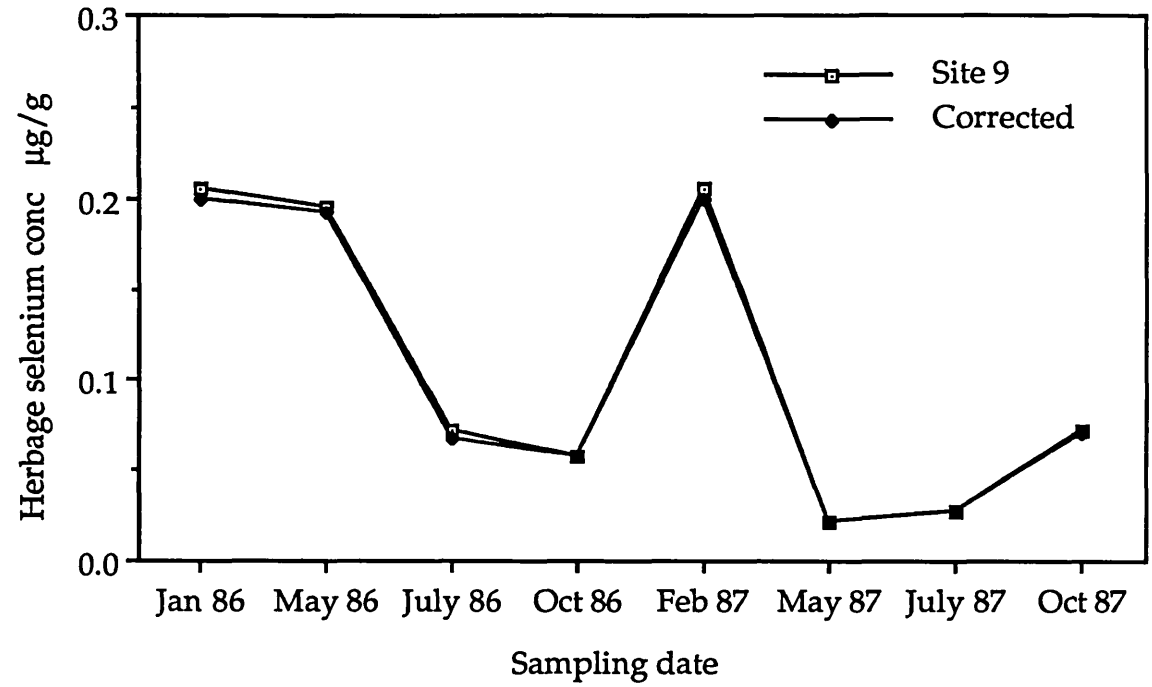


Figure 6.14 Seasonal variation in herbage Se concentration at Site 10 before and after correction to remove the contribution from soil contamination

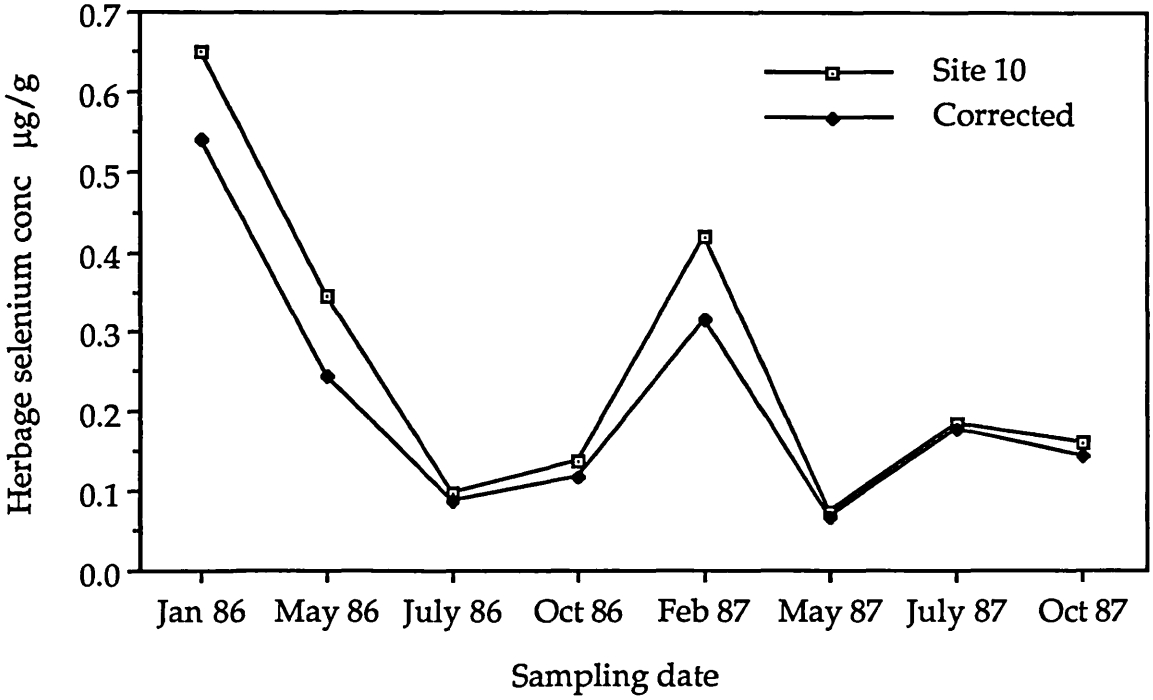


Figure 6.15 Seasonal variation in herbage Se concentration at Site 11 before and after correction to remove the contribution from soil contamination

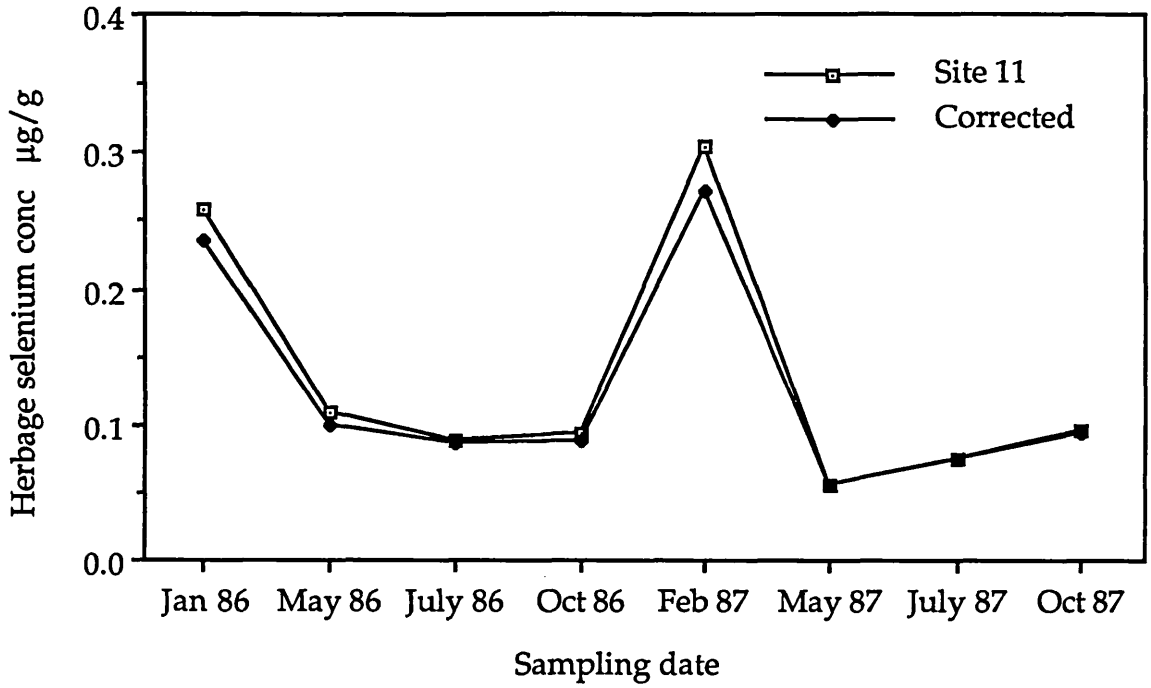
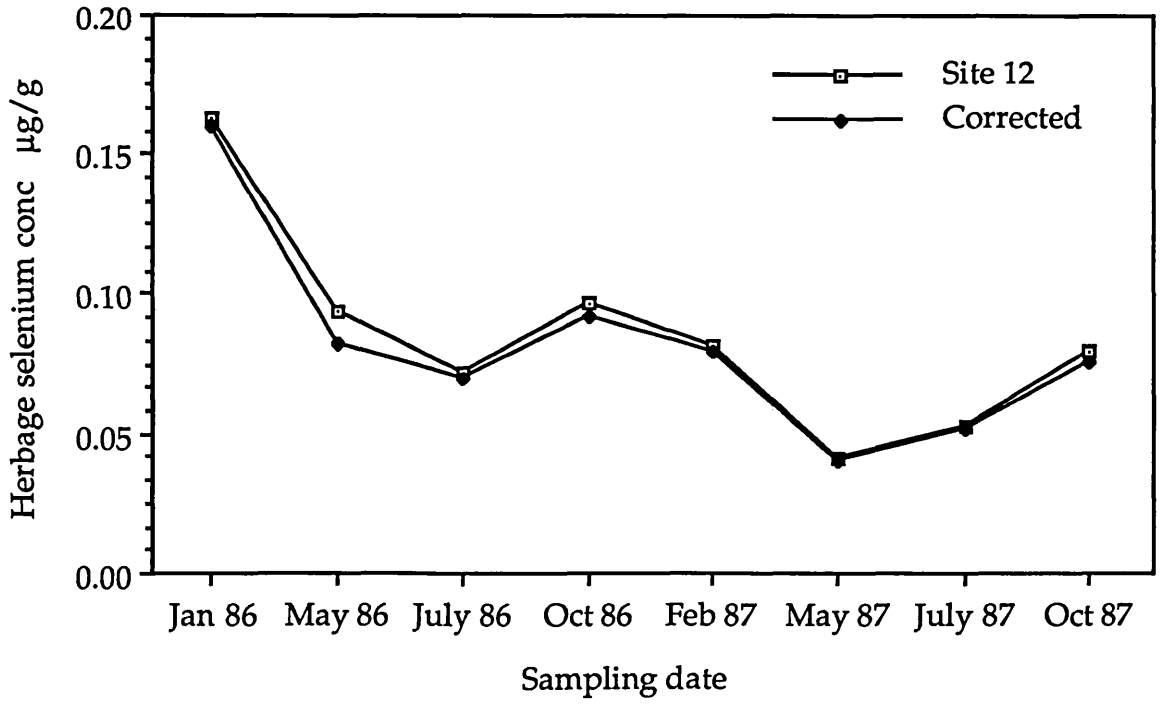


Figure 6.16 Seasonal variation in herbage Se concentration at Site 12 before and after correction to remove the contribution from soil contamination



6.4 SOIL FACTORS INFLUENCING THE SELENIUM CONTENT OF SOIL AND HERBAGE

The soil factors measured in this research which have some influence on the selenium content of soil and herbage include soil pH, soil organic matter content, soil particle size especially clay content, soil iron content and the pyrophosphate extractable iron fraction, soil sulphur content, cation exchange capacity of the soil and soil moisture content. The relationship of each of these with the measured selenium concentrations of soil and herbage is discussed individually below. The influence of climatic conditions and rainfall at each site is also considered.

6.4.1 The Influence of Soil pH on Selenium in Soil and Herbage

Measurements of pH were made on the collected topsoils and subsoils in both DIW and $CaCl_2$ solution (see section 4.3.3). The measurements made using $CaCl_2$ solution produced lower results in each case than with DIW but there was excellent correlation between the two methods for topsoil ($r= 0.991$ Figure 6.17) and subsoil ($r= 0.997$ Figure 6.18). The topsoil and subsoil measurements also correlated well with each other ($r= 0.995$ Figure 6.19) for both methods. No significant seasonal or annual variation was found in the soil pH measurements (section 6.3.2). The complete set of topsoil pH measurements (DIW) is given in Table 6.13, and the average values and standard deviations (σ) obtained from both methods of measuring topsoil and subsoil pH are given in Table 6.14.

Selenium in the herbage shows a significant ($p<0.05$) negative correlation with topsoil pH ($r= -0.215$ Figure 6.20) and subsoil pH ($r= -0.285$ Figure 6.21). The selenium concentration in the soil shows a stronger negative correlation with the soil pH levels, $r= -0.401$ (Figure 6.22) for topsoil and $r= -0.284$ (Figure 6.23) for the subsoil. Table 6.15 shows the correlation matrix for soil pH and selenium in soil and herbage.

Table 6.13 The pH measurements (DIW) of all the topsoil samples

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	5.63	5.69	6.16	6.12	5.92	6.08	—	6.31
2	5.44	5.44	5.78	—	—	—	—	—
3	5.83	6.18	5.82	6.06	6.06	6.15	6.14	6.16
4	5.19	5.21	5.11	5.21	5.40	5.32	5.42	5.26
5	3.93	4.12	3.98	4.01	4.02	4.10	4.12	4.17
6	4.02	3.96	4.15	4.00	3.93	4.00	3.98	4.13
7	4.07	4.19	4.43	4.11	4.13	4.23	4.05	4.32
8	—	—	—	—	5.65	5.58	5.54	5.69
9	5.96	6.03	5.81	5.94	6.18	5.76	5.95	6.02
10	5.67	5.73	5.55	5.73	5.81	5.77	5.67	5.76
11	5.73	5.91	5.63	5.87	5.44	5.91	—	—
12	7.88	7.97	7.68	8.03	7.98	7.84	8.08	8.18
13	7.43	—	7.38	7.26	7.35	7.02	7.25	7.18
14	4.16	—	4.01	4.13	4.24	4.03	4.19	4.35
15	—	—	5.82	6.06	6.34	6.02	6.10	6.29
16	—	—	5.12	4.99	5.21	5.04	4.91	5.19

Table 6.14 **The average pH values of the topsoil and subsoil samples from each site (DIW and $CaCl_2$) with their standard deviations**

Site No.	DIW Method				$CaCl_2$ Method			
	Topsoil pH		Subsoil pH		Topsoil pH		Subsoil pH	
	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.
1	5.99	0.25	---	---	5.50	0.20	---	---
2	5.55	0.19	5.49	---	4.91	0.19	4.69	---
3	6.05	0.14	6.19	0.05	5.48	0.07	5.50	0.06
4	5.26	0.11	5.35	0.10	4.60	0.04	4.66	0.03
5	4.06	0.83	4.28	0.13	3.33	0.06	3.55	0.09
6	4.02	0.08	4.05	0.05	3.42	0.11	3.41	0.07
7	4.19	0.13	4.08	0.06	3.54	0.21	3.45	0.05
8	5.62	0.07	5.50	0.06	4.89	0.06	4.82	0.04
9	5.96	0.13	5.82	0.09	5.36	0.11	5.25	0.05
10	5.71	0.08	5.82	0.09	5.12	0.80	5.14	0.06
11	5.75	0.19	5.87	0.46	5.18	0.19	5.24	0.54
12	7.96	0.15	8.11	0.08	7.49	0.10	7.61	0.05
13	7.27	0.14	7.27	0.24	6.79	0.15	6.66	0.20
14	4.16	0.12	4.17	0.14	3.88	0.19	3.94	0.13
15	6.10	0.19	6.14	0.15	5.65	0.08	6.01	0.07
16	5.08	0.12	5.06	0.11	4.69	0.21	4.57	0.16

Figure 6.17 The relationship between topsoil pH measured by DIW and by $CaCl_2$

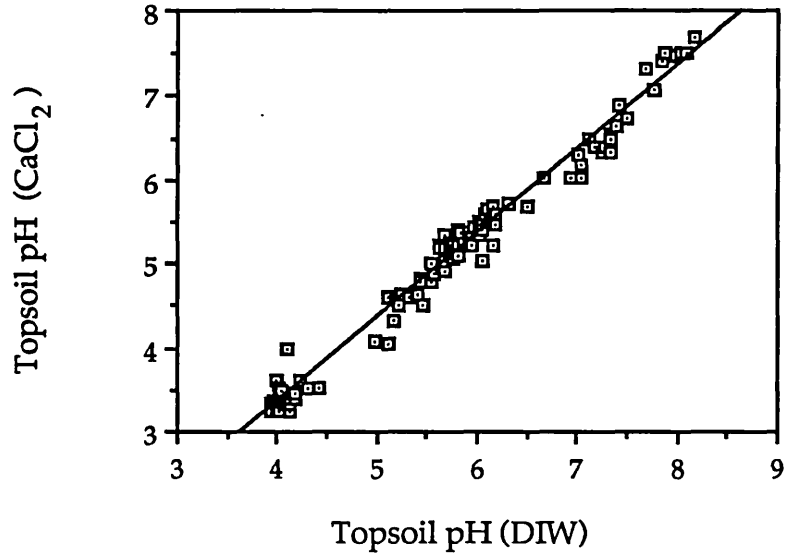


Figure 6.18 The relationship between subsoil pH measured by DIW and by $CaCl_2$

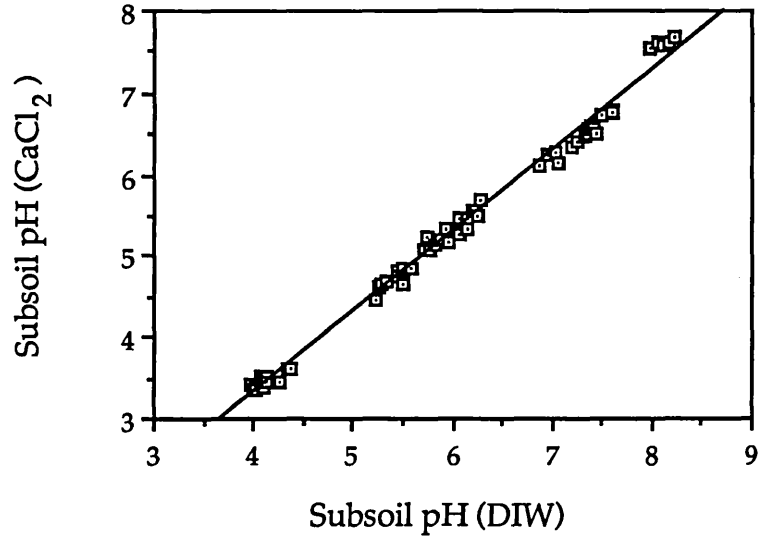


Figure 6.19 The relationship between the pH of the topsoil and the pH of the subsoil

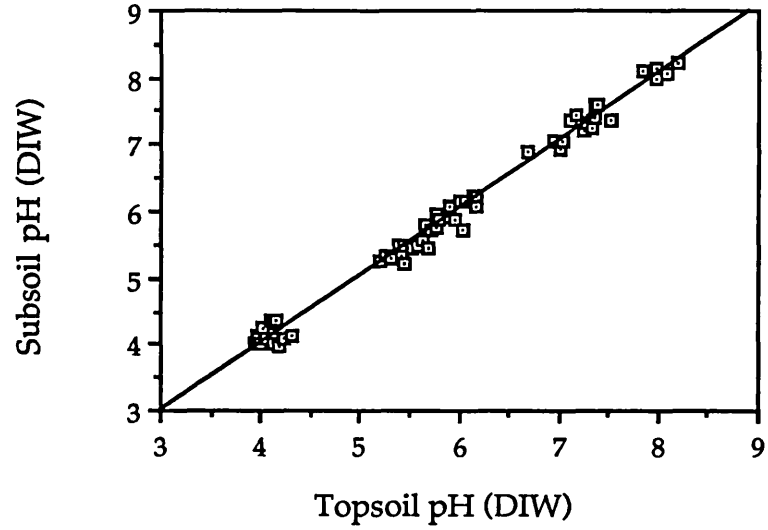


Table 6.15 The correlation coefficients (r) between soil pH (DIW) and selenium concentrations in soil and herbage

	Topsoil pH	Subsoil pH
Selenium in herbage	- 0.215 p<0.04	- 0.285 p<0.03
Selenium in topsoil	- 0.401 p<0.001	- 0.437 p<0.001
Selenium in subsoil	- 0.296 p<0.02	- 0.284 p<0.02

Figure 6.20 The relationship between topsoil pH and selenium concentration in the herbage

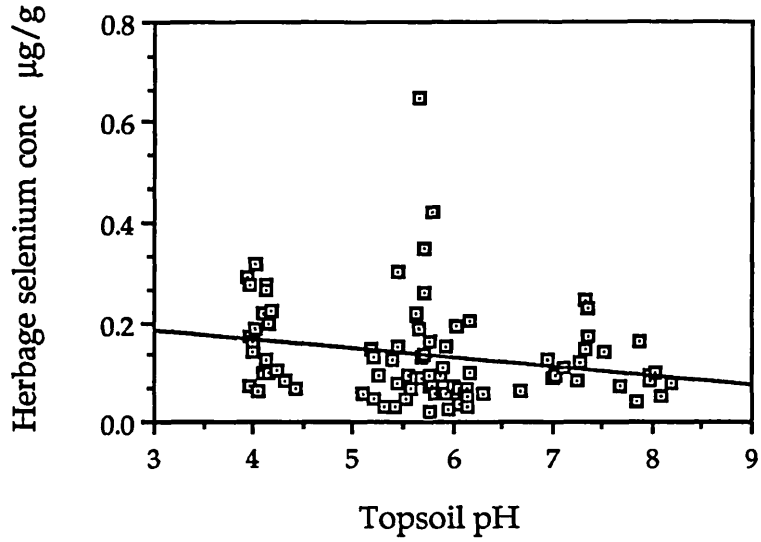


Figure 6.21 The relationship between subsoil pH and selenium concentration in the herbage

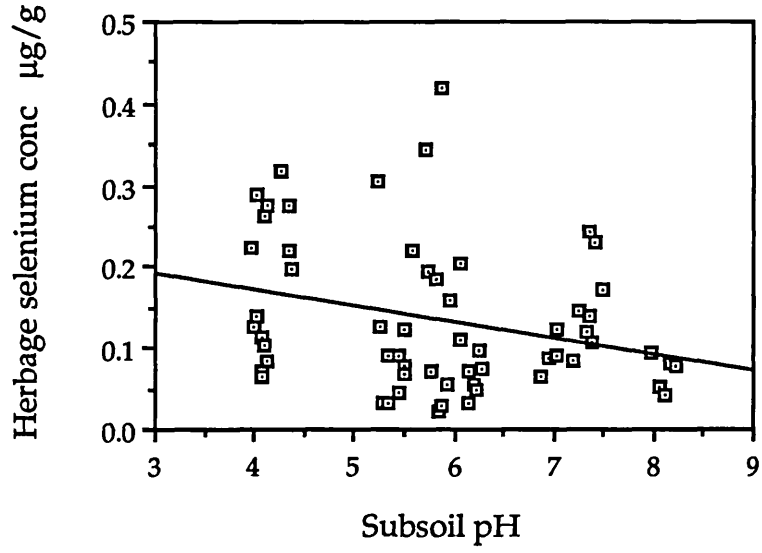


Figure 6.22 The relationship between topsoil pH and selenium concentration in the topsoil

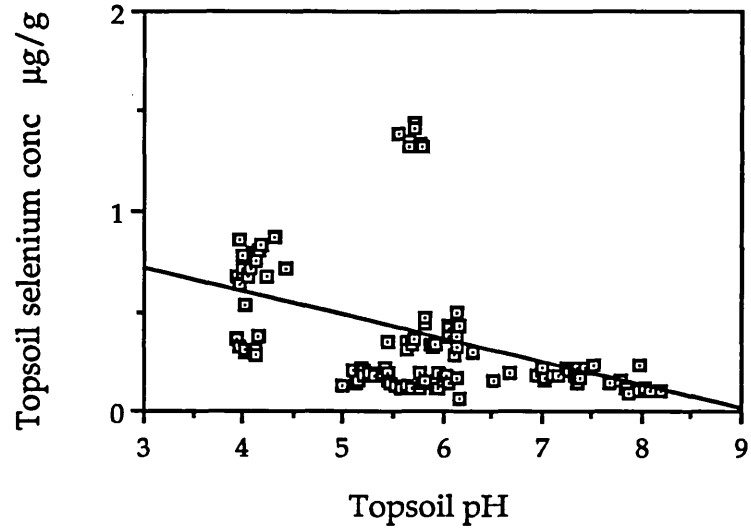
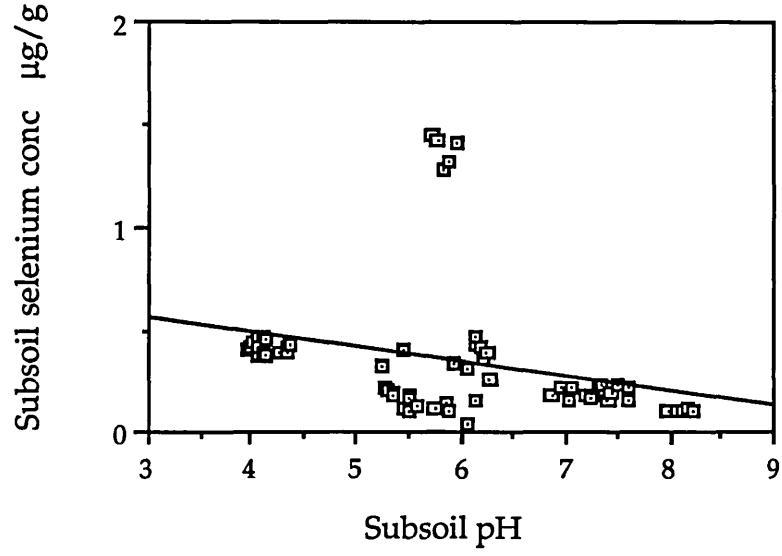


Figure 6.23 The relationship between subsoil pH and selenium concentration in the subsoil



The negative correlation of soil pH and soil selenium concentrations for both topsoil and subsoil is quite obvious from Figures 6.22-6.23. However in both plots, one group of results lies away from the rest of the population and these are all from the samples collected at site 10 which has high soil selenium levels derived from the high selenium content of the marine black shale parent material. If the results from this site are considered outliers and removed from the data sets, the correlation coefficient becomes r= -0.651 for the topsoil and r= -0.687 for the subsoil, and these corrected plots are shown in Figures 6.24-6.25.

The total selenium content of the soil tends to decrease with increasing soil pH in these sites, with the acid peat soils of the North Wales moorland containing more selenium than the neutral sandy soils of Brecon and Woburn and the calcareous soil on Romney Marsh. This inverse relationship may actually be due to the variation in organic matter amongst the sites. The highly organic peat soils are invariably acidic and contain higher levels of selenium associated with the organic matter than the neutral sandy soils which contain very little organic matter and have low selenium levels.

Since the pH value of the soil is, to some extent, determined by the organic matter content in the soil, it is very difficult to consider the individual effect of these two variables on the selenium content of the soil and the uptake of selenium into herbage.

The selenium concentration in herbage also increases with decreasing soil pH, reflecting the increase in soil selenium concentration and the decrease in pH with the high organic matter content of the peat soils.

In contrast, the uptake of sulphur into herbage shows a very strong positve correlation with pH in both topsoil (r= 0.685 Figure 6.26) and subsoil (r= 0.617 Figure 6.27) even though the soil sulphur content has a strong negative correlation with pH (r= -0.604 topsoil, r= -0.654 subsoil) like soil selenium. The sulphur content of the soil is increased with increasing organic matter since the decaying plant material contains large amounts of sulphur, and in this respect is similar to the association between selenium and organic material in the soil. Plants actively accumulate sulphur, absorbed as the sulphate ion, and plant sulphur concentrations are often many times higher than the soil sulphur

Figure 6.24 The relationship between topsoil pH and selenium concentration in the topsoil (without Site 10 results)

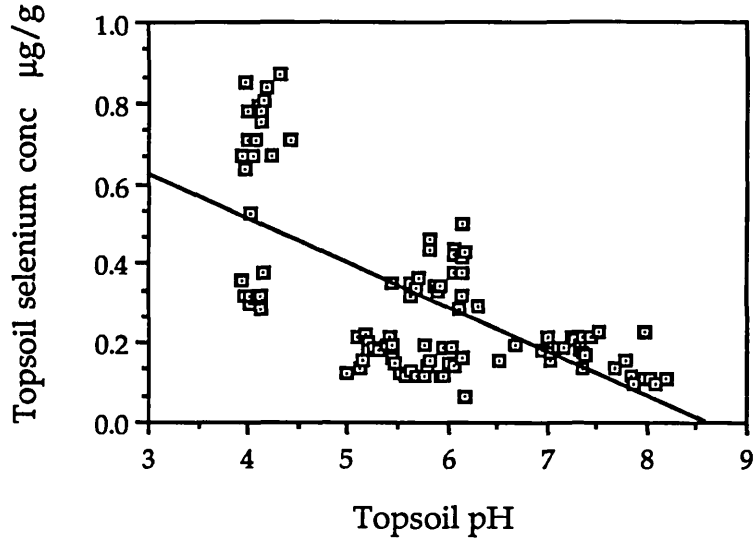


Figure 6.25 The relationship between subsoil pH and selenium concentration in the subsoil (without Site 10 results)

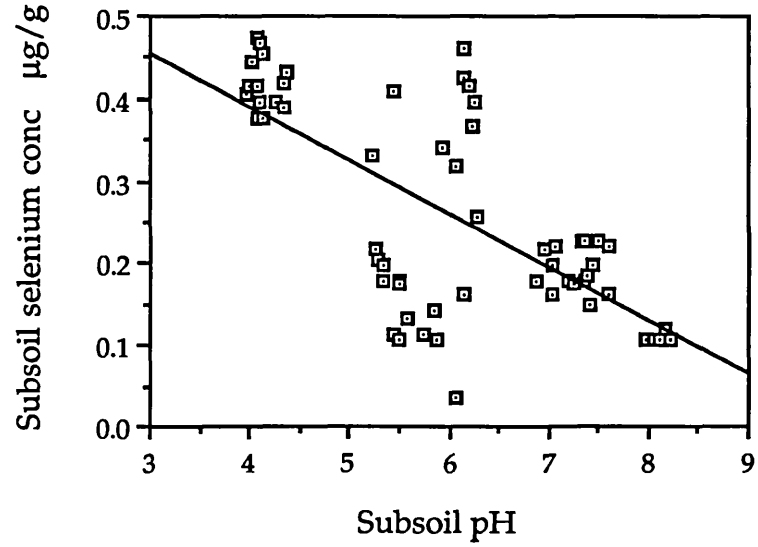


Figure 6.26 The relationship between topsoil pH and sulphur concentration in herbage

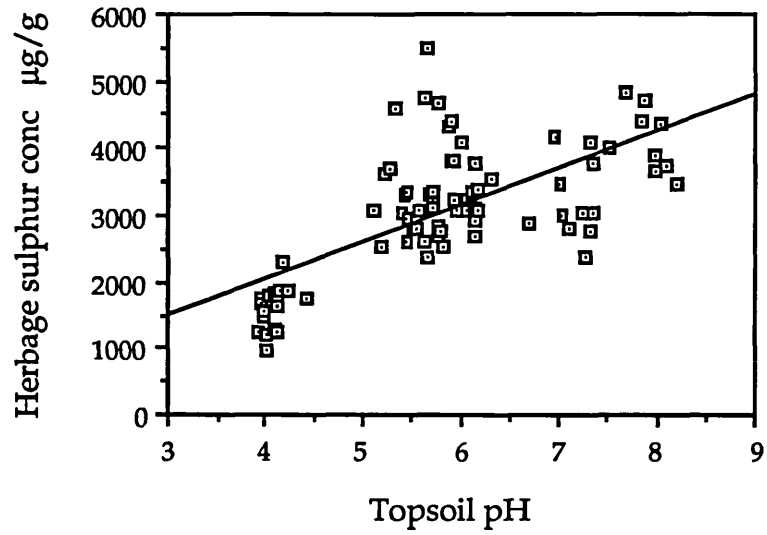
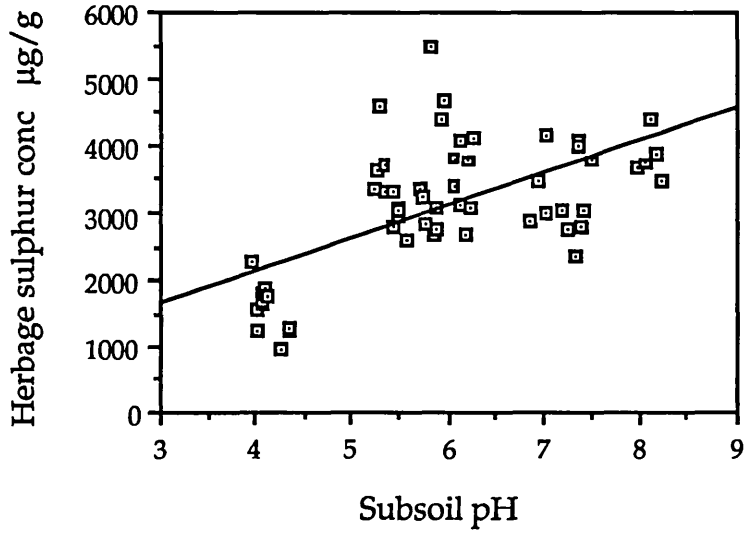


Figure 6.27 The relationship between subsoil pH and sulphur concentration in herbage



concentration. More neutral to alkaline soil pH will favour the formation of the sulphate ion and consequently the availability of soil sulphur to plants is enhanced in alkaline conditions. In soils of lower pH, the sulphite ion is likely to be more prevalent and since this is less readily absorbed by the plants, the positive correlation between plant sulphur levels and soil pH is easily explained.

However for selenium there has been no conclusive evidence of active uptake by plants, although some similarities between sulphate ion and selenate ion absorption by plants have been noted (section 2.4.2). Plant uptake of selenium is hence more heavily dependent upon the solubility of the selenium species present in the soil.

In mineral soils, inorganic selenium speciation is strongly influenced by pH, with alkaline conditions favouring the formation of the more oxidised species, especially the selenate ion. The selenite ion is more prevalent in acid to neutral, well-drained soils. In waterlogged or strongly reducing conditions, insoluble elemental selenium and selenides may be formed.

Whereas the selenate ion remains soluble in the soil and is therefore available for plant uptake, the solubility of the selenite ion is governed by its association with ferric oxides (section 2.3.2). The selenite-ferric adsorption complexes are relatively insoluble especially at acid pH levels, although above pH 7.5-8.5 the stability of these complexes is decreased, releasing more selenite ions into solution which may then become oxidised to selenate ions.

In the peat soils studied in this research, organic forms of selenium and sulphur may predominate, formed from the breakdown of plant proteins. Organic selenium compounds are thought to be freely available to plants, and this may account for the enhanced uptake of selenium from these peat soils compared with the mineral soils (section 6.2). Another possible explanation is that the organic material acts as a weak ion exchange medium for selenite, with the selenite ion readily released into solution and therefore available for plant uptake. This would be in contrast to the rather insoluble iron hydroxide - selenite complexes found in mineral soils with greater iron concentrations.

So, in mineral soils, the uptake of selenium by plants increases with increasing pH of the soil, since the solubility of the inorganic selenium species is

increased at higher pH. Therefore, where organic material is not a variable factor, higher pH levels in the soil produce greater uptake of selenium in the herbage (Gissel-Nielsen, 1971b). The fact that this situation is reversed in the sites studied here reveals the importance of the organic matter content of the soil in influencing the total soil selenium concentration. Paasikallio (1981) also found that increase in pH decreased the plant uptake of Se^{75} from peat soils of low iron content in Finland. Soils with a higher iron content produced an abrupt increase in Se^{75} uptake when the pH increased above 7.

6.4.2 The Influence of Soil Organic Matter on Selenium in Soil and Herbage

The organic matter content of the collected soils was determined using loss on ignition measurements (section 4.3.2). The organic matter content of the soils was not shown to change with the seasons and hence the average values for the organic matter content of the topsoil and subsoil at each site are given in Table 6.16. The original data is given in Appendix A. The organic matter content is greater in the topsoil soil than the subsoil at almost every site with the peat soils from Sites 6 and 7 showing the largest differences between topsoil and subsoil.

Organic matter in the soil shows a very poor correlation with selenium in the herbage ($r= 0.134$ topsoil, Figure 6.28 ; $r= 0.233$ subsoil, Figure 6.29). These correlations are not significant at the 95% level for either topsoil ($p<0.3$) or subsoil ($p<0.08$). However soil organic matter is strongly correlated with the soil selenium levels for the topsoil ($r= 0.489$ $p<0.001$, Figure 6.30) and significantly, but not as strongly, for the subsoil ($r= 0.272$ $p<0.03$, Figure 6.31). As with the pH measurements, the results from site 10 are seen as outliers on these graphs due to the high selenium levels in the soil and if these values are removed the correlation between soil organic matter and soil selenium concentrations becomes $r= 0.899$ for the topsoil (Figure 6.32) and $r= 0.676$ for the subsoil (Figure 6.33).

Table 6.16 **The average organic matter contents of the topsoil and subsoil samples with their standard deviations (σ)**

Organic Matter Content %

Site No.	Topsoil		Subsoil	
	Mean	S. D.	Mean	S. D.
1	14.3	2.5	---	---
2	8.8	3.6	6.6	---
3	11.2	1.1	8.7	0.8
4	9.1	1.8	7.1	0.5
5	30.7	15.6	12.7	2.9
6	75.2	11.7	27.5	4.8
7	74.8	6.8	32.9	7.5
8	6.4	0.9	5.3	0.3
9	7.6	0.5	5.5	0.6
10	14.5	1.1	10.9	0.8
11	11.5	1.1	8.7	0.7
12	8.1	2.0	7.5	1.0
13	2.6	0.1	2.6	0.1
14	2.6	0.3	2.8	0.2
15	2.6	0.2	2.7	0.3
16	2.3	0.7	2.0	0.1

Figure 6.28 The relationship between topsoil organic matter content and herbage selenium concentration

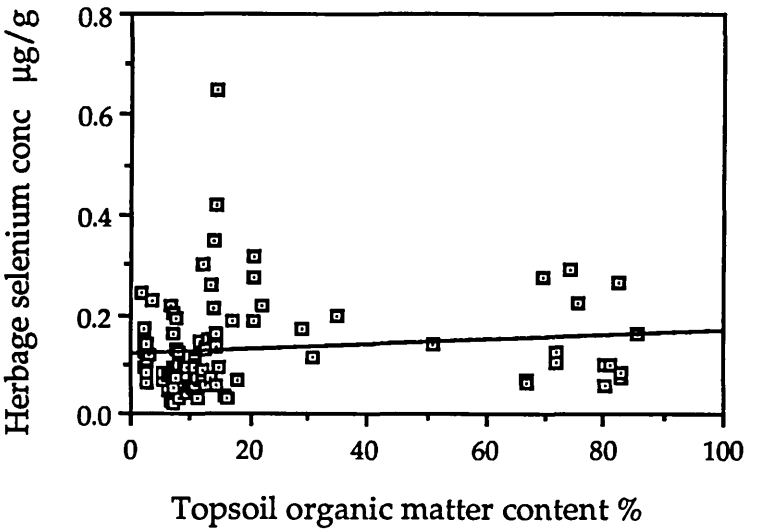


Figure 6.29 The relationship between subsoil organic matter content and herbage selenium concentration

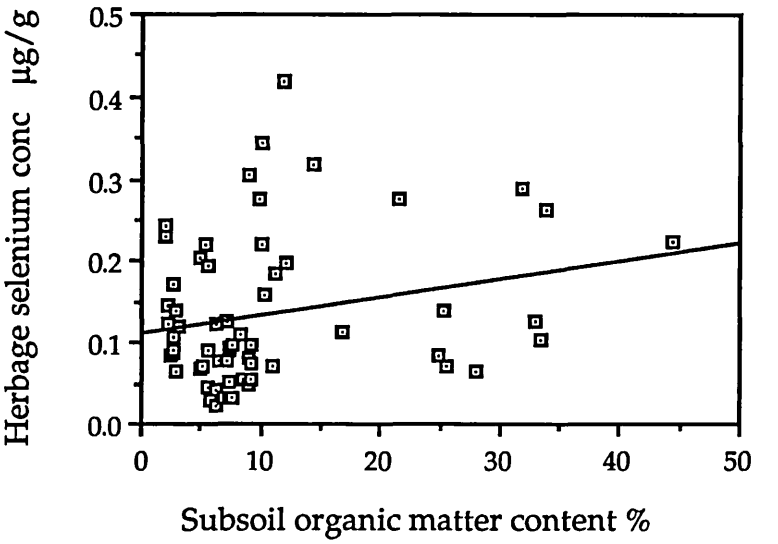


Figure 6.30 The relationship between organic matter content and selenium concentration of the topsoil

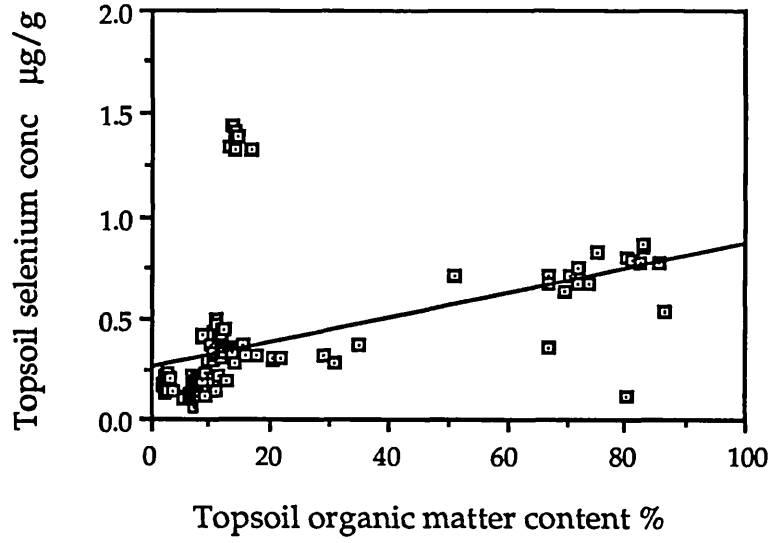


Figure 6.31 The relationship between organic matter content and selenium concentration in the subsoil

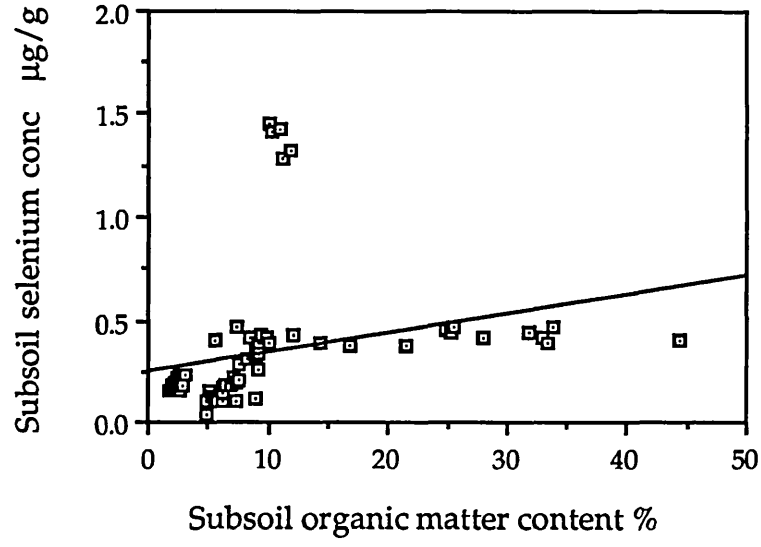


Figure 6.32 **The relationship between organic matter content and selenium concentration in the topsoil (without Site 10 results)**

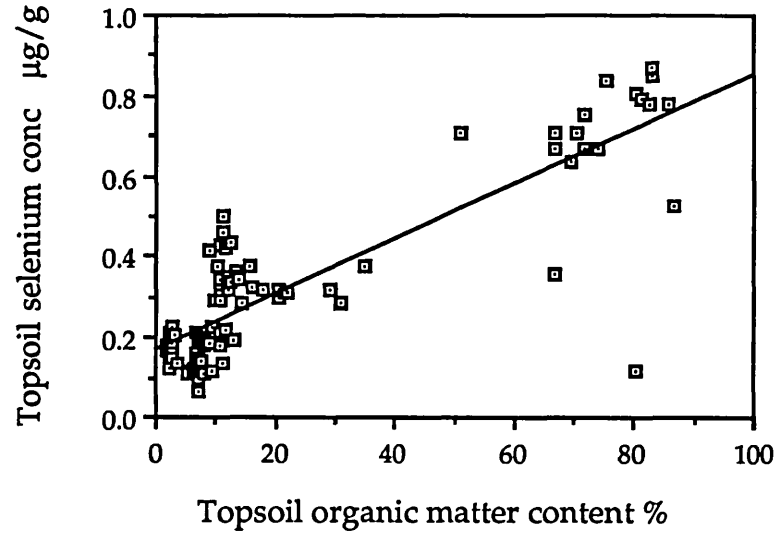
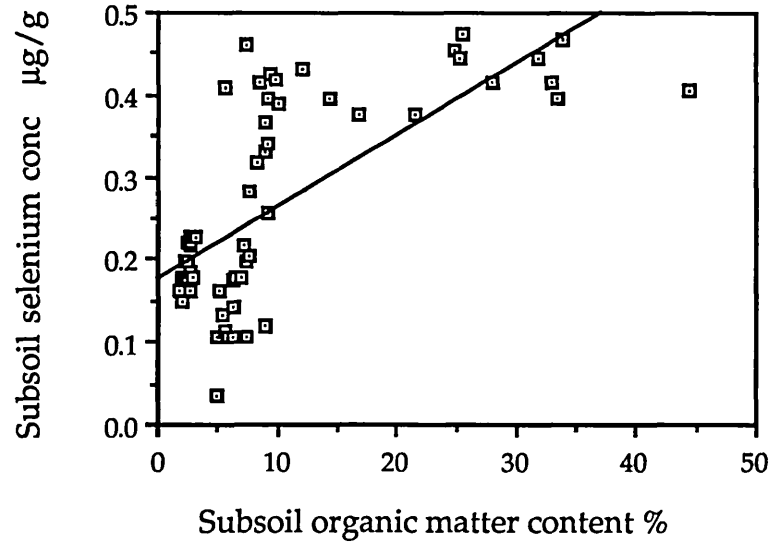


Figure 6.33 **The relationship between organic matter content and selenium concentration in the subsoil (without Site 10 results)**



Organic material contains elevated levels of selenium and so greater organic matter in the soil will increase the total selenium content of the soil. However due to the poor relationship between soil organic matter and herbage selenium levels it would appear that this selenium from the organic matter is not directly available to the plants, possibly as it is present in large undecomposed organic molecules such as proteins which cannot be taken up by plants.

The presence of organic matter does however appear to be important in influencing the pH of the soil which in turn affects the uptake of selenium into herbage.

6.4.3 The Influence of Iron on Selenium in Soil and Herbage

Selenite in soils has been shown to be associated with iron as ferric selenides (Geering et al., 1968, Howard, 1972) and because of this the association between iron and selenium concentrations was investigated in this research.

The average iron levels in the herbage, topsoil and subsoil are given in Table 6.17. Iron in herbage shows a slight positive correlation with iron in topsoil ($r= 0.2214$, $p<0.05$, Figure 6.34) and in the subsoil ($r= 0.1923$, not significant, Figure 6.35) although the majority of this may be due to soil contamination (section 6.3.2).

The amount of iron which could be extracted from the soil using sodium pyrophosphate solution was also measured (see section 4.x). Table 6.18 shows the average pyrophosphate extractable iron (%) levels in the soil. This pyrophosphate extractable iron shows no relationship with the total iron in the soil for either topsoil or subsoil, and there is no correlation between herbage iron concentration and the pyrophosphate extractable iron from either topsoil or subsoil as can be seen from Table 6.19. The pyrophosphate extractable iron measurement is therefore not a useful indicator for the herbage uptake of iron.

Table 6.20 shows the correlations between selenium in herbage and soils with total iron concentration and the pyrophosphate extractable iron content of the soils.

Table 6.17 **The average iron concentration of herbage, topsoil and subsoil at the field sites**

Site No.	Herbage Fe µg/g		Topsoil Fe µg/g		Subsoil Fe µg/g	
	Mean	S. D.	Mean	S. D.	Mean	S. D.
1	879	995	39830	3730	—	—
2	712	231	32930	399	33370	—
3	366	202	39540	1090	40890	663
4	1058	1103	41200	666	42240	904
5	333	271	21980	3830	28900	1152
6	208	109	10150	1360	15530	2333
7	230	74	11380	2870	12990	7149
8	1316	1560	39430	629	39210	1287
9	517	826	34270	1860	34890	2751
10	782	910	37540	1680	44050	1352
11	924	1103	31050	1030	32060	796
12	589	369	26550	445	27550	420
13	202	123	36820	509	37010	1330
14	186	110	38420	5032	42290	3064
15	173	105	35930	520	36830	3362
16	222	168	32760	934	31590	898

Figure 6.34 The relationship between iron concentration in herbage and in topsoil

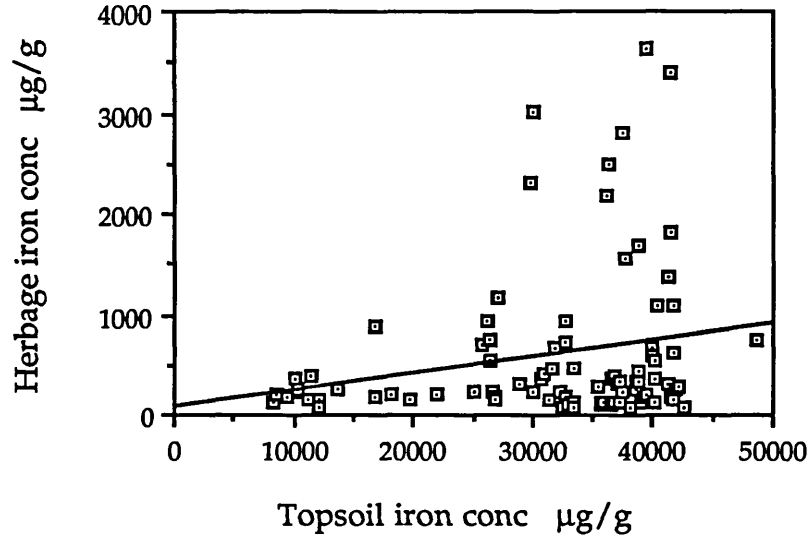


Figure 6.35 The relationship between iron concentration in herbage and in subsoil

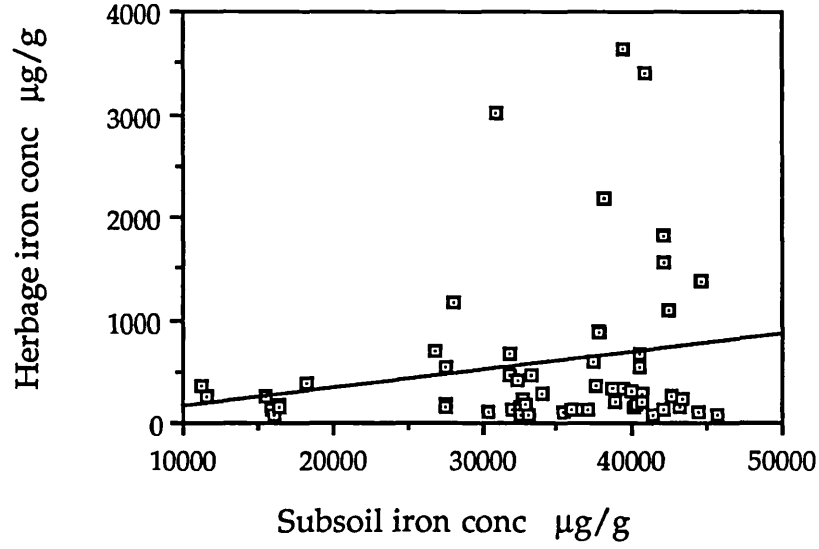


Table 6.18 **The pyrophosphate extractable iron content (%) of the topsoil and subsoil from the field sites**

Pyrophosphate Extractable Iron %

Site No.	Topsoil Fe_{ex}		Subsoil Fe_{ex}	
	Mean	S. D.	Mean	S. D.
1	0.673	0.039	—	—
2	0.148	0.029	0.142	—
3	0.832	0.036	0.883	0.057
4	0.536	0.035	0.508	0.028
5	0.723	0.100	1.227	0.029
6	0.726	0.153	0.622	0.049
7	0.767	0.064	0.577	0.199
8	0.382	0.040	0.332	0.006
9	0.345	0.007	0.340	0.015
10	0.436	0.034	0.403	0.022
11	0.382	0.030	0.316	0.008
12	0.065	0.008	0.067	0.004
13	0.052	0.009	0.059	0.011
14	0.069	0.021	0.054	0.004
15	0.064	0.018	0.063	0.005
16	0.069	0.025	0.053	0.000

Table 6.19 **The correlation matrix of total iron concentration and pyrophosphate extractable iron concentration in the samples**

	Total iron in herbage	Total iron in topsoil	Total iron in subsoil
Pyrophosphate extractable iron in topsoil	- 0.044 (p = 0.75)	- 0.167 (p = 0.15)	- 0.236 (p = 0.17)
Pyrophosphate extractable iron in subsoil	- 0.210 (p = 0.27)	- 0.363 (p = 0.05)	- 0.018 (p = 0.92)

Table 6.20 **The correlation matrix of selenium concentration, iron concentration and pyrophosphate exctractable iron concentration in the samples**

	Herbage Se (fluorimeter)	Herbage Se (ICPAES)	Topsoil Se	Subsoil Se
Herbage Fe	0.472*	0.392*	- 0.029	- 0. 035
Topsoil Fe	- 0.166	- 0.193	- 0.223*	- 0.018
Subsoil Fe	- 0.057	- 0.017	- 0.159	- 0.160
Extractable Fe in topsoil	0.028	0.100	0.424*	0.315
Extractable Fe in subsoil	0.177	0.342	0.288	0.268

* Significant correlation ($p<0.05$)

Apart from a significant positive correlation between iron in herbage and selenium in herbage ($r= 0.4027$, $p<0.001$, Figure 6.36) which is due to soil contamination of the herbage, and a significant negative correlation between selenium in the topsoil and iron in the topsoil ($r= -0.2230$, $p<0.05$, Figure 6.37), there are no significant correlations with total iron concentrations and total selenium concentrations in the soil or herbage. This negative relationship between selenium and total iron in the topsoil reflects the high iron concentration of the Brown Earth soils at Sites 1-4, 9-11, 13-16 which have generally low selenium concentrations. In contrast, the peat soils of North Wales have rather low iron concentrations and higher selenium concentrations.

The pyrophosphate extractable iron content (%) shows positive correlations with soil selenium concentrations in both topsoil (significant, $p<0.001$) and subsoil suggesting that the soil selenium is associated with the iron fraction extractable by sodium pyrophosphate, presumably as the selenite ion (see Figures 6.38-6.39). Pyrophosphate extractable iron in the subsoil also shows a positive correlation with selenium in the herbage measured by ICPAES ($r= 0.342$, $p<0.05$) although this correlation is not significant. The herbage selenium does appear to have a closer relation with the pyrophosphate extractable iron in the subsoil than in the topsoil, perhaps reflecting the main plant rooting zone which is below 15 cm depth, in the subsoil.

Figure 6.36 The relationship between iron concentration and selenium concentration in the herbage

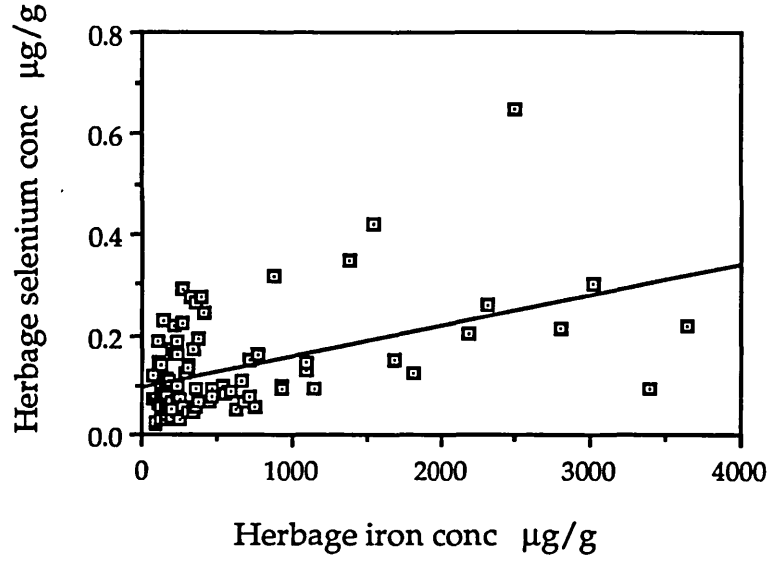


Figure 6.37 The relationship between iron concentration and selenium concentration in the topsoil

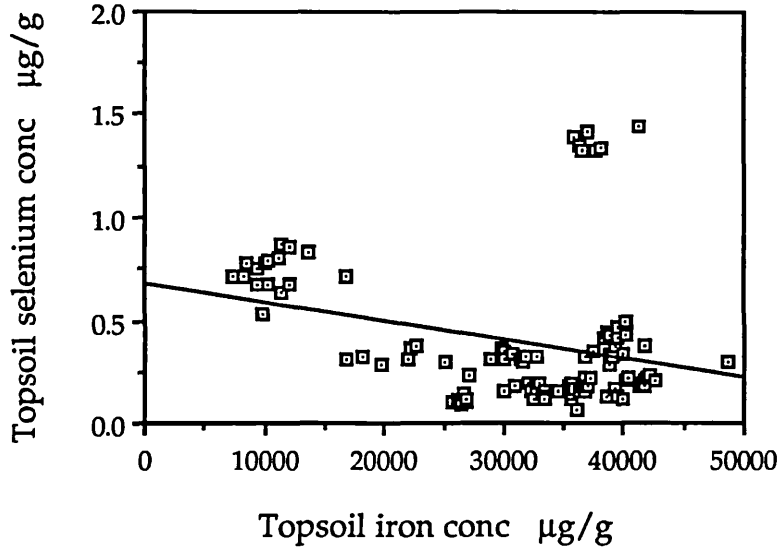


Figure 6.38 The relationship between pyrophosphate extractable iron (%) and selenium concentration in the topsoil

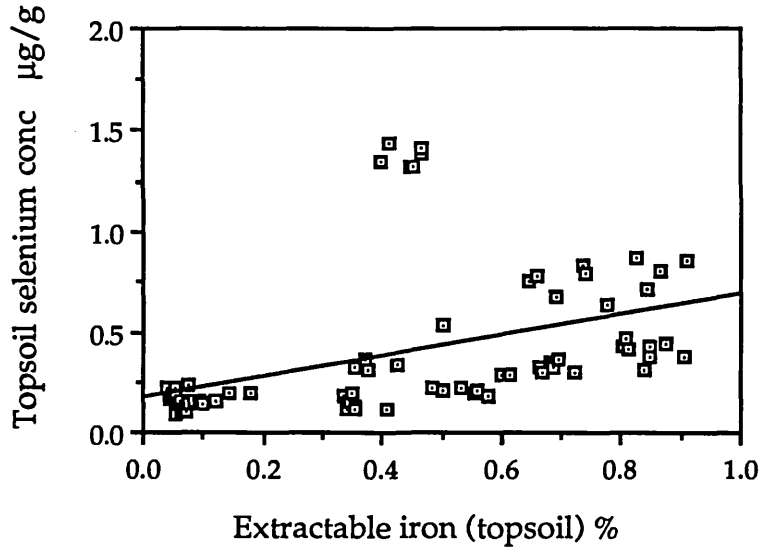
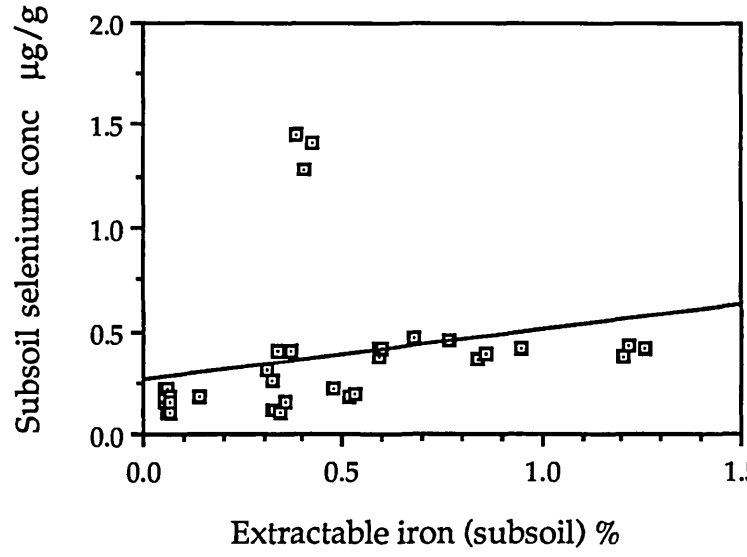


Figure 6.39 The relationship between pyrophosphate extractable iron (%) and selenium concentration in the subsoil



6.4.4 The Influence of Soil Particle Size on Selenium in Soil and Herbage

After decomposition of organic material the particle size distribution of the remaining mineral fraction of the collected soils was determined (section 4.3.4) using both the International and the Soil Survey of England and Wales (SSEW) classification system. The main differences in the two systems is that the International system uses a smaller size range for classifying the silt fraction (0.02-0.002 mm) than the SSEW (0.05-0.002 mm). The United States Department of Agriculture (USDA) uses a similar size range for measuring the particle size distribution in soils as the SSEW, but has a different system of naming the soil textural classes. Table 6.21 lists the soil descriptions on the basis of particle size using the triangular soil texture diagram for both the British (SSEW) and American (USDA) systems. Table 6.22 gives the sand, silt and clay fractions as a percentage of the mineral portion of the soil for both of these classifications and also for the International system.

The mineral fraction of the soil is dependent upon the organic matter content of the soil and hence shows an inverse correlation with soil selenium content ($r = -0.447$, $p<0.001$). Table 6.23 shows the correlation matrix for soil and herbage selenium concentrations and the soil particle size fractions.

Table 6.21 The British and American soil textural classifications on the basis of particle size distribution for the soils from the field sites

Site No.	S. S. E. W.		U. S. D. A	
	Topsoil	Subsoil	Topsoil	Subsoil
1	Sandy silt loam	----	Loam	-----
2	Silty clay loam	Silty clay loam	Silty clay loam	Silty clay loam
3	Sandy silt loam	Sandy silt loam	Silt loam	Silt loam
4	Clay loam	Clay loam	Clay loam	Loam
5	Silty clay loam	Sandy silt loam	Silty clay loam	Silt loam
6	Clay loam	Silty clay loam	Clay loam	Silty clay loam
7	Clay	Silty Clay loam	Clay loam	Silty clay loam
8	Clay loam	Sandy loam	Loam	Sandy loam
9	Clay loam	Sandy silt loam	Loam	Silt loam
10	Silt loam	Silty clay loam	Silt loam	Silt loam
11	Sandy silt loam	Silt loam	Silt loam	Silt loam
12	Sandy silt loam	Silt loam	Silt loam	Silt loam
13	Sandy loam	Sandy loam	Sandy loam	Sandy loam
14	Sandy loam	Sandy loam	Sandy loam	Sandy loam
15	Sandy loam	Loamy sand	Sandy loam	Loamy sand
16	Sandy loam	Loamy sand	Sandy loam	Loamy sand

Table 6.22 **The particle size distribution for the mineral fraction of the soils from the field sites**

Site No.	S. S. E. W. (U. S. D. A) Topsoil %			S. S. E. W. (U. S. D. A) Subsoil %			International* Topsoil %		International* Subsoil %	
	Sand	Silt	Clay	Sand	Silt	Clay	Sand	Silt	Sand	Silt
1	32.2	46.7	16.6	—	—	—	44.2	37.4	—	—
2	14.6	51.3	32.7	13.5	51.3	34.8	8.4	38.3	25.5	39.3
3	20.1	70.1	4.7	18.7	70.5	5.5	31.1	59.1	29.7	59.5
4	31.5	37.0	26.8	32.7	41.3	20.1	39.9	28.6	41.7	32.3
5	7.5	41.8	29.6	19.3	59.1	11.9	15.5	33.8	26.3	52.1
6	11.5	29.6	21.8	8.5	42.4	26.2	18.5	22.6	18.5	32.4
7	13.3	22.7	24.8	10.2	49.3	22.9	15.3	20.7	19.2	40.3
8	40.7	35.7	19.6	57.7	24.4	15.5	54.7	21.7	65.7	16.4
9	31.8	47.1	19.5	22.0	57.1	14.1	44.8	34.1	48.0	31.1
10	7.5	69.9	10.8	9.8	48.3	18.0	24.5	52.9	28.8	29.3
11	29.5	58.7	9.2	13.4	69.0	7.9	48.5	39.7	46.4	36.0
12	20.8	71.5	3.3	12.7	75.9	1.8	39.8	52.5	35.7	52.9
13	78.7	10.6	13.3	78.8	8.9	9.8	82.7	6.6	81.8	5.9
14	76.4	11.6	13.4	78.1	9.7	11.3	81.4	6.6	81.1	6.7
15	79.6	8.7	13.3	80.5	8.4	11.2	83.6	4.7	83.5	5.4
16	83.6	2.9	15.3	84.0	8.2	7.8	85.6	0.9	86.0	6.2

Particle size classes:

SSEW/USDA

Clay < 0.002 mm

Silt 0.002-0.05 mm

Sand 0.05-2.0 mm

International*

Clay < 0.002 mm

Silt 0.002-0.02 mm

Sand 0.02-2.0 mm

Table 6.23 The correlation matrix of selenium in soil and herbage and the soil particle size fractions

	Selenium in herbage n=100	Selenium in topsoil n=100	Selenium in subsoil n=100	Selenium in soil soln. n=16	Selenite in soil soln. n=16
Mineral fraction	- 0.067	- 0.447*	- 0.180	- 0.040	- 0.281*
% Sand in topsoil	- 0.159	- 0.529*	- 0.456*	- 0.470*	- 0.713*
% Sand in subsoil	- 0.088	- 0.490*	- 0.390*	- 0.547*	- 0.701*
% Silt in topsoil	- 0.003	0.295*	0.385*	0.649*	0.751*
% Silt in subsoil	- 0.079	0.183	0.157	0.451	0.576*
% Clay in topsoil	0.086	- 0.021	- 0.098	- 0.454	- 0.309
% Clay in subsoil	0.131	0.409*	0.280*	- 0.071	0.051

* Significant correlation ($p<0.05$)

There are no significant correlations between any of the size fractions and the selenium concentration in the herbage. This suggests that the particle size distribution of the soil mineral fraction has no effect on the availability of soil selenium to the plants. In contrast, the soil selenium levels show strong negative correlations with the percentage of sand in the soil for both topsoil and subsoil, sandy soils tend to be low in selenium often due to leaching of selenium through the soil profile. It is noticeable that the topsoil selenium levels show a stronger

negative correlation with the sand fraction than subsoil suggesting leaching of selenium from the topsoil soil.

The silt fraction in the topsoil shows a positive correlation with selenium levels in the soil although the correlation between the subsoil silt percentage and soil selenium concentration is not significant. This positive relationship may be due to the fact that the silt fraction of the soil increases as the sand fraction decreases in general so that this correlation is merely showing the antagonistic relationship between the sand and silt fractions. Alternatively the soil selenium may be associated with the silt size fraction to some extent.

The topsoil clay fraction shows no correlation with selenium in the soil, however, the subsoil clay fraction does show a positive correlation with the selenium levels in both topsoil and subsoil. The reason for this association with subsoil clay alone is not clear, although possibly the selenium is leached from the surface soil but is retained by the clay fraction in the subsoil.

Table 6.23 also shows the correlations between the extractable selenium and the particle size fractions. The relationship between extractable selenium and other soil factors is discussed in detail in section 6.6, however since the correlations with particle size tend to follow those of total soil selenium they were included in this table. Correlations for both the extractable selenium and extractable selenite are similar, showing negative correlations with the sand fraction and positive correlations with the silt fraction and no significant relationship with the clay fraction. The fact that the extractable selenium is strongly correlated with the silt fraction suggests that this fraction may be the site for readily exchangeable selenium in the soil system. Any selenium associated with the clay fraction may be more strongly bound, less soluble and hence less extractable. The sand fraction shows the same correlation trends for extractable selenium as for total selenium highlighting the lack of affinity of selenium for sand in the soil system.

6.4.5 The Influence of Cation Exchange Capacity on Selenium in Soil and Herbage

The cation exchange capacity of the soils was measured as described in section 4.3.5, to provide an estimate of the ability of the soil to adsorb ions from solution. A method of estimating the anion exchange capacity of the soils may have been more appropriate for the study of selenium in soils, however this was not attempted.

Table 6.24 shows the correlation matrix between the soil cation exchange capacity and soil and herbage selenium concentrations. The cation exchange capacity of the soil shows a significant positive correlation with selenium for both topsoil (r= 0.566 Figure 6.40) and subsoil (r= 0.365 Figure 6.41), although there is no significant correlation between herbage selenium and soil cation exchange capacity.

It can be seen from Figures 6.40-6.41 that there are some outlying values of high selenium content and low cation exchange capacity. These values correspond to the samples from site 10 with high selenium in the parent material, if these values are removed from the data set the correlation coefficients become r= 0.912 for topsoil (Figure 6.42) and r= 0.721 for subsoil (Figure 6.43).

Cation exchange capacity correlates very strongly with soil organic matter since the organic material in the soil provides a large proportion of the cation exchange sites in the soil system. Cation exchange capacity also shows large positive correlations with the pyrophosphate extractable iron concentration and negative correlations with the pH of the soils. Table 6.25 shows the correlation matrix for soil cation exchange capacity, soil organic matter, pyrophosphate extractable iron content and pH. These factors are to a large extent dependent upon one another and the correlations provide some indication of this.

Figure 6.40 The relationship between cation exchange capacity and selenium concentration in the topsoil

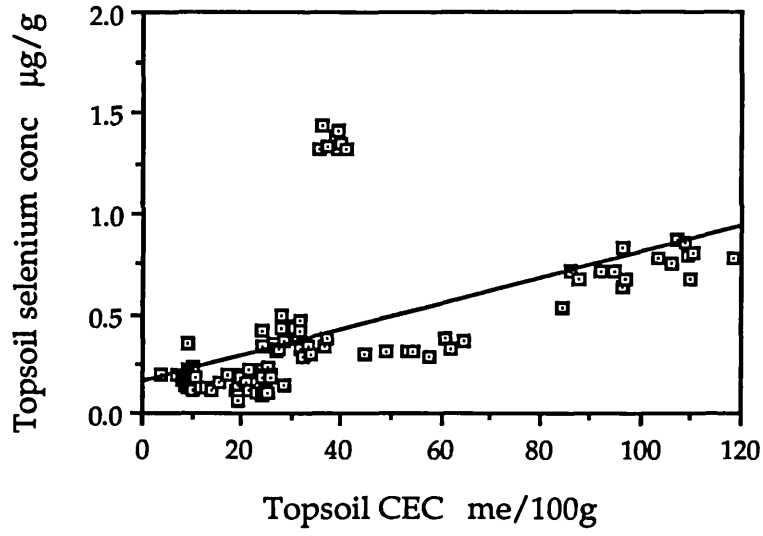


Figure 6.41 The relationship between cation exchange capacity and selenium concentration in the subsoil

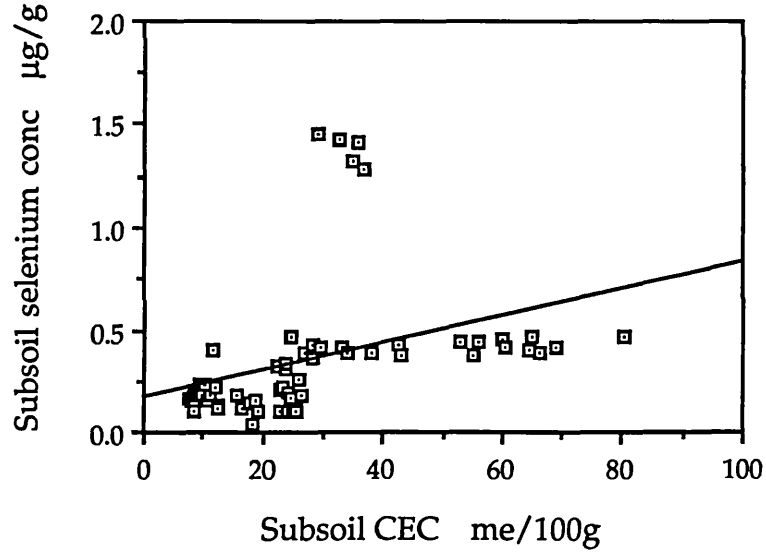


Figure 6.42 The relationship between cation exchange capacity and selenium concentration in the topsoil (without site 10 results)

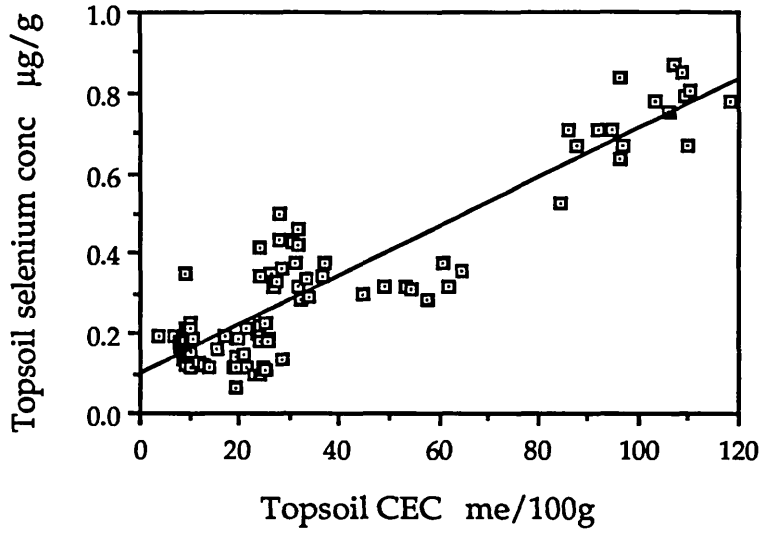


Figure 6.43 The relationship between cation exchange capacity and selenium concentration in the subsoil (without site 10 results)

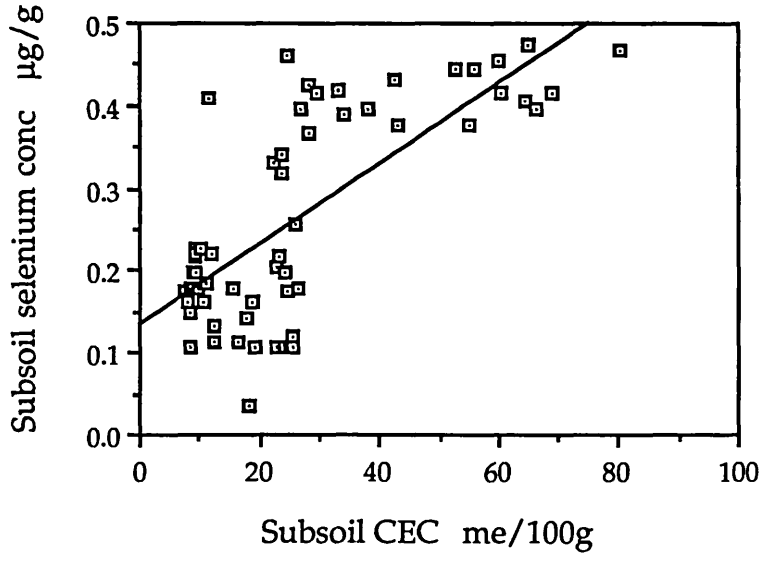


Table 6.24 **The correlation matrix of soil cation exchange capacity and soil and herbage selenium concentrations**

	Herbage selenium	Topsoil selenium	Subsoil selenium
Topsoil CEC	0.153	0.566*	0.297*
Subsoil CEC	0.224	0.617*	0.365*

* Significant correlation ($p<0.05$)

Table 6.25 **The correlation matrix of cation exchange capacity, organic matter content, pyrophosphate extractable iron content and pH in soil**

	Organic matter		Extractable iron		PH	
	topsoil	subsoil	topsoil	subsoil	topsoil	subsoil
Topsoil CEC	0.964*	0.950*	0.657*	0.531*	- 0.720*	- 0.773*
Subsoil CEC	0.935*	0.944*	0.748*	0.567*	- 0.757*	- 0.753*

* Significant correlation ($p<0.05$)

6.4.6 The Influence of Soil Sulphur on Selenium in Soils and Herbage

The average sulphur content of herbage, topsoil and subsoil is shown in Table 6.26. The selenium content of herbage shows a significant negative correlation with sulphur in the herbage (r= -0.286 Figure 6.44), possibly suggesting competitive uptake between the two elements, however there is no negative relationship with sulphur in soil and selenium in the herbage. This suggests that the competition between sulphur and selenium which has been noted by some workers (eg. Asher, Butler and Peterson, 1977) is primarily due to plant uptake and that soil sulphur levels have no direct effect on plant selenium levels at low levels of soil selenium.

Table 6.27 shows the correlation between sulphur and selenium in soil and herbage samples. There is a significant positive correlation between topsoil selenium and soil sulphur levels although this correlation is not significant for the subsoil selenium levels. Both soil selenium and soil sulphur concentrations are strongly correlated with soil organic matter and hence would be expected to show some correlation with each other.

For sulphur alone, there is a significant negative correlation for herbage sulphur with soil sulphur concentrations (r= - 0.496 topsoil, r= - 0.350 subsoil), unlike selenium in herbage which shows a positive correlation with soil selenium. Plants accumulate sulphur and usually have higher levels in the plant than the soil, whereas selenium uptake by plants is considered to be a passive process.

Table 6.26 **The average values of sulphur in herbage, topsoil and subsoil samples from the field sites**

Site No.	Herbage S µg/g		Topsoil S µg/g		Subsoil S µg/g	
	Mean	S. D.	Mean	S. D.	Mean	S. D.
1	3275	402	875	124	—	—
2	2766	177	847	66.6	860	—
3	3041	438	613	47.7	474	55.5
4	3441	615	616	72.7	514	53.2
5	1352	285	821	77.5	532	46.6
6	1597	211	2950	494	1340	163
7	1895	204	3100	260	1380	162
8	2950	318	520	39.2	494	86.1
9	3280	464	459	27.0	402	105
10	3429	1081	909	45.8	628	53.6
11	4033	527	929	60.4	834	52.6
12	4133	513	1050	117	850	61.6
13	3427	376	170	14.1	155	23.8
14	3057	846	177	22.9	168	53.8
15	3347	721	165	18.7	158	17.1
16	3306	706	143	29.4	135	33.2

Figure 6.44 The relationship between sulphur concentration and selenium concentration in the herbage

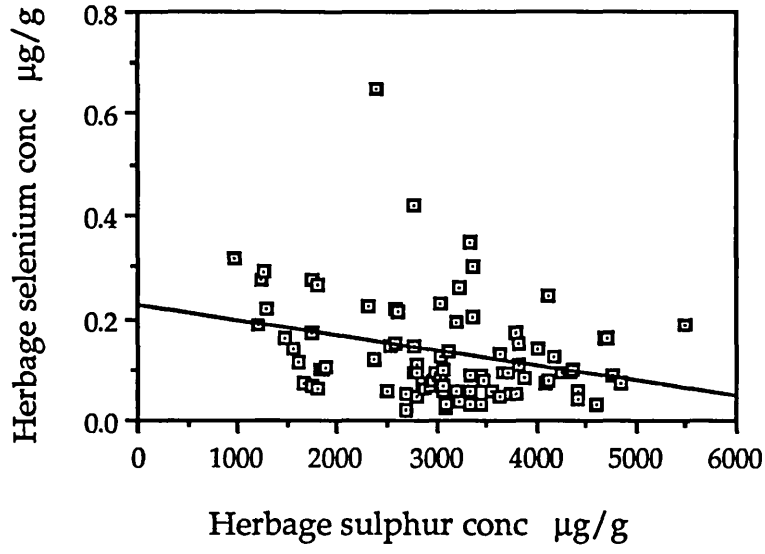


Table 6.27 The correlation matrix of selenium and sulphur in soil and herbage

	Herbage selenium	Topsoil selenium	Subsoil selenium
Herbage sulphur	- 0.286*	- 0.198	0.031
Topsoil sulphur	0.079	0.515*	0.219
Subsoil sulphur	0.118	0.478*	0.218

* Significant correlation ($p<0.05$)

6.4.7 The Influence of Rainfall, Climate and Soil Moisture Content on Selenium in Soil and Herbage

The moisture content of the topsoil and subsoil was measured immediately on returning from the field sampling, and the seasonal values are given in Table 6.28-6.29. Surprisingly the soil moisture content does not show significant variation between the seasons.

The correlations between selenium in the soil and herbage and soil moisture content are shown in Table 6.30. There are significant positive correlations with soil moisture for selenium in the soil (r= 0.234 Figure 6.45) and herbage (r= 0.496 Figure 6.46) although these positive correlations are probably due to the soil organic matter content. Soils of high organic matter content hold water well and also have a higher selenium concentration so consequently a positive link between soil moisture content and soil selenium will be found. The positive relationship between herbage selenium concentration and soil moisture content may be explained by the rise in soil moisture content in the winter months, when the herbage selenium concentrations are highest.

Table 6.30 also shows the correlations between soil and herbage selenium concentrations and the rainfall and temperature data obtained for each sampling area. The monthly rainfall figures, obtained from the Meteorological Office, have been used to provide a three monthly total rainfall value for the time preceding each sampling. The temperatures recorded for each site at the time of sampling have been used rather than a three monthly average. These values are given in Tables 6.31-6.32. The herbage selenium concentrations show a significant positive correlation with the rainfall figures and a negative correlation with the temperature figures. This reflects the increase in herbage selenium concentration in the winter months, when the rainfall is high and the temperature low. No correlation is seen between the climate data and the soil selenium concentration. In some situations, rainfall may have an influence on soil selenium concentrations, especially leaching of selenate from soil, however insufficient sites were studied in this research to provide any conclusions on the effect of climate on soil selenium concentrations.

Table 6.28 The moisture content (%) of the topsoil samples at each collection

	Sampling Date							
Site No.	1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	42.0	30.9	20.5	30.3	32.6	29.7	33.5	29.8
2	49.1	33.7	31.8	—	—	—	—	—
3	38.3	—	30.6	34.9	37.9	34.9	30.8	35.4
4	40.2	—	23.6	20.8	25.7	25.9	20.1	29.3
5	59.1	—	46.8	51.0	47.5	47.1	52.9	55.8
6	75.5	—	70.9	79.2	73.0	65.1	75.4	79.0
7	71.9	—	62.4	73.8	75.3	71.1	67.6	74.5
8	—	—	—	—	35.6	30.5	34.8	36.7
9	35.6	—	17.5	28.8	28.9	30.5	28.4	33.5
10	46.7	40.2	27.2	34.9	43.0	31.5	41.3	39.7
11	46.2	—	26.1	33.6	38.6	31.6	33.2	37.8
12	—	—	17.2	25.4	23.8	19.4	23.8	25.5
13	21.3	—	4.0	12.2	14.2	9.0	19.9	13.2
14	20.4	—	4.5	12.4	14.9	11.6	14.6	14.0
15	—	—	4.4	12.0	14.0	8.7	12.5	14.5
16	—	—	6.4	11.2	12.0	9.2	6.7	13.4

Table 6.29 The moisture content (%) of the subsoil samples at each collection

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	—	—	—	—	—	—	—	—
2	—	33.1	—	—	—	—	—	—
3	—	33.2	—	—	35.7	31.3	31.8	33.7
4	—	24.5	—	—	25.1	22.3	21.9	27.1
5	—	36.6	—	—	44.6	36.0	44.9	40.6
6	—	58.0	—	—	64.3	57.4	56.7	70.0
7	—	64.6	—	—	60.5	58.5	54.9	53.6
8	—	—	—	—	31.3	28.2	29.8	37.0
9	—	24.7	—	—	24.2	27.4	26.3	28.9
10	—	31.4	—	—	36.0	29.9	31.9	31.9
11	—	30.0	—	—	31.9	27.7	28.4	31.9
12	—	—	—	—	29.0	18.4	25.3	27.1
13	—	—	—	—	14.5	8.8	13.6	13.9
14	—	—	—	—	15.4	10.9	13.3	14.7
15	—	—	—	—	14.1	8.6	12.9	14.5
16	—	—	—	—	12.4	9.1	12.5	13.1

Figure 6.45 The relationship between the soil moisture content and the herbage selenium concentration

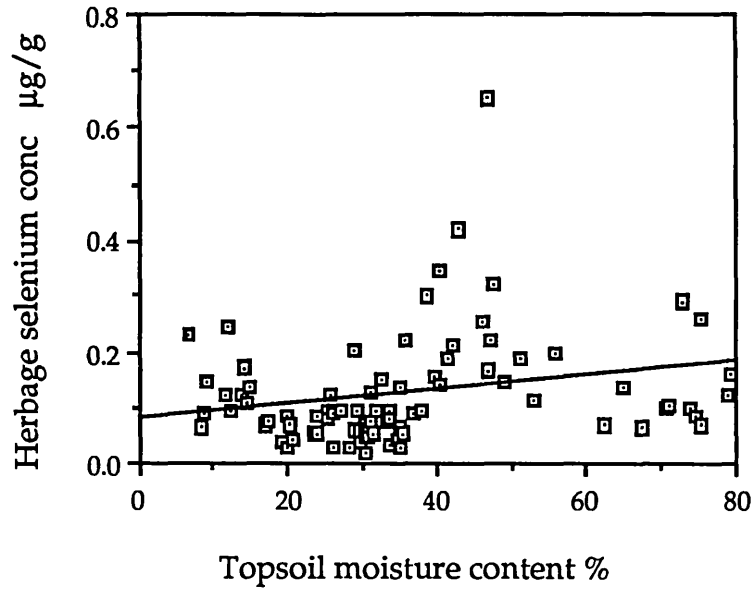


Figure 6.46 The relationship between the soil moisture content and the topsoil selenium concentration

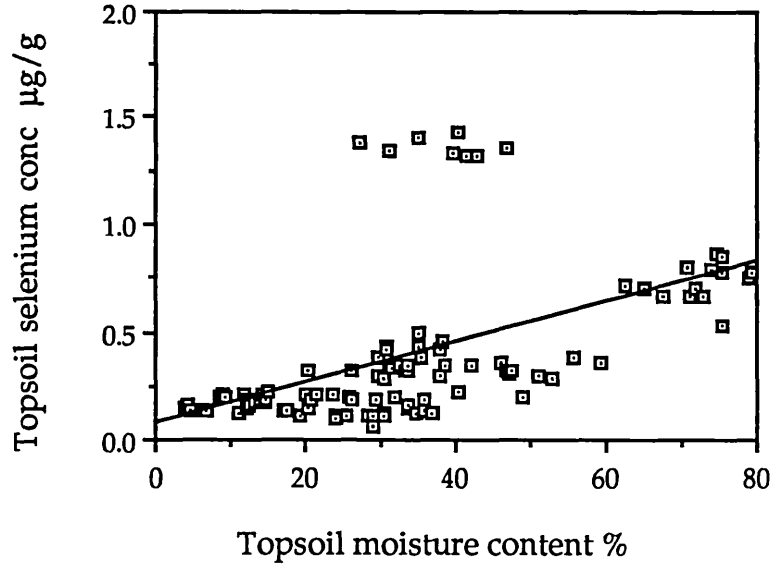


Table 6.30 **The correlation matrix of soil moisture content, rainfall and temperature, and selenium concentration in soil and herbage**

	Topsoil moisture content	Subsoil moisture content	Total seasonal rainfall	Average temperature
Herbage selenium	0.234*	0.251*	0.336*	- 0.498*
Topsoil selenium	0.496*	0.528*	0.064	- 0.043
Subsoil selenium	0.311*	0.299*	0.076	- 0.007

* Significant correlation ($p<0.05$)

Table 6.31 **The total rainfall (mm) during the three months preceeding the sampling date**

	Sampling Date							
Site No.	1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1-8	512	371	112	314	525	364	217	487
9	541	350	98	351	731	329	227	453
10	336	308	76	190	352	380	215	248
11	459	389	123	311	505	318	304	383
12	345	208	71	213	273	161	176	279
13-16	244	228	60	222	210	167	151	299

Table 6.32 The average air temperature (°C) recorded for each sampling date

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1-8	1.9	8.5	12.9	8.7	1.5	7.9	13.3	7.5
9	1.9	9.0	13.4	9.5	2.5	8.7	13.9	8.0
10	2.3	10.6	15.3	10.3	3.3	9.9	15.5	8.8
11	1.5	9.7	13.9	8.9	2.1	8.4	14.1	7.7
12	4.0	11.1	16.7	11.7	3.7	10.6	16.5	11.7
13-16	2.9	10.7	16.0	11.1	3.1	9.6	15.5	9.7

6.4.8 Multiple Regression for Factors Affecting Selenium in Soil and Herbage

The relationships between soil and herbage selenium concentrations and the measured soil factors were further explored using multiple regression analysis. The initial choice of independent variables was made with reference to the simple correlation measurements discussed earlier in this section. Only the topsoil measurements have been used throughout these multiple regression analyses.

The relationship between those factors affecting herbage selenium concentration was of particular interest in the context of this study. The soil factors which were considered to have some influence on the selenium content of the herbage included, soil selenium concentration (r=0.405*), soil pH (r= -0.215*), soil moisture content (r=0.234*), soil organic matter content (r=0.134), pyrophosphate extractable iron content (r=0.079) and soil sulphur concentration (r=0.028). Some of these factors did not show significant correlation with selenium concentration in herbage, however they were still included in the multiple regression analysis.

Regression of soil selenium concentration, soil pH, soil moisture content and pyrophosphate extractable iron accounted for only 22% of the variance in the herbage selenium concentration. The equation obtained from this multiple regression is given below:

$$\text{Herbage Se } (\mu g/g) = 0.143 + 0.130\ \text{Soil Se } (\mu g/g) - 0.112\ \text{Pyrophosphate Ex. Fe } (\%) - 0.0076\ \text{Soil pH} + 0.0003\ \text{Soil Moisture } (\%)$$

If soil organic matter was also included in the multiple regression calculation, then 35% of the variance in herbage selenium was accounted for. This was given by the following equation:

$$\text{Herbage Se } (\mu g/g) = 0.019 + 0.123\ \text{Soil Se } (\mu g/g) - 0.089\ \text{Pyrophosphate Ex. Fe } (\%) + 0.0044\ \text{Soil Moisture } (\%) - 0.0033\ \text{Soil Organic Matter } (\%) - 0.0022\ \text{Soil pH}$$

However the 'best' model available from this data accounted for 36.6% of the variation in herbage selenium concentration, and was given by the multiple regression of soil selenium concentration, soil moisture content, soil organic matter and pyrophosphate extractable iron content.

$$\text{Herbage Se } (\mu g/g) = 0.0015 + 0.128 \text{ Soil Se } (\mu g/g) - 0.085 \text{ Pyrophosphate Ex. Fe } (\%) + 0.0044 \text{ Soil Moisture } (\%) - 0.0033 \text{ Soil Organic Matter } (\%)$$

A similar analysis was carried out for the factors affecting soil selenium concentration. Strong correlations were seen with many of the soil factors mentioned above and those considered to influence the soil selenium concentration included; soil organic matter (r=0.489*), soil pH (r= -0.401*), pyrophosphate extractable iron content (r=0.424*), soil moisture content (r=0.533*), soil sulphur concentration (r=0.515*) and soil cation exchange capacity (r=0.566*). Despite strong individual correlations for these factors the 'best' equation obtained from multiple regression analysis accounted for only 35.7% of the variance in soil selenium concentration. Some of the soil factors mentioned above accounted for over 25% of the variance in soil selenium concentration when considered individually. The equation given below accounts for 35.7% of the variance in soil selenium concentration, and was obtained from the multiple regression of soil pH, soil organic matter content, soil cation exchange capacity and soil moisture content on soil selenium concentration.

$$\text{Soil Se } (\mu g/g) = -0.198 + 0.025 \text{ Soil pH} - 0.014 \text{ Soil Organic Matter } (\%) + 0.014 \text{ Soil CEC } (me/100g) + 0.0066 \text{ Soil Moisture Content } (\%)$$

Some of the factors used for this multiple regression analysis are not independent of one another, and other statistical techniques may have been more suitable for the analysis of this type of data. However, due to the specific nature of the field sites chosen and the consequent groups of data seen in the results, it was felt that more detailed statistical analysis on this data set was not justified.

6.5 THE SELENIUM CONTENT OF DIFFERENT PLANT SPECIES

Different plant species are known to accumulate various amounts of selenium from any given soil although only limited investigations have been carried out on soils of low selenium content. The majority of the fields studied in this research had a sward composed of one dominant grass species (usually perennial ryegrass, *Lolium perenne*) with a few scattered individual plants of other species (see Table 3.3). So in these cases the total herbage selenium value given is a measure of the selenium content of the dominant species. However, site 10 had a large proportion, about 25%, of buttercup (*Ranunculus acris*) growing in addition to the perennial ryegrass (*Lolium perenne*), and the three moorland sites 5, 6 and 7 had a very wide range of species growing on them, including large proportions of moss species, lichens, heathers and *Carex* species in addition to the grass species.

On the last sampling visit samples of separate species were collected from sites 5, 6, 7 and 10 in addition to the normal bulked herbage sample. At site 10 samples of buttercup (*Ranunculus acris*), perennial ryegrass (*Lolium perenne*) and clover (*Trifolium repens*) were collected. At sites 5, 6 and 7 there was a large range of species present, especially many lichen and moss varieties and it would have been difficult to collect sufficient material of each species for analysis from within the sampling grid. So samples of mixed lichens, mosses, grasses, *Carex* species and heathers were collected from each site where present and were individually analysed for selenium and other trace element content. The selenium concentrations found in these samples are shown in Table 6.33.

Although this was a very limited study the results indicate that lichens and mosses have a higher selenium content than grasses, *Carex* species and heather species growing on the same soil. The buttercup sample had a higher selenium content than the grasses and clovers, with clover lower in selenium concentration than grasses.

The fact that clover species take up less selenium than grass species growing under the same conditions has been noted by several other workers in the past (Davies and Watkinson, 1966; Bisbjerg and Gissel-Nielsen, 1969).

Table 6.33 The species variation in field herbage selenium concentrations

Selenium Concentration μg/g

Site No.	Moss	Lichen	Heather	Carex	Grass	Butter-cup	Clover	Mixed herbage
5	0.223	0.235	0.089	0.094	---	---	---	0.199
6	0.154	---	---	0.097	0.097	---	---	0.126
7	0.142	---	---	0.050	0.064	---	---	0.085
10	---	---	---	---	0.153	0.182	0.101	0.160

Wheat was growing at Sites 13-16 in 1987, and in July 1987, samples of both the leaves and the ears of the plants were taken. These were analysed for selenium and the results are shown in Table 6.34. The ears of wheat generally contained lower selenium concentrations than the leaves of the plants.

Table 6.34 The selenium concentration (μg/g) in wheat ears compared with wheat leaves collected from Sites 13-16 in July 1987

Selenium Concentration μg/g

Site No.	Wheat ears	Wheat leaves
13	0.071	0.085
14	0.114	0.107
15	0.065	0.091
16	0.128	0.231

6.6 WATER SAMPLES

6.6.1 Rainwater Samples

Rainwater was collected at sites 4, 9, 11, 12 and 13 during some of the three month periods between sampling times, this gave samples of rainwater from each of the areas of the country under study, North Wales, Brecon, Derbyshire, Romney Marsh and Woburn. Site 8 bordered a small stream and a sample of this stream water was also collected for analysis.

It has been suggested (Lag, 1978) that one source of selenium in the soil may be from rainwater, originating either from the burning of fossil fuels as is the case with sulphur, or as natural emissions of marine origin. If any selenium could be detected in these collected rainwaters then additions from this source to the environment would have to be taken into account.

The pH of the collected waters is given in Table 6.35. The rainwaters were all acidic with no particular variation across the country, except the stream water collected in North Wales at site 8 which was neutral.

Table 6.35 The pH of the rainwater and stream water samples collected at the sampling sites

Site No.	Sampling time					
	3	4	5	6	7	8
4	5.19	5.04	4.72	4.10	5.44	_ _
8	_ _	_ _	_ _	7.08	_ _	_ _
9	4.75	5.35	4.93	_ _	_ _	_ _
11	4.32	5.35	4.72	_ _	_ _	_ _
12	5.44	4.69	5.72	4.79	4.63	_ _
13	_ _	4.47	4.62	4.46	_ _	5.11

Unfiltered and filtered (0.45 μm nucleopore) rainwaters were analysed for total selenium and for selenite only (see section 4.3.7v). The remaining collected water was acidified to 0.1 M using hydrochloric acid and left for 2 days in order to mobilise any selenium which may have become bound to the surface of the bottle or suspended particles during the collection period. The acidified samples were then analysed for total selenium on both filtered and unfiltered samples. The results of these analyses are given in Tables 6.36-6.41. The detection limit for the analysis of selenium in aqueous solution was found to be 0.122 μg/l Se and many of the values obtained from the rainwaters lie very close to or just under this detection limit and therefore no confidence can be given to these values. So very little information can be gained from these results except that the average selenium content of rainwater in the areas studied is below 0.122 μg/l Se. Any further investigation into selenium in rainwater would require some preconcentration techniques.

The rainwaters were also analysed for a suite of elements using ICPAES. For most of the elements, the concentrations measured were below the detection limit. Only sodium, potassium, magnesium, calcium and sulphur provided results significantly above the detection limits. Of these elements, only sulphur was of interest in this research, and the results for the sulphur concentration in the unfiltered rainwater samples are given in Table 6.42. From this table it can be seen that Site 12 on Romney Marsh and Site 13 in Woburn have slightly higher sulphur concentrations in the rainwater than the sites in Derbyshire and Wales.

Table 6.36 The total selenium concentration (μg/l) in filtered rainwaters

Site No.	Sampling time					
	3	4	5	6	7	8
4	0.444	0.158	0.144	0.008	0.036	—
8	—	—	—	0.048	—	—
9	—	0.104	0.022	—	—	—
11	0.104	0.226	0.076	—	—	—
12	0.158	0.376	0.036	0.076	0.062	—
13	—	0.090	0.076	—	—	0.348

Table 6.37 The total selenium concentration (μg/l) in unfiltered rainwaters

Site No.	Sampling time					
	3	4	5	6	7	8
4	—	—	0.052	0.106	—	—
8	—	—	—	—	—	—
9	0.066	0.364	0.066	—	—	—
11	0.078	0.242	0.106	—	—	—
12	0.106	0.024	—	0.270	0.106	—
13	—	—	0.120	0.038	—	0.065

Table 6.38 The selenite ion concentration (μg/l) in filtered rainwaters

Site No.	Sampling time					
	3	4	5	6	7	8
4	0.140	0.100	0.032	0.086	0.318	—
8	—	—	—	0.018	—	—
9	0.222	0.174	0.018	—	—	—
11	0.046	0.100	0.304	—	—	—
12	0.194	0.194	0.114	0.072	0.344	—
13	—	0.140	0.114	0.018	—	0.466

Table 6.39 The selenite ion concentration (μg/l) in unfiltered rainwaters

Site No.	Sampling time					
	3	4	5	6	7	8
4	0.002	0.327	—	—	0.032	—
8	—	—	—	0.002	—	—
9	—	0.124	0.056	—	—	—
11	0.278	0.029	—	—	—	—
12	0.206	—	—	—	0.634	—
13	—	0.082	—	0.262	—	0.358

Table 6.40 **The total selenium concentration (μg/l) in filtered, acidified rainwaters**

Site No.	Sampling time					
	3	4	5	6	7	8
4	0.276	0.126	0.398	0.290	0.344	—
8	—	—	—	0.058	—	—
9	0.140	0.126	0.114	—	—	—
11	0.072	0.466	0.440	—	—	—
12	0.038	0.304	0.182	0.440	0.466	—
13	—	0.684	0.072	0.196	—	0.412

Table 6.41 **The total selenium concentration (μg/l) in unfiltered, acidified rainwaters**

Site No.	Sampling time					
	3	4	5	6	7	8
4	0.222	0.114	0.262	0.114	0.058	—
8	—	—	—	0.072	—	—
9	0.072	—	—	—	—	—
11	—	—	—	—	—	—
12	—	—	—	—	0.194	—
13	—	—	—	—	—	0.386

Table 6.42 The sulphur concentration (μg/ml) of the unfiltered rainwaters

Site No.	Sampling time					
	3	4	5	6	7	8
4	2.2	1.0	1.2	2.1	1.4	—
8	—	—	—	3.5	—	—
9	1.7	1.5	0.3	—	—	—
11	2.4	3.2	1.6	—	—	—
12	3.2	2.8	3.7	3.7	2.9	—
13	—	2.1	1.7	3.3	—	2.6

6.6.2 The Selenium Content of Extracted Soil Water

Soil waters extracted from freshly collected soils (section 4.2.4) were analysed for both total soluble selenium and the selenite fraction using spectrofluorimetry. The extracted waters were filtered using 0.45 μm nucleopore filters in order to remove all suspended solids and colloidal material prior to analysis. This filtration procedure was considered to remove the majority of selenium which was present in organic combinations and that associated with colloidal particles, so that no measure of the organic fraction of selenium present in the soil water could be made. Analysis of total selenium on the unfiltered samples was unsuccessful due to the formation of insoluble precipitates during the complexation process.

The results are shown in Table 6.43 and are the average of duplicates. The total selenium concentration of the extracted filtered waters and the selenite concentration is given in μg/l (ppb), the selenite concentration is also given as a percentage of the total selenium in solution. The remaining selenium in solution will presumably be as the selenate ion in the absence of organic selenium, and since no oxidative digestion process was employed, any selenide or elemental selenium would not be detected by the fluorimetric method.

Table 6.43 The results of the selenium speciation study on extracted soil solutions

Site No.	Total selenium in soil water µg/l	Selenite in soil water µg/l	% Selenite in soil water	Se in pyrophosphate extract µg/l
1	1.88	0.75	39.9	0.76
2	0.86	0.62	72.1	--
3	1.13	0.86	76.1	0.87
4	1.26	0.56	44.3	0.61
5	0.38	--	0	--
6	--	--	--	--
7	--	--	--	--
8	0.75	0.52	68.9	0.26
9	0.76	0.37	48.4	0.74
10	4.06	1.93	47.6	0.99
11	2.74	1.24	45.4	--
12	2.34	0.81	34.7	0.12
13	0.66	0.28	42.8	0.14
14	0.71	0.29	40.5	0.35
15	0.59	0.23	38.2	0.16
16	0.74	0.31	41.9	0.24

No water could be extracted from the peat soils from sites 6 and 7 using the arklone displacement method since the majority of the soil floated above the water and solvent and so the water could not be separated. The normal centrifuge method was therefore used to obtain the soil waters for these analyses.

The amount of total selenium in the extracted soil waters roughly reflects the selenium content of the soil (r=0.750), although the ratio of soil to water varied depending on the moisture content of the collected soil. The percentage of selenite in the solution varied from 0% in the acidic organic soil from site 5, to 72% in the waterlogged clay soil at site 2 and 76% at site 3, the improved moorland site.

The solutions obtained for the pyrophosphate extractable iron measurements were also analysed for total selenium content. This analysis was also hindered by the formation of precipitate during the complexation process, especially with samples from sites with high organic matter content, and apart from a few preliminary results (Table 6.43), this analysis was not continued.

The values for the extracted total selenium and selenite ion concentrations in the soil solutions have been used to provide a correlation matrix between these values and herbage selenium concentrations, total soil selenium concentrations and a number of other soil factors. This correlation matrix is given in Table 6.44. The most important relationship noticed from this correlation matrix is that the herbage selenium concentration shows a stronger relationship with the selenite ion concentration in the soil solution than with the total selenium concentration in the soil solution. This suggests that the selenite ion in the soil solution may be more readily available for plant uptake than the other forms of selenium present in the soil solution. However, no separation of selenate ion and organic selenium compounds was made in this analysis, and therefore no estimate of the relative availability of these compounds to plants could be made.

Table 6.44 **The correlation matrix of total selenium and selenite in soil solution with total soil and herbage selenium concentrations and other soil measurements**

	Total Se in soil soln.	Selenite in soil soln.	% Selenite in soil soln.	Total Se in extract (P)
Herbage Se	0.252	0.400*	- 0.253	0.188
Topsoil Se	0.750*	0.868*	0.084	0.652*
Subsoil Se	0.715*	0.847*	0.030	0.651*
Extractable Fe	0.021	0.359*	0.020	0.783*
Organic matter	0.122	0.766*	- 0.461*	0.790*
Cation Exchange	0.153	0.812*	- 0.460*	0.758*
% Clay	0.086	- 0.309*	- 0.130	0.066
% Silt	- 0.003	0.751*	0.230	0.618*

P = pyrophosphate extractable solution

CHAPTER 7

THE EFFECT OF FERTILISERS AND ORGANIC MATTER ON THE UPTAKE OF SELENIUM IN PASTURE PLANTS GROWN UNDER GREENHOUSE CONDITIONS

7.1 INTRODUCTION

The provision of greenhouse space at the botanical gardens of Royal Holloway College in Egham has enabled selenium uptake experiments to be carried out on pot-grown pasture plants during two consequetive summers. These two experiments were designed to further investigate some of the major factors controlling selenium uptake in pasture plants which were being studied as part of the field survey.

The first experiment aimed to study the interaction of sulphur in the soil with selenium uptake by plants and was extended to include the use of both sulphur containing fertilisers and nitrogen fertilisers.

The second experiment was designed to examine the effect of additions of organic matter to various soils, on native and applied selenium uptake by plants.

Sulphur, nitrogen fertilisers and organic matter have been shown to affect selenium uptake at high levels of selenium; however at lower selenium levels these factors have not been conclusively shown to alter the uptake of selenium, either native or applied, and consequently both these experiments were carried out using relatively low concentrations of added selenium solutions.

7.2 THE EFFECT OF FERTILISERS ON SELENIUM UPTAKE BY PLANTS GROWN IN GREENHOUSE CONDITIONS

Due to the chemical similarity between selenium and sulphur, the effects of sulphur in the soil on plant uptake of selenium have been widely studied. At high levels of soil selenium, sulphur (present as sulphate) has been shown to reduce the uptake of selenium by plants. This has been useful in some areas where the soils support vegetation containing toxic levels of selenium. However, at lower levels of soil selenium, the relationship between sulphur in the soil and plant uptake of selenium is not so clear. Much research has been carried out on the effects of sulphur on the uptake of selenium by plants and this has been reviewed in Chapter 2. The results found by earlier researchers have not shown a definite inhibition of plant selenium uptake by soil sulphur at lower selenium levels. Consequently this greenhouse experiment was devised to further investigate the relationship between soil sulphur concentrations and selenium uptake by plants.

The effect of other fertilisers on plant selenium uptake has also been investigated by other workers. Carter et al. (1972) found that phosphate additions increased plant selenium contents of plants grown on six out of fourteen soils, for both native and added selenium, and they considered that the effect might be sufficient to induce adequate levels of selenium in marginal pastures. Other work with N, P, S and Se additions to soils has shown that the effects of phosphate depended upon the level and interaction with other nutrients (Gissel-Nielsen, 1974).

In the case of superphosphate applications, the presence of both sulphur and phosphate in this fertiliser has so far prevented any comprehensive study of its effects on plant selenium uptake.

Little is known of the effects of nitrogenous substances on the uptake of selenium by plants. Cary and Gissel-Nielsen (1973) noted that nitrogen fertiliser application may reduce the uptake of selenium but that this is probably due to differences in uptake at the plant root, rather than an effect on the solubility of selenium in the soil.

7.2.1 Experimental Design

Sulphur is naturally present in soils at varying concentrations, but one of the most prevalent sources of sulphur addition to soils, both currently and in the past, is the use of sulphur containing fertilisers, such as ammonium sulphate and superphosphates. It was decided to add the sulphur required for this experiment in the forms in which it is used on pasture fields. Ammonium sulphate and superphosphate fertilisers were used as two treatments and, for comparison, ammonium nitrate was also included as a non-sulphate containing nitrogenous fertiliser.

Four different species of pasture plant were used in this experiment, two commonly occurring grass species and two clover species.

It was decided to grow the plants in vermiculite rather than in soil, in order to produce conditions similar to those of a solution culture. Vermiculite is assumed not to adsorb ions from soilution to any great extent. Therefore the plant uptake mechanisms could be studied independently without taking into consideration the interaction between soil and the ions in solution. The use of this artificial growth-medium also removed the problem of establishing the levels of nitrogenous material already present in the soil as a 'buffer' of fertiliser.

Dilute sodium selenite and sodium selenate solutions were added as teatments to the pots of plants which had been treated with the various fertilisers. Every combination of plant species (4), fertiliser type (3), selenium treatment (2) and control was included, in triplicate, in the experiment and several harvests from the pots were taken.

7.2.2 Experimental Procedure

The aim of the experiment was to investigate the effect of various fertilisers on selenate and selenite uptake in four grassland species.

The species chosen were ones commonly sown for permanent pasture in this country and which also thrive under greenhouse conditions.

The 4 species used were:

A.	*Trifolium repens*	S184 3/PB/500/310	(White Clover)
B.	*Trifolium pratense*	S123 9/PB/346/589	(Red Clover)
C.	*Dactylis glomerata*	Cambria O/PB/500/8	(Cock's-foot grass)
D.	*Lolium perenne*	S23 3/PB/500/302	(Perennial Rye grass)

The seeds for this experiment were obtained from:
Welsh Plant Breeding Station, (Bridfa Blanhigian Cymru), Plas Gogerddan, Aberystwyth, Dyfed,SY23 3EB. WALES.

The seeds were sown in medium grade vermiculite contained in 7" plastic pots with trays. Thirty-six pots of each species were sown with an even distribution of seed (fine sprinkling) on the surface of each pot. The seeds had all germinated within a week, although both grass species were more advanced than the clovers.

Since the seeds were growing in pure vermiculite, nutrients had to be provided, and for consistancy a nutrient solution was given regularly to all the pots after germination, even those receiving fertilizer applications as part of the experiment.

The Arnold-Hoagland nutrient solution (Peterson, 1969) was used as a reference mixture and this was modified for this experiment by the removal of sulphate ions.

Modified Arnold-Hoagland nutrient solution

	Concentration	Stock solution ml/dm^3
KNO_3	1 M	50
$Ca(NO_3)_2.4H_2O$	1 M	50
KH_2PO_4	1 M	30
$Mg(NO_3)_2.6H_2O$	1 M	20
NaFeEDTA	0.1 M	10
H_3BO_3		
$MnCl_2.4H_2O$	10^{-3} M	10
$CuCl_2.2H_2O$	of each	
$ZnCl_2$		
H_2MoO_4		

10 ml of this stock solution was added to each pot at each application.

Using 36 pots of each of 4 species (144 pots), 12 different treatments were administered, with triplicates of each for comparison. For statistical purposes, all the pots were randomised within the greenhouse to reduce any effects of sunlight, temperature and watering variations.

The 12 treatments given to each species were:

1.	Control	+ Nutrient Solution (N.S.) only
2.	Selenite solution	+ N.S.
3.	Selenate solution	+ N.S.
4.	Ammonium sulphate solution	+ N.S.
5.	Ammonium nitrate solution	+ N.S.
6.	Superphosphate fertilizer	+ N.S.
7.	Selenite solution + ammonium sulphate solution	+ N.S.
8.	Selenite solution + ammonium nitrate solution	+ N.S.
9.	Selenite solution + superphosphate fertilizer	+ N.S.
10.	Selenate solution + ammonium sulphate solution	+ N.S.
11.	Selenate solution + ammonium nitrate solution	+ N.S.
12.	Selenate solution + superphosphate fertilizer	+ N.S.

All treatments were watered with tap water and given nutrient solution as the controls required.

The concentrations of the selenium solutions given were low, similar to the concentrations in pasture soils marginally deficient in selenium, which are those of interest in this work. The fertiliser concentrations were calculated from ADAS recommended levels for permanent pasture (M.A.F.F., 1981). About 1/4 of the annual recommended dose was used and converted from kg/ha to g/7" pot. The concentrations of the solutions added as treatments are given below.

Treatment solutions:-

Sodium selenite solution

100 ml of 0.5 μg/ml solution of Na_2SeO_3 to each pot.

Sodium selenate solution

100 ml of 0.5 μg/ml solution of Na_2SeO_4 to each pot.

Ammonium sulphate solution

$(NH_4)_2SO_4$	22% N	To provide 40 kg/ha N
3.2 g per pot	10 ml of solution (321 g/l) per pot	

Ammonium nitrate solution

NH_3NO_3	34% N	To provide 40 kg/ha N
2.1 g per pot	10 ml of solution (208 g/l) per pot.	

Superphosphate solution.

$Ca(H_2PO_4)_2CaSO_4$	17% P_2O_5	To provide 20 kg/ha P_2O_5
2.0 g per pot	10 ml of solution (201 g/l) per pot.	

The first treatment solutions, both selenium and fertiliser, were given as soon as the plants were greater than 2 cm in height, and 3 harvests were taken about 1 month apart, cutting herbage to 2.5 cm above the vermiculite using stainless steel scissors. After 3 harvests a second treatment was given and two further harvests were taken of the mature plants. The dates of the treatments and harvests are given below.

Seeds sown	15/1/86
Seeds germinated	22/1/86
Nutrient solution added (N.S.)	4/2/86 + 24/2/86
1st treatment + N.S.	4/3/86
Harvest grasses only	17/3/86
1st harvest + N.S.	14/4/86
2nd harvest + N.S.	20/5/86
3rd harvest + N.S.	19/6/86
2nd treatment + N.S.	3/7/86
4th harvest	17/7/86
5th harvest	24/7/86

The herbage from the harvests was dried at 30 °C and stored in paper sample bags. The dry weight from each pot at each harvest was recorded.

7.2.3 Experimental Results

Five harvests were taken during the course of this experiment, three following the addition of the first treatments and two more following a second addition of the same treatments. The results from the triplicate pots have been averaged and the standard deviation is shown as error bars on the histograms.

The results displayed in Figures 7.1-7.12 have been divided into herbage selenium concentration, herbage dry weight and selenium uptake for each of the four species, and all five harvests are shown together in each histogram.

The same results have then been arranged harvest by harvest for all four species in order to show the differences between the species more clearly (Figures 7.13-7.27).

Selenium was added to treatments 2, 3 and 7-12 before harvests 1 and 4 and in all these pots there is a gradual decrease in selenium content from harvest 1-3, then an increase again with harvest 4 after the second selenium addition which falls off once more with the subsequent harvest 5 (Figures 7.1-7.4). This is merely a

response to the length of time after the addition of selenium. The plants were growing much more strongly by the time the second selenium treatment was added and consequently the concentration found in the herbage is lower than that of the first harvest since the selenium is diluted by an increase in dry weight. The **uptake** of selenium however is greatly increased for all species and treatments in the fourth harvest when the strong growth of the plants allowed a greater amount of the added selenium to be taken up.

In Figures 7.1-7.4 it will be noticed that the selenium concentration of those plants given selenite treatment (Treatments 2, 7-9) is higher than that of the selenate treated plants (Treatments 3, 10-12). This is most noticeable in the early harvests but the effect is still discernable in the later harvests. When plants are grown in soil, selenate is usually taken up to a much greater extent than selenite, in direct contrast to the situation here. In soils the differences in plant selenium accumulation can probably be accounted for by the adsorption of selenite ions onto ferric-oxide complexes which renders the selenite unavailable for plant uptake, unlike selenate ions which remain in solution (Geering et al., 1968). Since the plants were growing in vermiculite which essentially approximates to a solution culture, the selenite ions are not bound to the particle surfaces since the iron provided is in the form of iron-EDTA, so the selenite is not sorbed and is therefore available to the plants. It would appear from these results that when either selenite or selenate ions are present in an available form, selenite is taken up more readily by plants. The smaller size of the selenite ion may partially explain this.

The addition of sulphate containing fertilisers (Treatments 4, 6, 7, 9, 10 and 12) does not appear to have any significant effect on the concentration of added selenium found in either the grass or the clover species. Also the phosphate fertiliser treatments have not produced plants with different selenium concentrations to those treated with nitrogenous fertilisers.

Figures 7.5-7.8 show the dry weight collected from the plants at each harvest. In all cases the dry weight increases with successive harvests and then falls off at harvest 4 or 5 when the plants were probably becoming limited by the nutrient solution added. Also there was a shorter time interval between the fourth and fifth harvests than between the others which accounts for some of the decline in

dry weight recorded.

The two clover species (A, *Trifolium repens* and B, *Trifolium pratense*) show a remarkably similar pattern of dry weight for all treatments, with only the control plants, which obviously received less fertiliser, showing any lower dry weight than all the other plants. The pattern for each treatment at each harvest is also very similar. However with the two grass species (C, *Dactylis glomerata* and D, *Lolium perenne*) there is a strong growth response in the plants which received nitrogenous fertiliser treatments. The grasses receiving phosphate fertiliser treatments showed only a slight increase in dry weight over the control plants. The lack of growth response of the clovers to the nitrogenous fertilisation suggests that they were fixing nitrogen despite being grown in vermiculite. This was also concluded from the nodulated appearance of the clover roots at the end of the experiment.

The strong growth increase shown by the grass species with nitrogen fertiliser additions has produced an increase in the total uptake of selenium by these plants, especially at the fourth harvest when the plants were better established Figures 7.9-7.12). However this increase in grass growth has produced a fall in the selenium concentration of the plants due to a dilution effect. The clovers show an increase in selenium uptake with all types of fertilisers over the uptake of the controls.

In all species the uptake of selenium falls off with successive harvests after the first selenium addition, except for the early harvest where the uptake is low due to the smaller amount of dry weight. The uptake of selenium cannot be said to be supressed by the addition of sulphate containing fertilisers at this level of selenium and sulphur additions.

One thing which is noticed in Figures 7.9-7.12 is a reasonably high uptake in harvest 4 for treatments 4, 5 and 6 with *Trifolium pratense* , *Dactylis glomerata* and *Lolium perenne* when these plants have not received any added selenium treatment. In some cases the uptake is equivalent to that seen in the plants which had received added selenium. This has only occurred at harvest 4 when the plants were growing very strongly and the second selenium treatment had just been administered. The only way in which selenium could have been transferred to

these control plants is aerially, by volatilisation from the other plants and taken up through the leaves or possibly absorbed from the surface of the pots. Interestingly species A (*Trifolium repens*) produces much smaller plants than any of the other three and so possibly is little affected by the uptake of volatilised selenium through the leaf surface. Also the control plants which received no fertilisers (Treatment 1) are less affected by this, and this may be connected to the fact that they produced smaller plants generally. This phenomenon would suggest that the control plants had taken up volatilised selenium from the treated plants, possibly absorbing it through the leaves since the effect seems to be dependant upon the amount of foliage present. This could not be proved with this experiment since it was not designed to take volatilisation of selenium into account, but if uptake from volatilised selenium does occur then it would have a profound impact on the interpretation of all greenhouse experiments involving selenium additions where the separate treatments are contained in the same part of the greenhouse.

With harvest 5 the levels of selenium uptake are back to those expected for control pots, however the question of volatilisation as a means of losing and accumulating selenium by plants requires further study.

Figures 7.13-7.27 show the same results as Figures 7.1-7.12, but in more detail, and have highlighted the differences between the four species used in the experiment.

The differences in dry weight produced by the clover and grass species were quite noticeable, especially once the plants were established. Both grass species produced a greater dry weight than clovers when treated with nitrogenous fertilisers, but in all other situations the clover species tended to produce a slightly greater dry weight.

The selenium concentrations of the clover species was lower than those of the grasses in most cases when established, especially *Dactylis glomerata* . The other grass species *Lolium perenne* occasionally had the highest selenium concentration when treated with nitrogenous fertilisers. The most noticeable difference in selenium concentration was between the two species of grass confirming that species differences in sward can greatly affect the selenium content

of a pasture on any given soil. However the selenium concentrations of the plants was very variable and depended inversely on the dry weight produced by the plants. The general concentrations of selenium in the different plant species tended to decrease in the order *Lolium perenne* > *T. repens* > *T. pratense* > *Dactylis glomerata* , although there are many exceptions to this order.

The differences in selenium uptake by the four species were strongly affected by the variations in dry matter produced. *Lolium perenne* produced the greatest selenium uptake when combined with nitrogenous fertilisation. *Dactylis glomerata* on the other hand had a lower uptake than the clovers in most cases. There were only slight differences in uptake between the two clover species, with *T. repens* producing a slightly greater uptake than *T. pratense* in the majority of cases. The general uptake of selenium by the different plant species tended to decrease in the order *Lolium perenne* > *T. repens* ≥ *T. pratense* > *Dactylis glomerata* . The increased uptake of *Lolium perenne* was somewhat reduced by the fifth harvest suggesting perhaps that this species has a quicker response to the fertiliser and selenium treatments than the others, but that the difference in uptake is not as great in the long term.

The experiment originally aimed to investigate whether sulphur in the form of sulphate would depress the uptake of selenate in pasture plants at low levels of sulphur and selenium additions. Generally there seems to be little, if any, interaction between sulphur and selenium throughout the experiment, although the results of the fourth and fifth harvests do show a very slight depression of selenate uptake in the sulphur containing fertiliser treated plants although the selenite uptake is not affected. However this depression is so small that it could easily lie within the boundaries of experimental error and in general sulphate is seen to have almost no effect on the uptake of selenium by pasture plants at these levels of selenium and sulphur additions.

Figure 7.1 **The average selenium concentration (μg/g) in *Trifolium repens* found for all 12 fertiliser treatments**

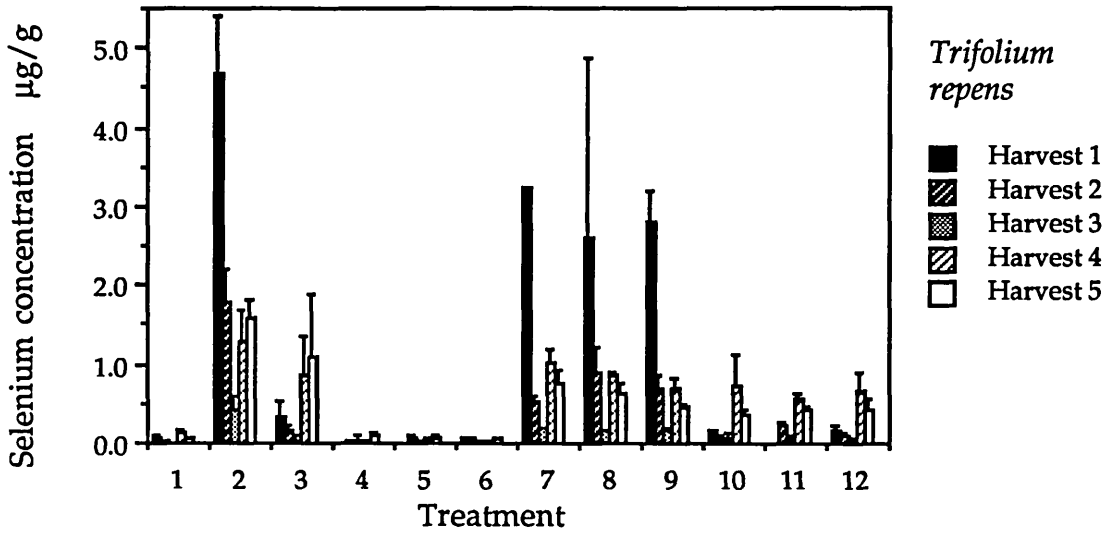


Figure 7.2 **The average selenium concentration (μg/g) in *Trifolium pratense* found for all 12 fertiliser treatments**

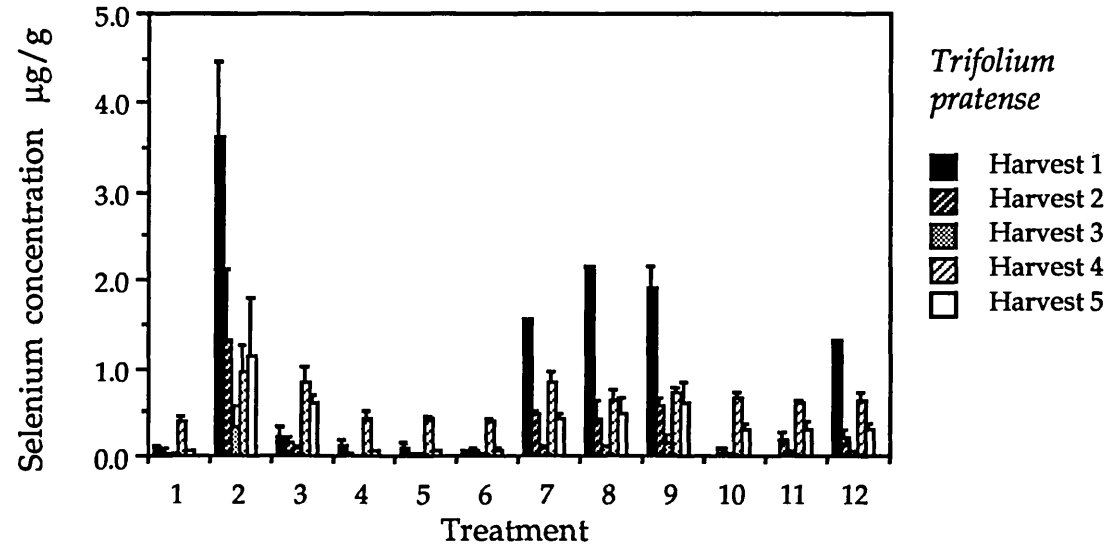


Figure 7.3 **The average selenium concentration (μg/g) in *Dactylis glomerata* found for all 12 fertiliser treatments**

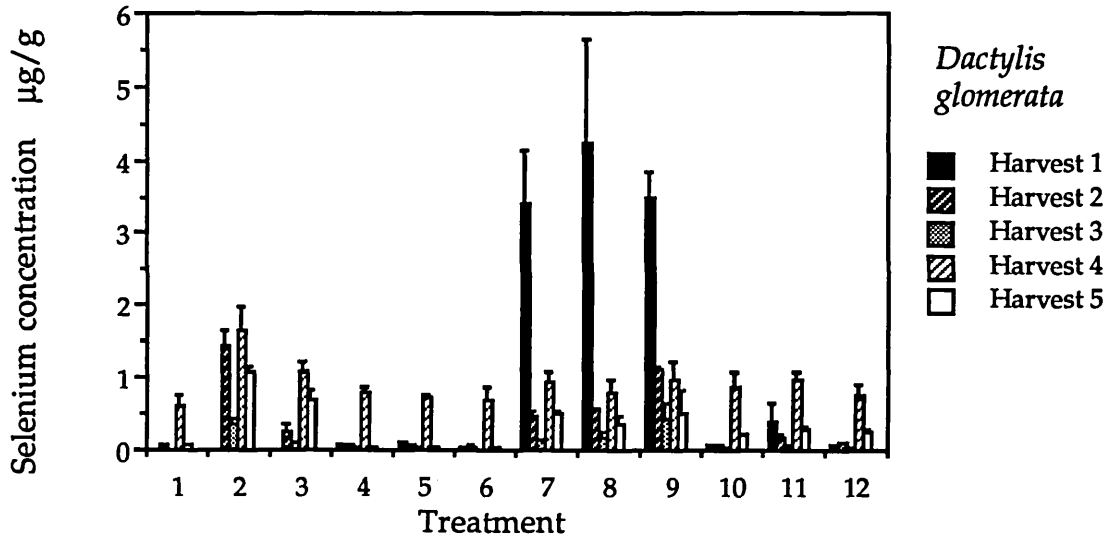


Figure 7.4 **The average selenium concentration (μg/g) in *Lolium perenne* found for all 12 fertiliser treatments**

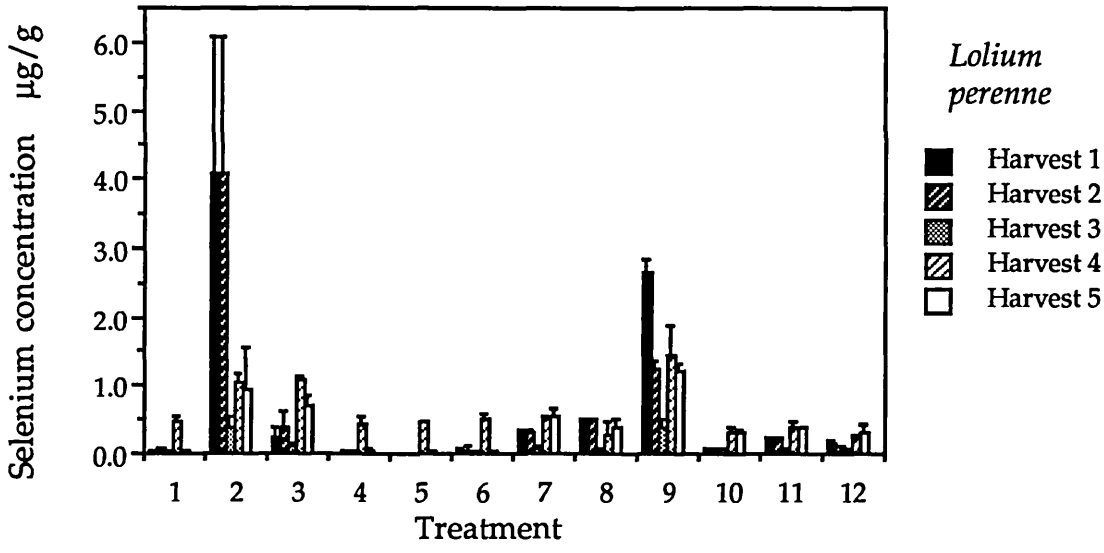


Figure 7.5 **The average dry weight (g/pot) in *Trifolium repens* found for all 12 fertiliser treatments**

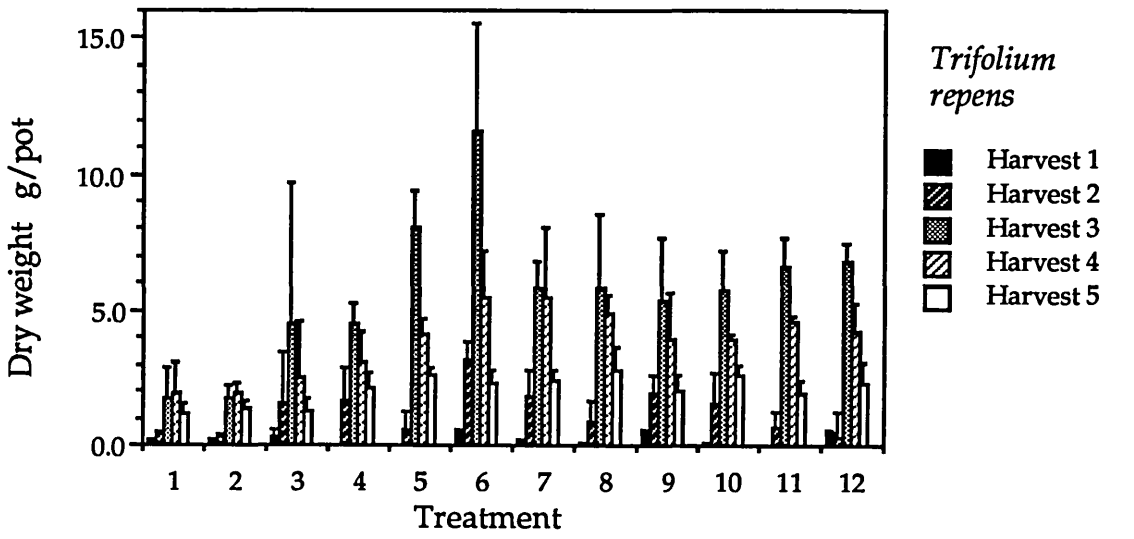


Figure 7.6 **The average dry weight (g/pot) in *Trifolium pratense* found for all 12 fertiliser treatments**

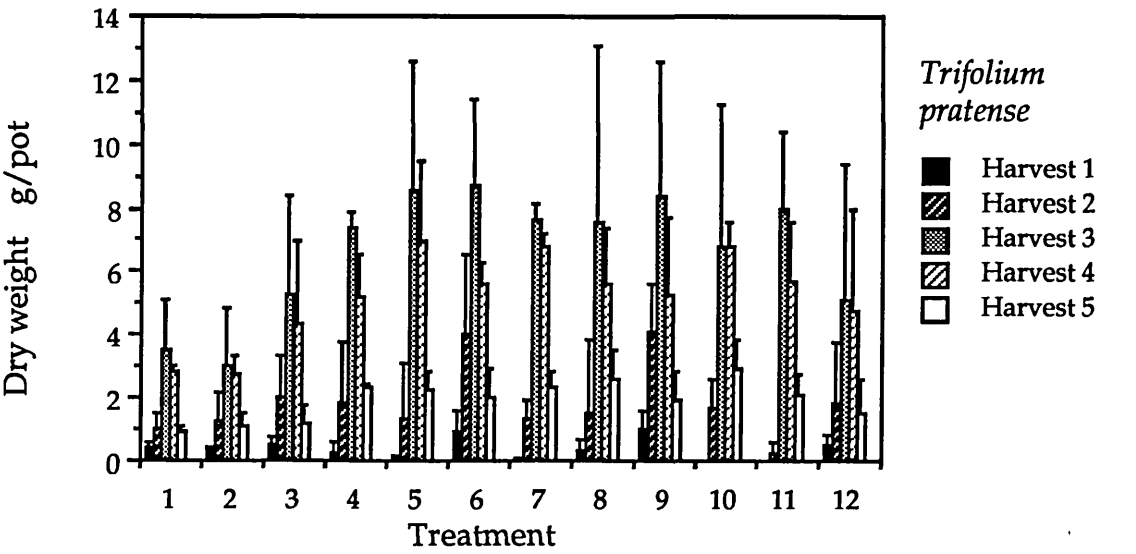


Figure 7.7 **The average dry weight (g/pot) in *Dactylis glomerata* found for all 12 fertiliser treatments**

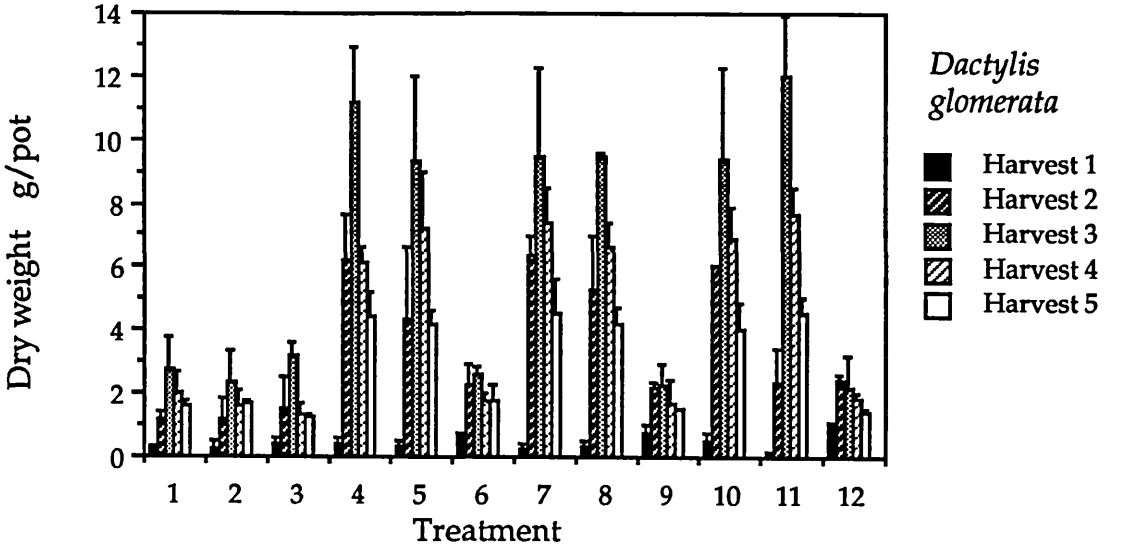


Figure 7.8 **The average dry weight (g/pot) in *Lolium perenne* found for all 12 fertiliser treatments**

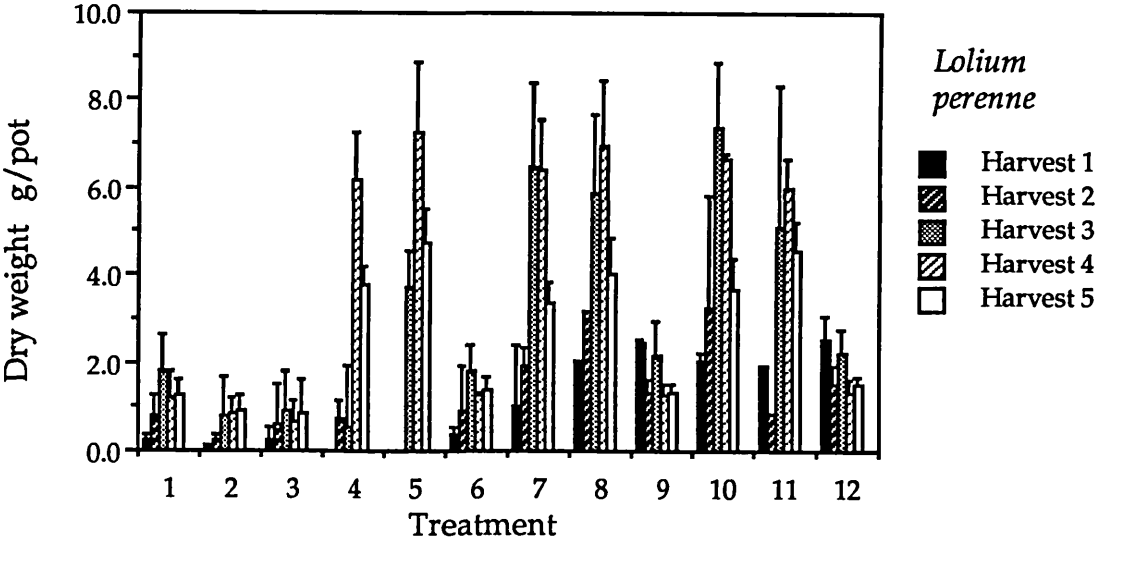


Figure 7.9 The average selenium uptake (μg/pot) in *Trifolium repens* found for all 12 fertiliser treatments

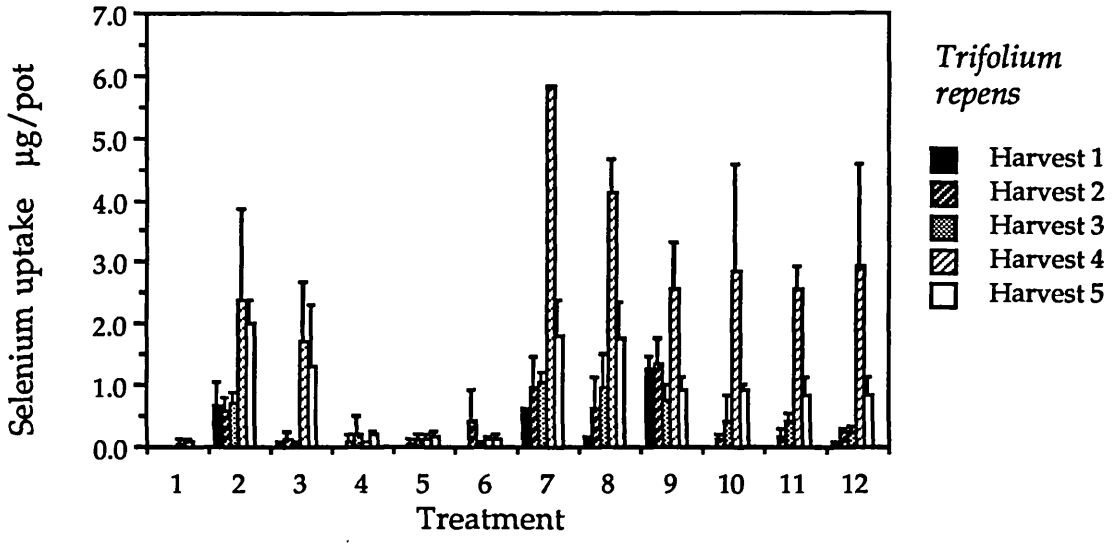


Figure 7.10 The average selenium uptake (μg/pot) in *Trifolium pratense* found for all 12 fertiliser treatments

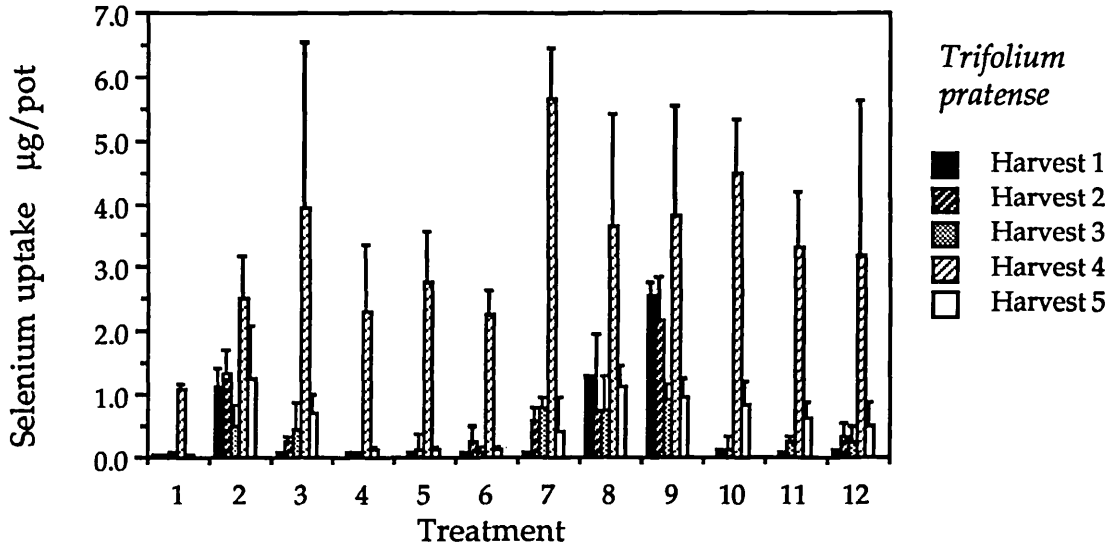


Figure 7.11 **The average selenium uptake (µg/pot) in *Dactylis glomerata* found for all 12 fertiliser treatments**

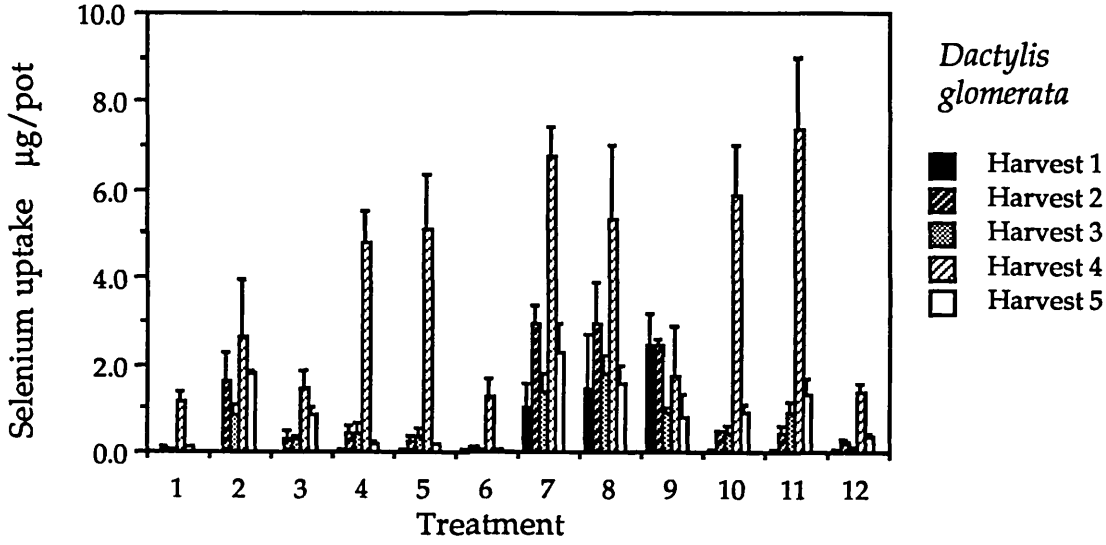


Figure 7.12 **The average selenium uptake (µg/pot) in *Lolium perenne* found for all 12 fertiliser treatments**

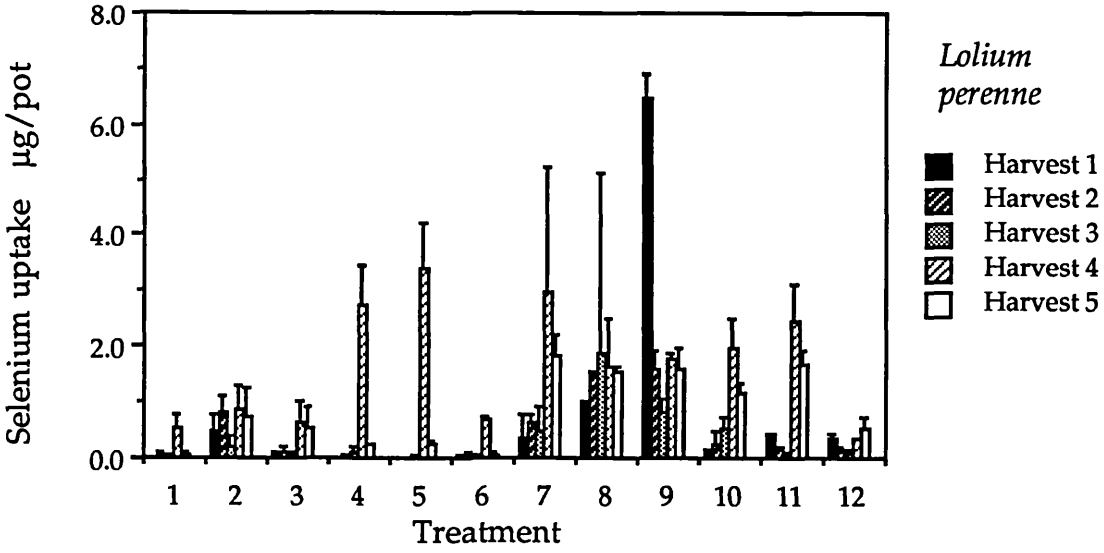


Figure 7.13 The average selenium concentration (μg/g) found at the first harvest in all plant species for the 12 fertiliser treatments

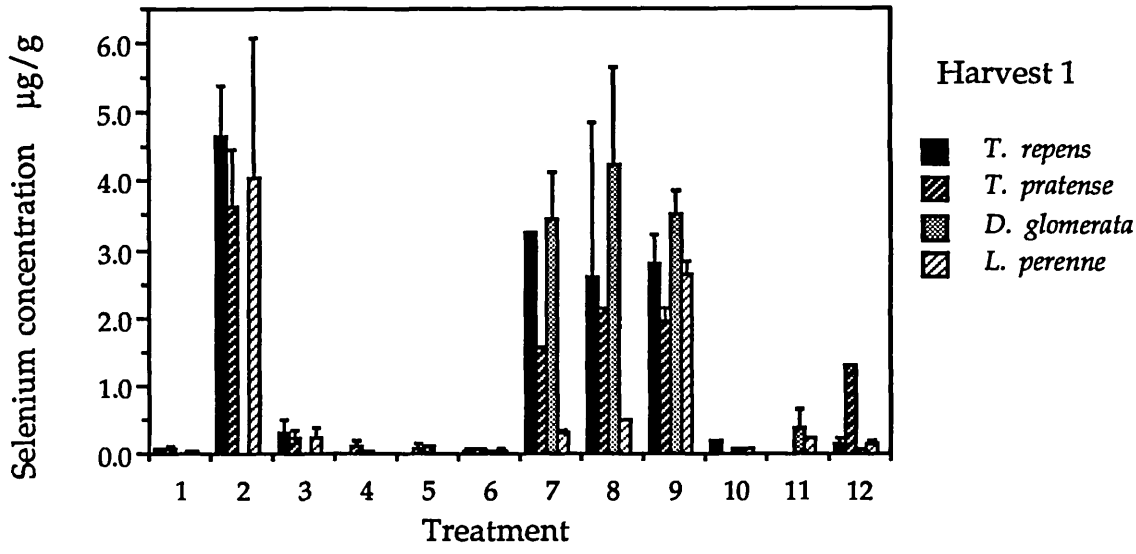


Figure 7.14 The average dry weight (g/pot) found at the first harvest in all plant species for the 12 fertiliser treatments

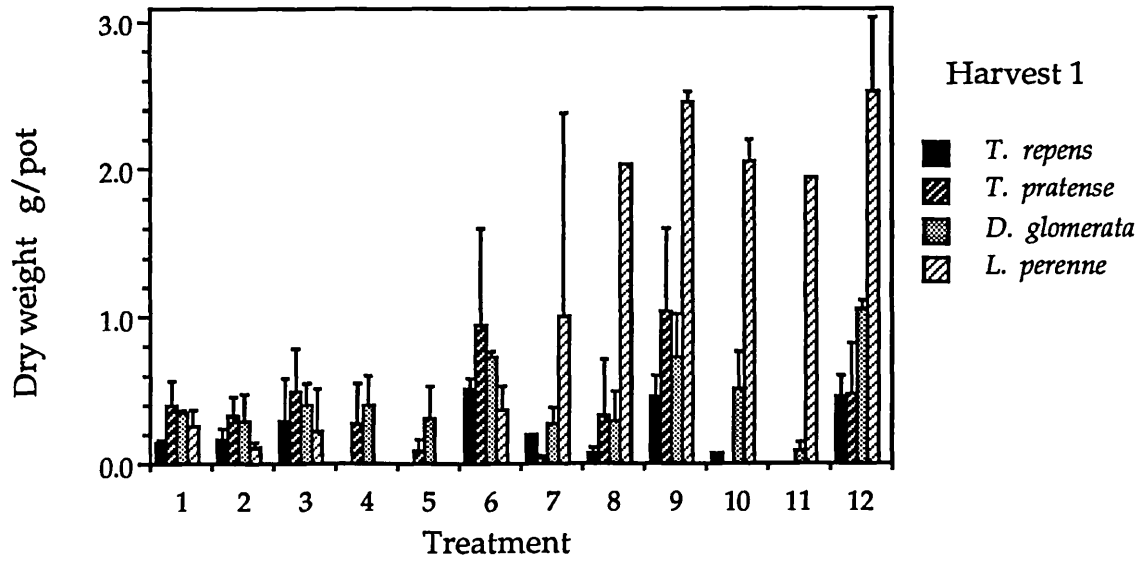


Figure 7.15 The average selenium uptake (μg/pot) found at the first harvest in all plant species for the 12 fertiliser treatments

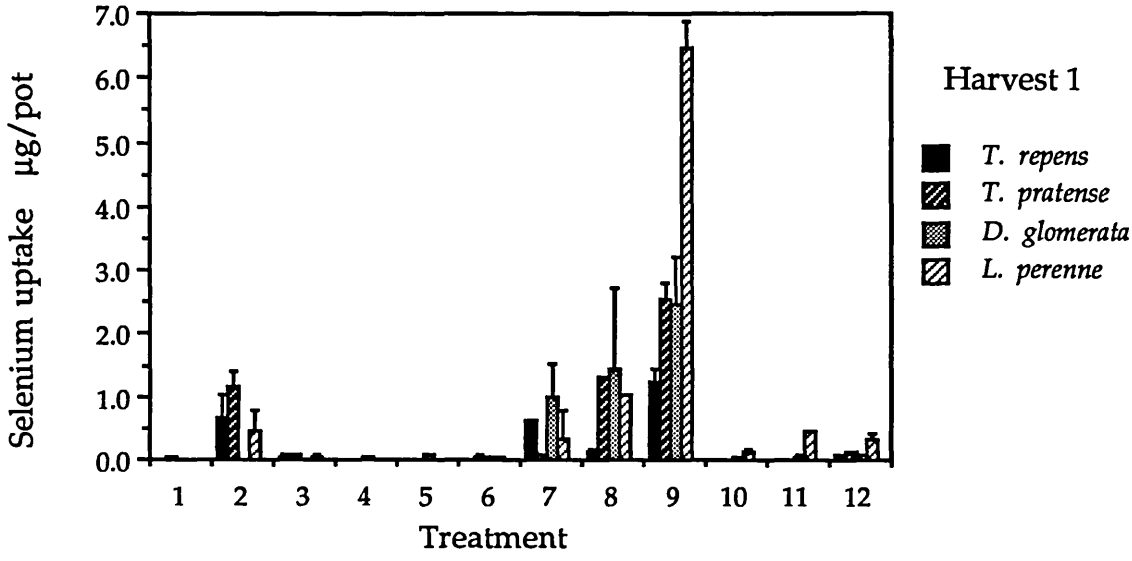


Figure 7.16 The average selenium concentration (μg/g) found at the second harvest in all plant species for the 12 fertiliser treatments

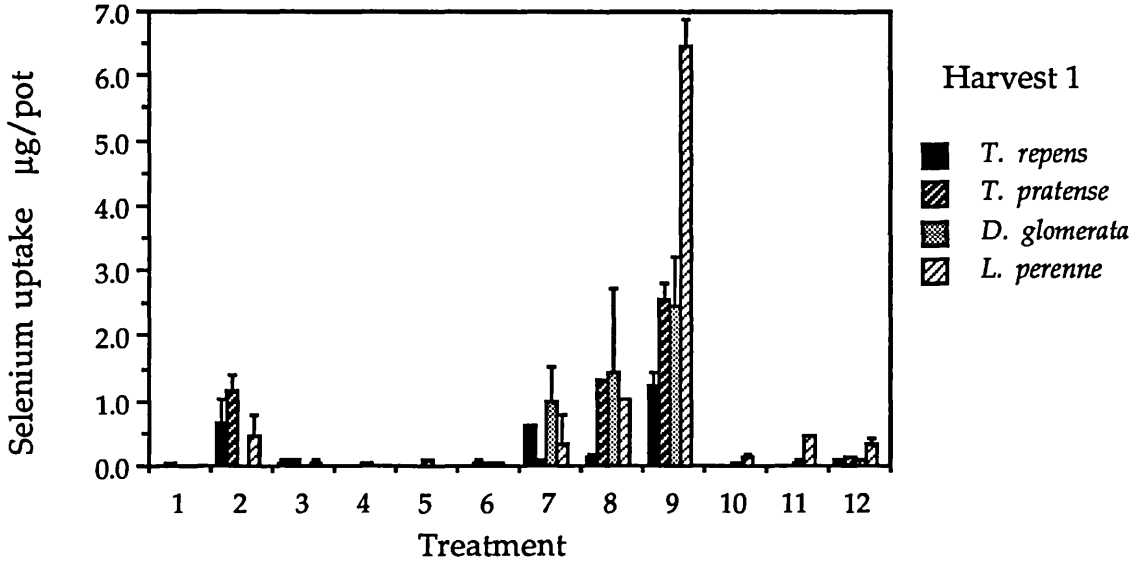


Figure 7.17 The average dry weight (g/pot) found at the second harvest in all plant species for the 12 fertiliser treatments

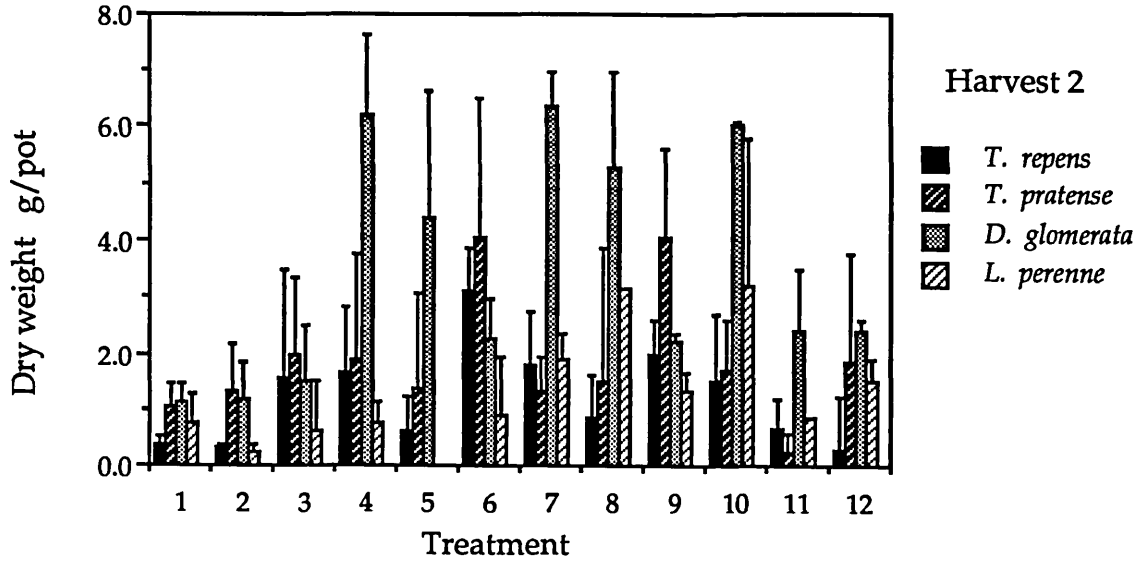


Figure 7.18 The average selenium uptake (µg/pot) found at the second harvest in all plant species for the 12 fertiliser treatments

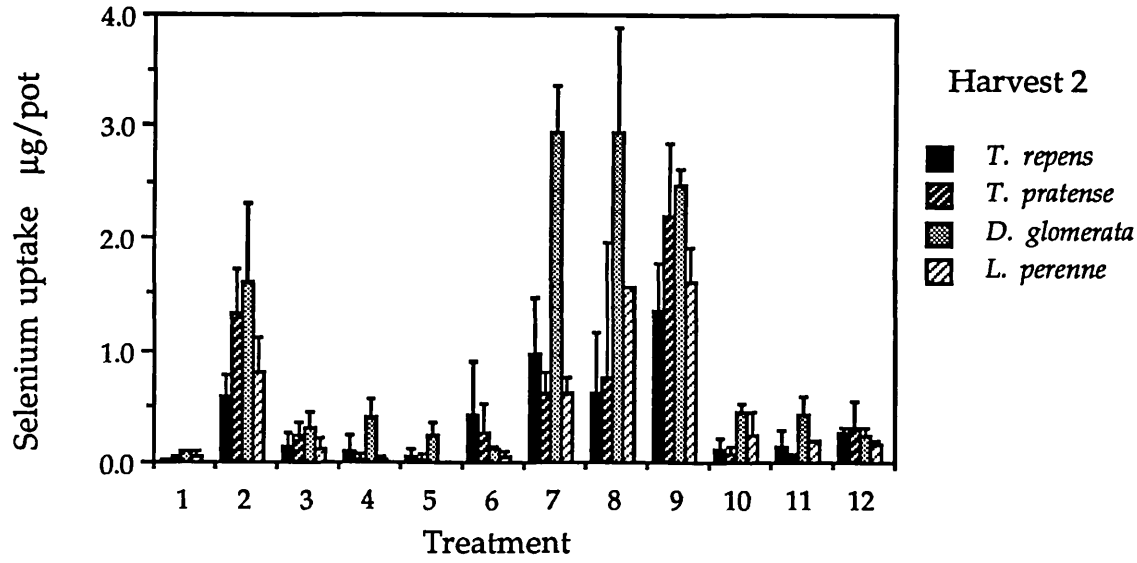


Figure 7.19 **The average selenium concentration (µg/g) found at the third harvest in all plant species for the 12 fertiliser treatments**

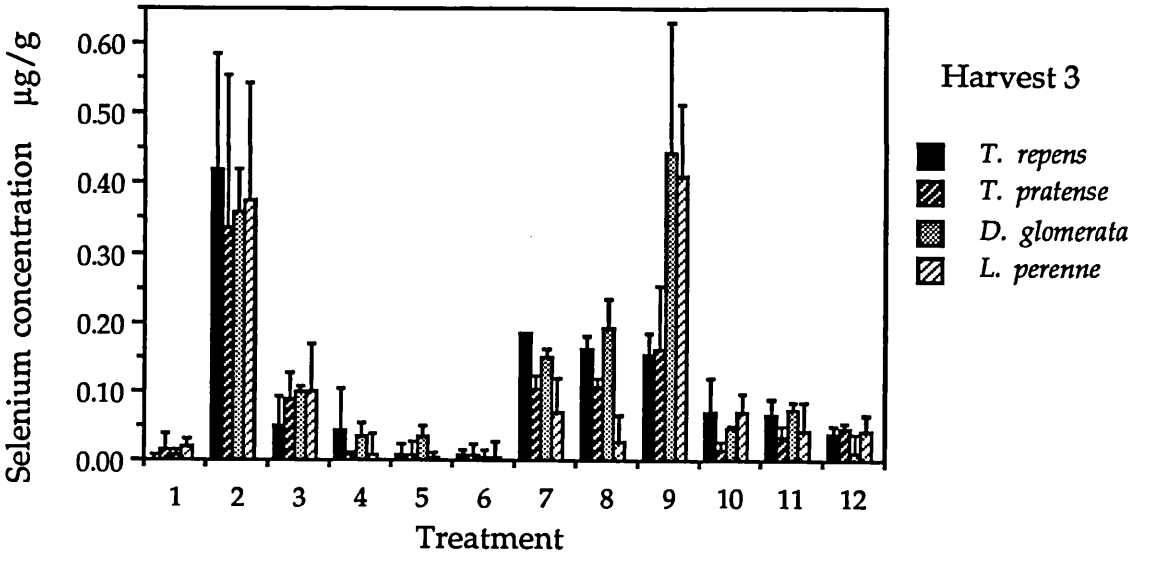


Figure 7.20 **The average dry weight (g/pot) found at the third harvest in all plant species for the 12 fertiliser treatments**

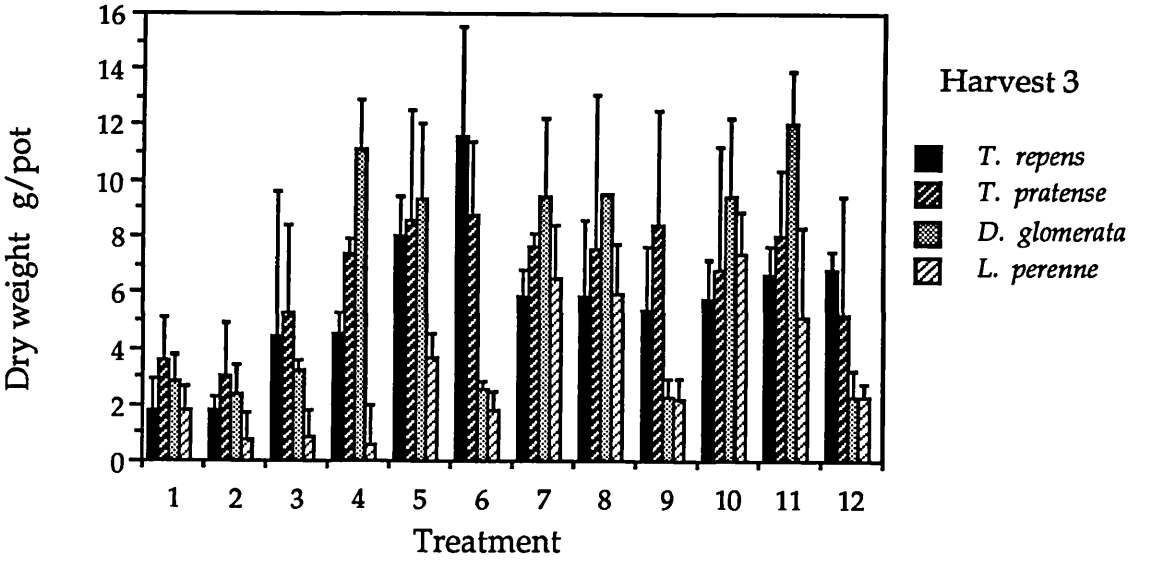


Figure 7.21 The average selenium uptake (µg/pot) found at the third harvest in all plant species for the 12 fertiliser treatments

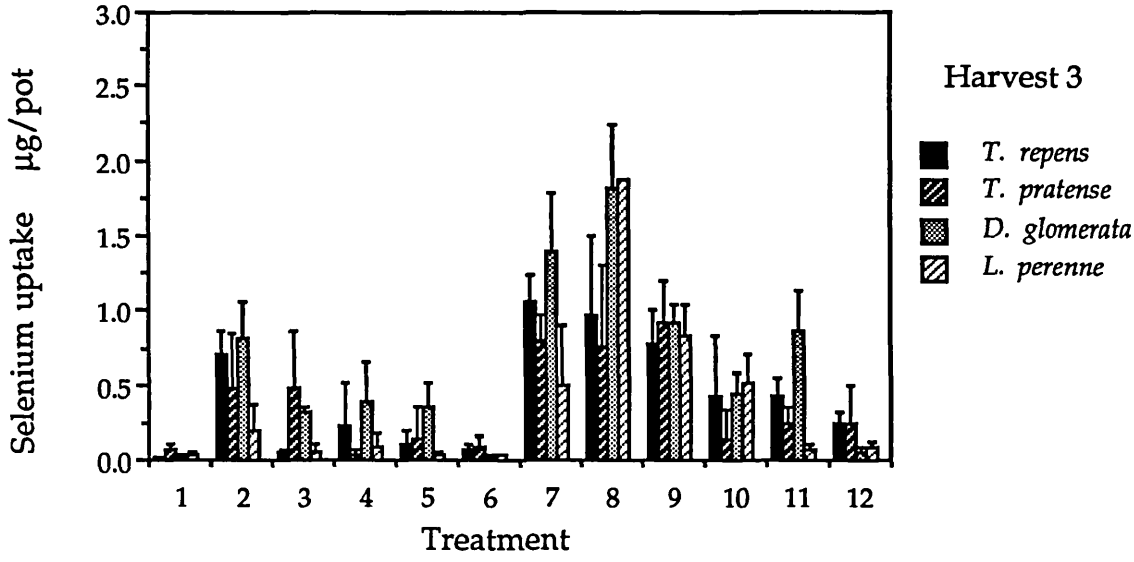


Figure 7.22 The average selenium concentration (µg/g) found at the fourth harvest in all plant species for the 12 fertiliser treatments

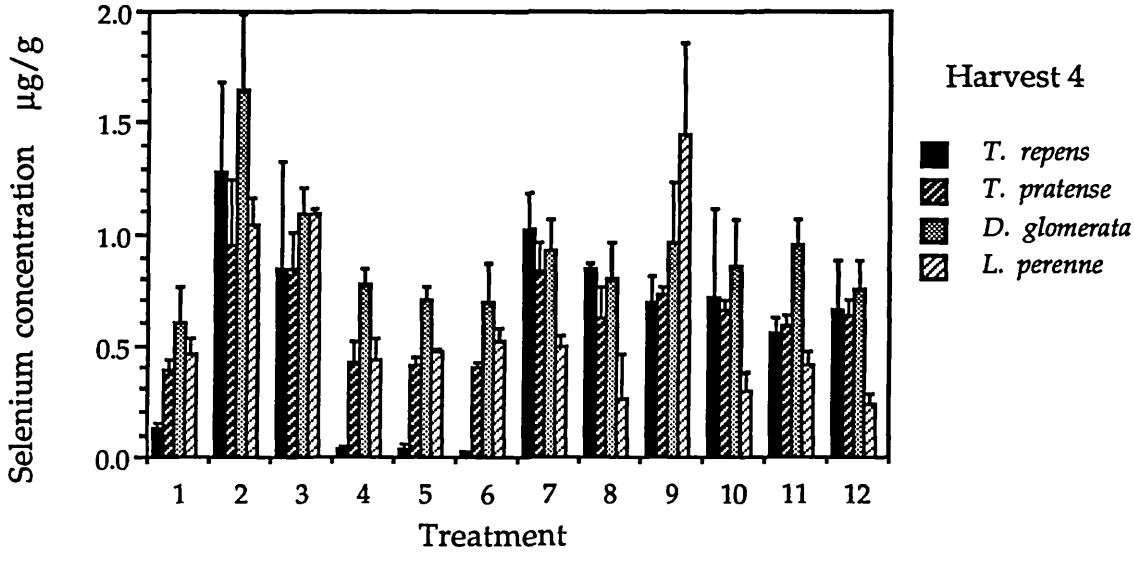


Figure 7.23 The average dry weight (g/pot) found at the fourth harvest in all plant species for the 12 fertiliser treatments

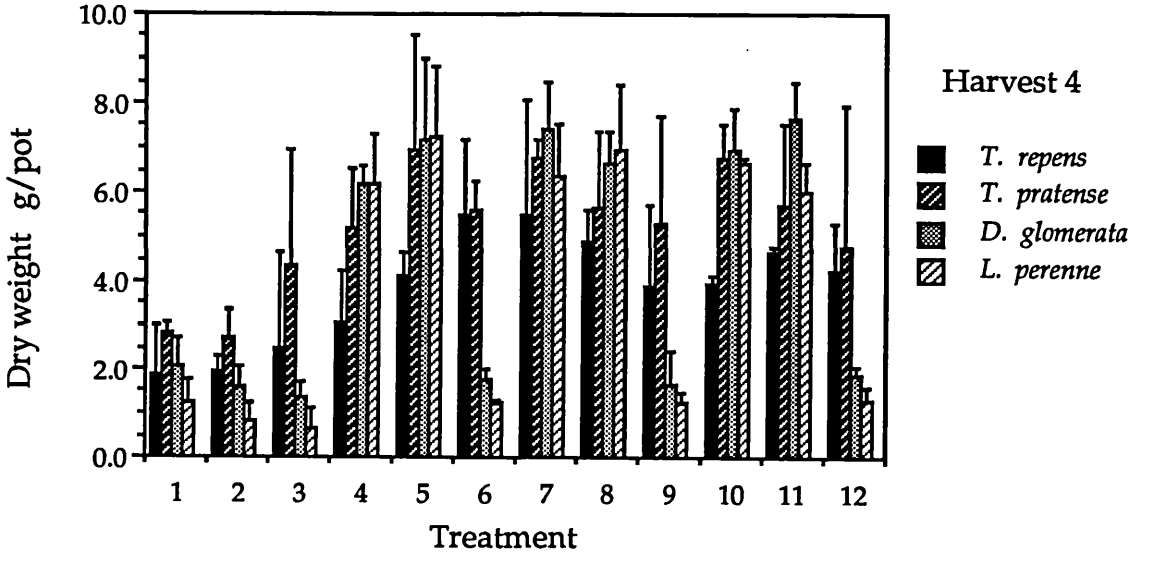


Figure 7.24 The average selenium uptake (µg/pot) found at the fourth harvest in all plant species for the 12 fertiliser treatments

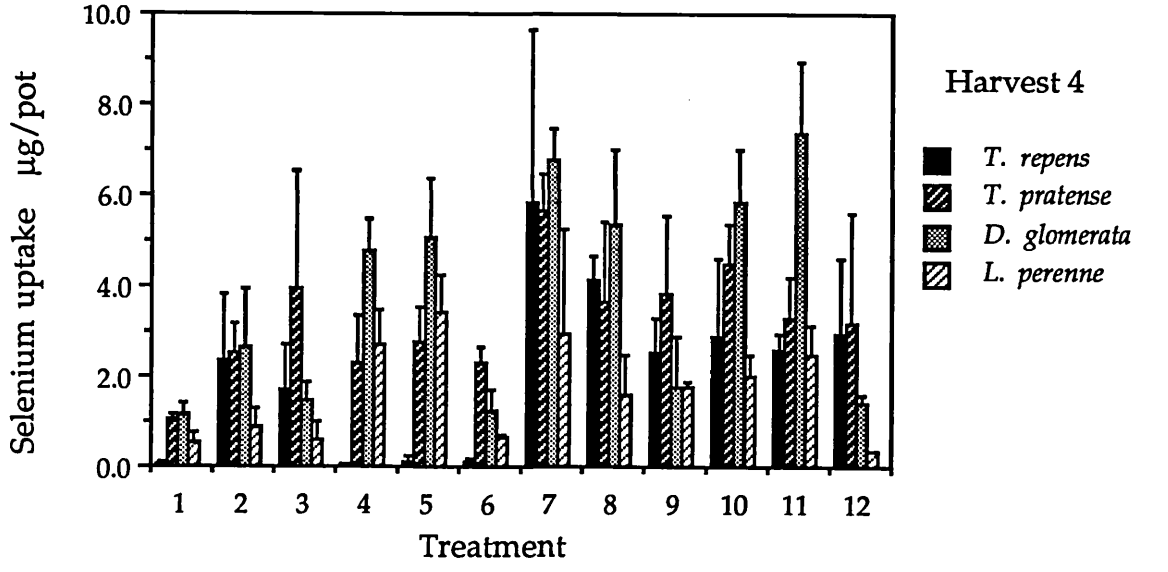


Figure 7.25 The average selenium concentration (μg/g) found at the fifth harvest in all plant species for the 12 fertiliser treatments

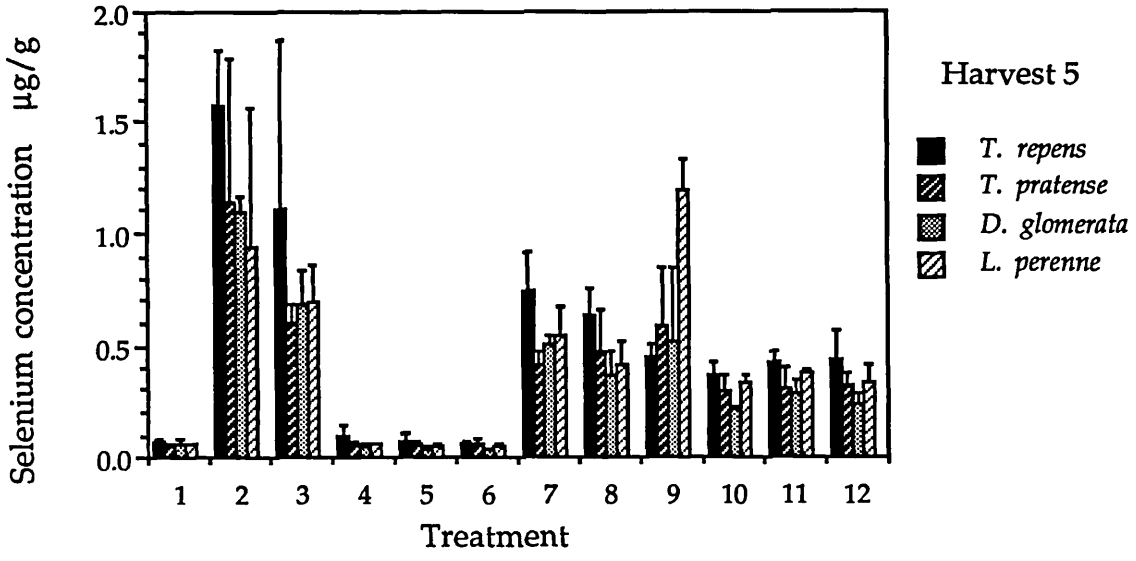


Figure 7.26 The average dry weight (g/pot) found at the fifth harvest in all plant species for the 12 fertiliser treatments

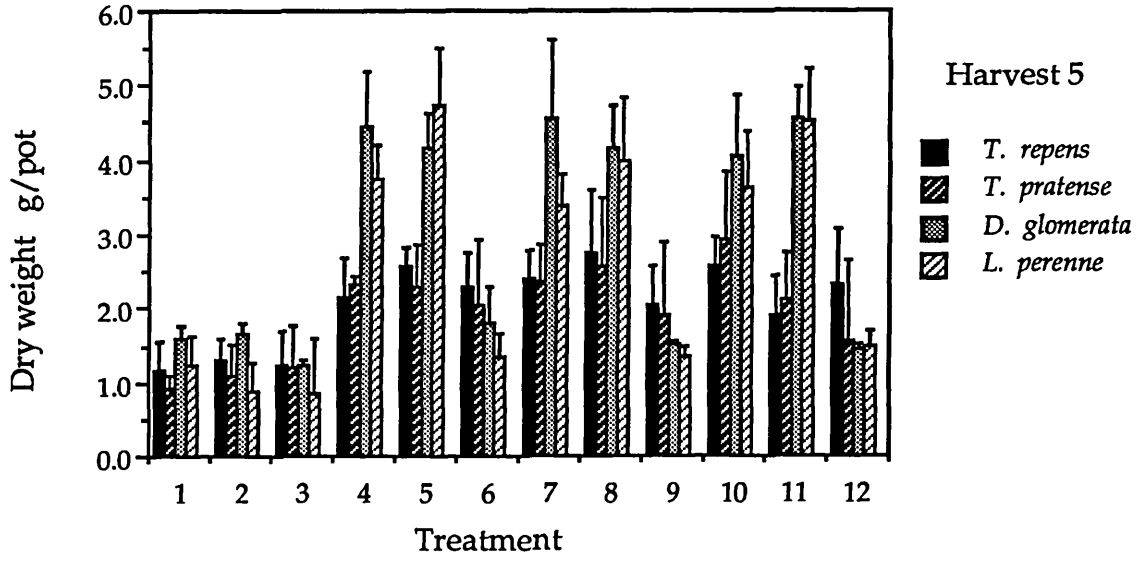
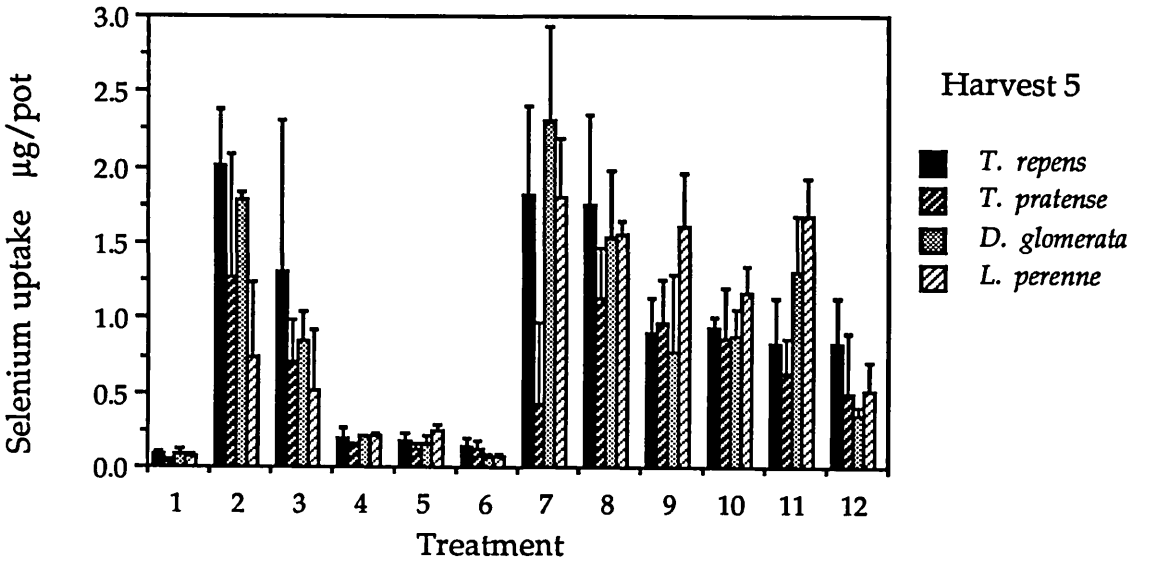


Figure 7.27 The average selenium uptake (µg/pot) found at the fifth harvest in all plant species for the 12 fertiliser treatments



7.3 THE EFFECT OF ORGANIC MATTER ON SELENIUM UPTAKE BY PLANTS GROWN UNDER GREENHOUSE CONDITIONS

The interaction of selenium with organic matter in the soil has been realised for some time, generally the greater the organic matter content of the soil the greater the total selenium levels. The organic matter content of a soil also affects the pH and drainage status of a soil.

Misra and Tripathi (1972) found a correlation between organic matter in soils and both total and water soluble selenium (r= +0.291 and r= +0.317 respectively). In a study of some British soils, the selenium content in both the soil and soil solution increased with increasing organic matter content (Van Dorst, 1984). Soils with high organic matter contents had low selenate levels and higher levels of selenite plus other selenium species, possibly including organic selenium compounds; soils low in organic matter showed the reverse effect. These trends were also found in the soil solution extracted from the same soils. Organic soils have been reported to retain selenium to a greater extent than mineral soils (Hupkens van der Elst and Tetley, 1970, Levesque, 1974a, Nye and Peterson, 1975, Gissel-Nielsen, 1976). Organic matter is also considered to impede leaching of selenium through soils and to diminish volatilisation of selenium from soil (Hamdy and Gissel-Nielsen, 1976a). Gissel-Nielsen and Hamdy (1977) found that leaching of selenite from Danish soils of low 'available' selenium was decreased by the addition of organic matter.

Organic material in the soil, derived from plant and animal detritus, often has relatively high levels of selenium compared to soil and there has been some postulation that the selenium contained in organic material may be in a form which is readily available to plants. An extract of organic selenium from the selenium accumulator *Astragalus racemosus* was rapidly accumulated by plants grown in culture solutions (Trelease and Disomma, 1944; Trelease and Greenfield, 1944). However there has been no recent work to confirm this suggestion, especially for non-accumulator species.

The interaction of organic matter in soils and selenium uptake by plants appears to involve two main factors. Firstly the organic material in the soil may

act as a chelating medium, binding selenium to the soil particles, impeding leaching and concentrating selenium in the upper soil layers and the rooting zone. In mineral soils, iron oxides form insoluble complexes with selenite in the soil, but in very organic soils the iron concentration may be low and adsorption of selenium onto the surface of organic material may replace the adsorption processes usually associated with iron oxides in the soil. Secondly the selenium content of the organic material itself is often higher than that of the soil and so increasing levels of organic matter may increase the total selenium content in the soil environment.

A greenhouse experiment was devised to investigate the interaction of different levels of organic matter in soils on the uptake of native and added selenium into perennial ryegrass, using soils collected from some of the field sites.

7.3.1 Experimental Design

Three field sites (Site 10, Derbyshire, 1.363 μg/gSe; Site 9, Brecon, 0.134 μg/g Se; and Site 3, North Wales, 0.434 μg/g Se) which have varying natural levels of selenium were chosen to reflect the range of selenium content found in the soils collected during the field investigation. Each soil was mixed with peat to produce three different ammendments; no added peat, 25% peat by volume and 50% peat by volume (X, Q, H).

In order to investigate the effect of different soils and differing levels of organic matter on selenium uptake, selenite and selenate solutions were added to some of the pots after the grass plants had become established. For each combination of soil type and organic matter ammendment, the selenium treatments given were; no selenium (control), sodium selenite solution (50 ml, 1 μg/g), and sodium selenate solution (50 ml, 1 μg/g) (0, 4, 6). A grass harvest was taken just before and one month after this addition of selenium.

Each type of treatment was repeated on three pots of grass and hence 81 pots were used for this experiment (3 soils x 3 peat mixtures x 3 selenium treatments x 3 replicates).

7.3.2 Experimental Procedure

In July 1987 enough soil was collected from the three chosen field sites (Sites 10, 9 and 3) to fill 27 x 7" plant pots each. The soil was taken from just underneath the turf and was predominantly topsoil (0-15 cm).

The soil was sieved while still moist through a 5 mm sieve to remove large stones and most of the plant root material.

The field soil was mixed with peat where required to produce mixtures of 25% and 50% peat in soil by volume. The volumes were measured using a 10 litre bucket and the soil and peat were mixed thoroughly by hand. The peat (Irish Moss Peat) was obtained from normal garden suppliers and a sample was retained for analysis.

The soil and soil-peat mixtures were placed in 7" plastic pots with trays, labelled, placed randomly on the greenhouse shelving and watered with distilled water. A fine sprinking of grass seed (*Lolium perenne* S 23) was placed on the surface of each pot, lightly mixed in with the soil of the pot and left to germinate. Throughout the experiment the soil was watered whenever necessary with distilled water only. The grass germinated well and grew evenly in all pots and a first harvest was taken about 1 month after sowing the seeds in order to keep the grass growing freely and prevent it from flowering. The grass was cut using stainless steel scissors to 2.5 cm above the soil surface and placed directly into preweighed paper sample bags.

Immediately after this first harvest the selenite and selenate treatments were added to the appropriate pots. Sodium selenite or selenate solution (50 ml of 1 μg/g) was carefully poured onto each pot receiving selenium treatment, giving an even amount of solution to all parts of the soil surface.

The plants were left for another month and watered with distilled water when necessary until there was sufficient growth for a second harvest. This second harvest was taken as before.

An attempt was made to remove the roots from the soil for analysis, however after very thorough washing and 60 minutes in an ultrasonic water bath soil partices were still visible on the root surface. Examination of the selenium

content of the roots would have been meaningless with this level of soil contamination and so this was not carried out.

The dates at which the treatment solutions were applied and the harvests were taken are given below.

3/8/87	Seed sown.
11/9/87	1st harvest, selenium solutions added.
8/10/87	2nd harvest.

The grasses from both harvests were dried at 30°C in the paper sample bags, and the dry weight from each pot at each harvest was recorded.

7.3.3 Experimental Results

The selenium concentration, organic matter content and pH of the soils and the peat used in this experiment are given below. The pH of all the soils was very similar, and the natural organic matter content of the soils did not vary greatly.

		Se µg/g	Organic matter %	pH
Soil 1	Site 10	1.363	14.5	5.71
Soil 2	Site 9	0.134	7.6	5.96
Soil 3	Site 3	0.434	11.2	5.71
Peat	—	0.410	100% ?	4.5

An explanation of the symbols used in the graphs to descibe the treatments used is given overleaf; X, Q, and H refer to the level of additional peat used, and 0, 4 and 6 refer to the selenium applications provided.

X - No added peat	**0** - No added selenium
Q - 25% added peat	**4** - Selenite solution added
H - 50% added peat	**6** - Selenate solution added

The first harvest was taken before the selenium treatments were added, so effectively there were 9 replicates of each sample. The results of these nine samples have been averaged and the standard deviation is shown as error bars.

Figure 7.28 shows both the selenium concentration of the herbage and the selenium uptake from this first harvest for the three soil types and all three different levels of peat incorporation. *Lolium perenne* was the only plant species used throughout this experiment.

Soil 1 is naturally high in selenium and the addition of peat has served only to dilute the selenium content of the growing medium and hence the selenium concentration of the herbage decreases with increasing levels of peat in the soil. This effect is just discernable in the second soil although to a much smaller extent, and is not noticed with the third soil at all.

Figure 7.29 shows the average dry weight of each sample type from the first harvest and these values have been used to produce the uptake values shown alongside the concentration in Figure 7.28. The dry weight is seen to increase with the addition of peat to the soil. Soil 1 has produced a regularly increasing dry weight in the herbage with the addition of 25% and then 50% peat, however both the other soils seem to produce most of the increase with the first addition of 25% peat and then to only increase slightly with the further addition of 50% peat. The additional organic material therefore appears to have a strong beneficial effect on the early plant growth in all these soil types. As a consequence of this increased dry weight, the total uptake of selenium for this harvest is increased in all three soils. However, the increase in uptake was small for Soil 1 where the addition of peat has diluted the naturally high selenium concentration of the soil.

The incorporation of peat of normal selenium concentration (0.410 μg/g) into agricultural soils may reduce selenium levels in herbage where toxicity is a problem, however in soils low in selenium it does not appear to increase the

concentration in the herbage at least in the short term. The increase in total uptake of selenium noted when peat is added to the soil is presumed to be due merely to the increased growth of the plants seen with the addition of peat to soils. A greater weight of grass and hence more productivity may be obtained by the addition of organic matter to soil but the selenium concentration in the herbage may not change and selenium deficiency in livestock could remain a problem. However this may be in contrast with and preferable to the dilution effect and reduction of selenium concentration noticed when inorganic fertilisers are used to increase productivity.

The sample variation was examined using one-way analysis of variance (ANOVA) and found to be low, well below the 95% confidence limit ($p<0.05$).

The plants were harvested a second time after the addition of 50 μg of selenite or selenate in solution to the pots of grasses. The most obvious result from this second harvest was that the selenate concentration and uptake found in the plants was far greater than that of the selenite. The total uptake of the selenate ions was around 10-15 μg/pot ; ie. about 20-30% of the selenium added to the pots was taken up into the herbage in just one month. In contrast, the uptake of the selenite ions lay in the range 0.2-0.4 μg/pot ; ie. less than 1% of the added selenite was taken up by the plants in the same time period (Figures 7.30-7.31). This difference in plant uptake between the selenate and selenite species is well documented and has been noticed by many workers in both greenhouse and field experiments (see Chapter 2). The addition of selenium to soil in order to supplement areas where herbage selenium is low has been proved difficult because of this difference in uptake between selenate and selenite ions. If selenium is added as selenate ions, which are readily available, the herbage selenium levels increase rapidly, perhaps even to toxic levels and the added selenium is quickly taken up by herbage from the soil so that the beneficial effects may only last for one growing season. Conversely, selenium added as selenite is taken up in such small quantities that the effect may be negligable, however if sufficient selenium is added the supplementation effect has been shown to last for many years (Watkinson, 1983, Whelan, pers. comm.).

The main aims of this experiment was however to study the efffect of the peat ammendments to the uptake of both native and applied selenium from the soil.

For the second harvest, the control pots with no selenium treatments produced results similar to those of the first harvest as would be expected. The selenium concentration of the herbage decreased with increasing peat in the soil (Figure 7.32), and the uptake also decreased in the second harvest rather than increasing as in the first harvest (Figure 7.32). This is due to the similarity of the dry weight for different treatments in the second harvest, so that the uptake reflects the concentration to a greater extent. The plants were established by this second harvest and the effect of the peat in the soil on their growth at this later stage appears to be minimal.

The effect of the peat ammendment on the uptake of the added selenium, both selenite and selenate, is shown in Figures 7.33-7.34. For all three soils the addition of peat has reduced the concentration of both the added selenite and selenate in the herbage; this may be due to exchange sites on the organic matter binding the selenium and making it slightly less available to the plants. However the overall uptake of selenium for both the added selenite and selenate is however slightly increased in the plants growing in the peat ammended soils. This is due to the increase in dry weight noticed in these plants.

The effect of the different soils on the uptake of selenium is also shown in Figures 7.32-7.34. Soil 1, which is naturally high in selenium, has given rise to higher levels of selenium in the herbage of the control pots, but after the addition of selenite or selenate solutions all three soils produce similar results since their natural selenium levels are low in comparison with the concentrations of added selenium. The selenium uptake produced by plants growing on Soil 3 was most affected by the increasing additions of peat, with a greater increase in selenium uptake in both the control situation and after the selenium additions, than noticed in the other soils.

Figures 7.35-7.38 present the same results as Figures 7.32-7.34, but highlights the exteme differences in selenite and selenate uptake produced by the plants.

The results of this experiment suggest that the incorporation of organic matter into soils of low selenium status may help to increase the total uptake of selenium from the soil. However it does not appear to affect the concentration of selenium in the herbage to any great extent.

The incorporation of organic matter in the soil has also increased the uptake of added selenium as either selenite or selenate in the three soils studied in this experiment.

Figure 7.28 **The average selenium concentration (µg/g) and uptake (µg/pot) found at the first harvest prior to selenium addition**

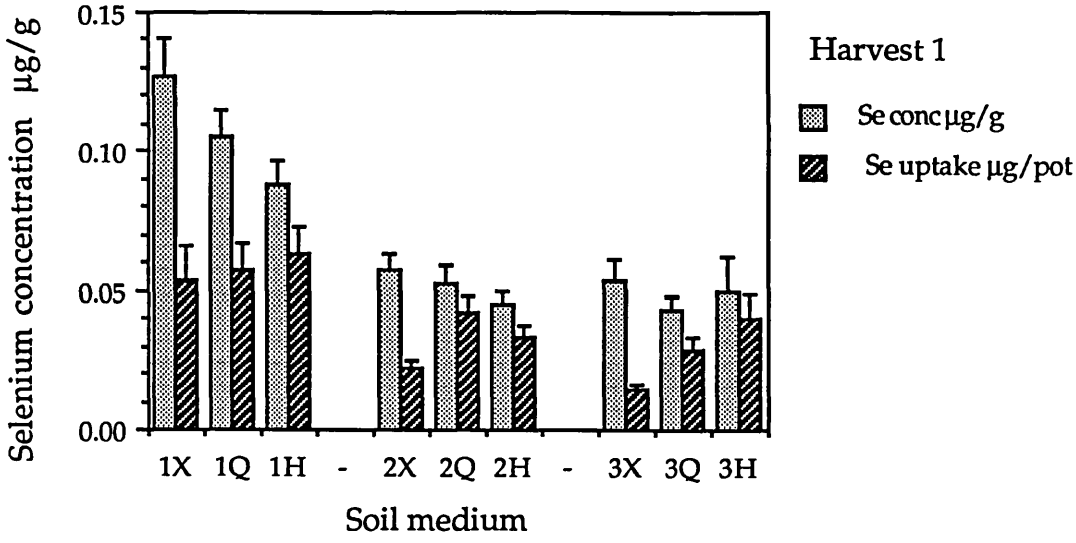


Figure 7.29 **The average dry weight (g/pot) found at the first harvest prior to selenium addition**

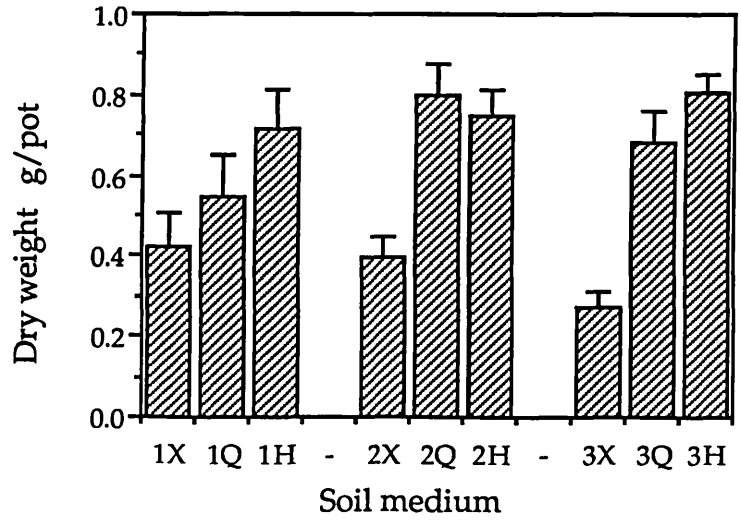


Figure 7.30 The average selenium concentration (μg/g) of the plants grown in ammended soils after treatment with selenium solution

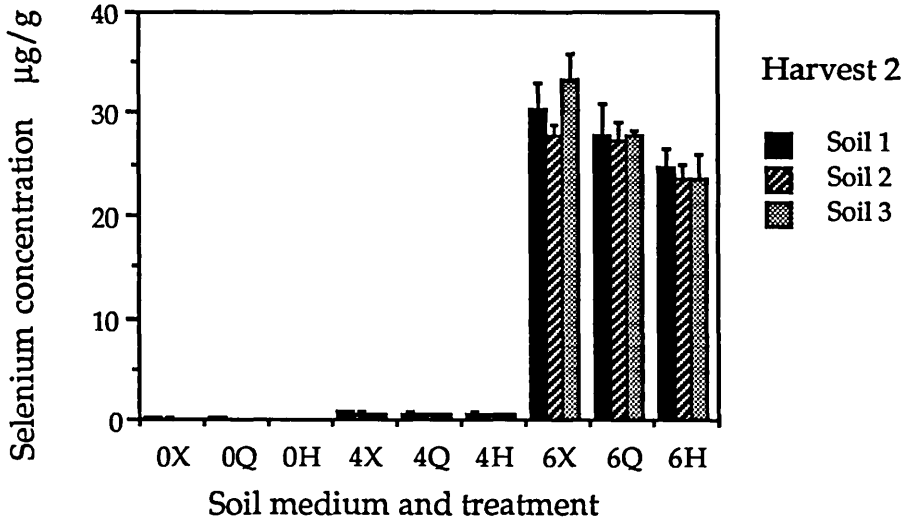


Figure 7.31 The average selenium uptake (μg/pot) of the plants grown in ammended soils after treatment with selenium solution

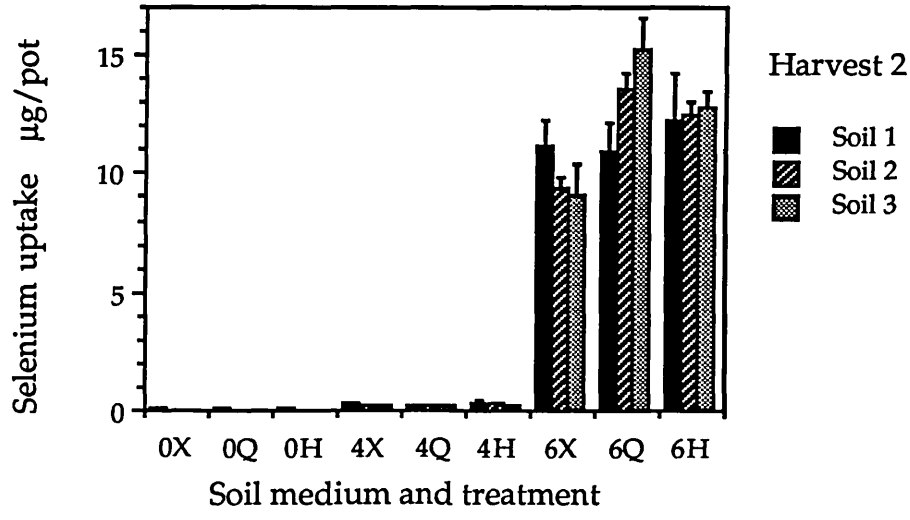


Figure 7.32 **The average selenium concentration (μg/g) and uptake (μg/pot) of the plants grown in ammended soils without addition of selenium solution**

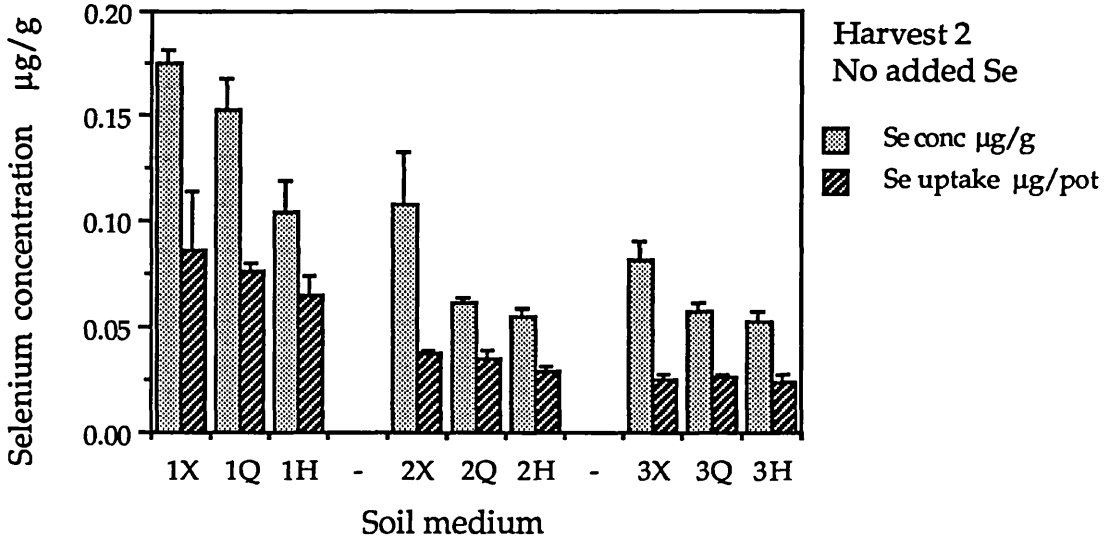


Figure 7.33 **The average selenium concentration (μg/g) and uptake (μg/pot) of the plants grown in ammended soils with addition of selenite solution**

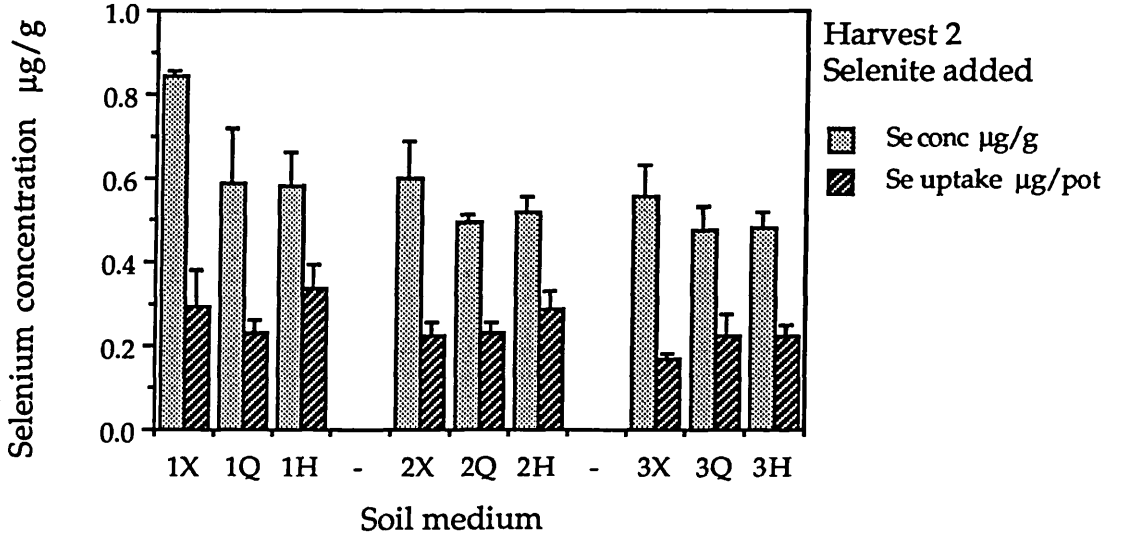


Figure 7.34 **The average selenium concentration (μg/g) and uptake (μg/pot) of the plants grown in ammended soils with addition of selenate solution**

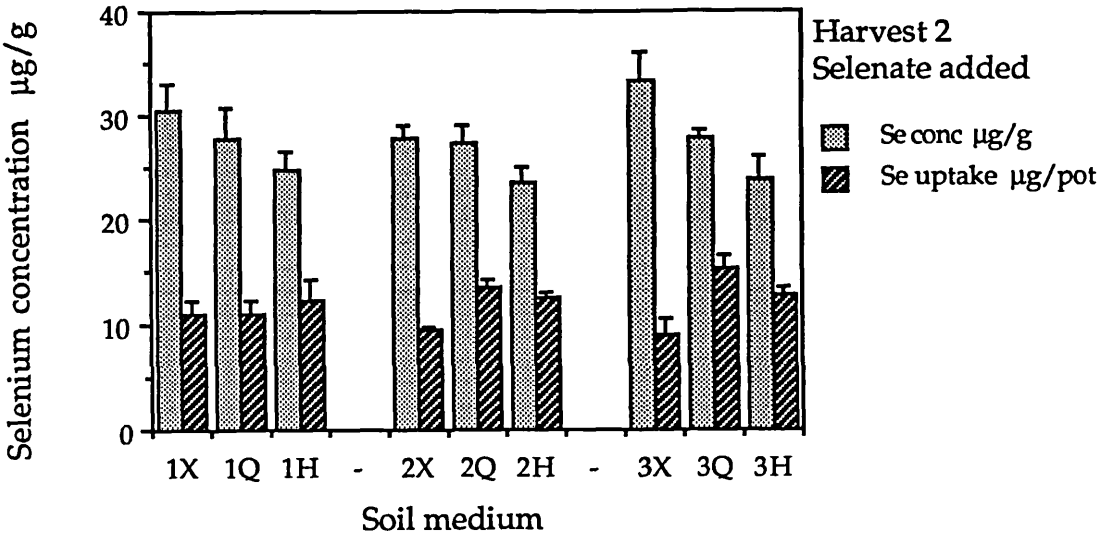


Figure 7.35 **The average selenium concentration (μg/g) of the plants grown in ammended soils without addition of selenium solution and with addition of selenite solution**

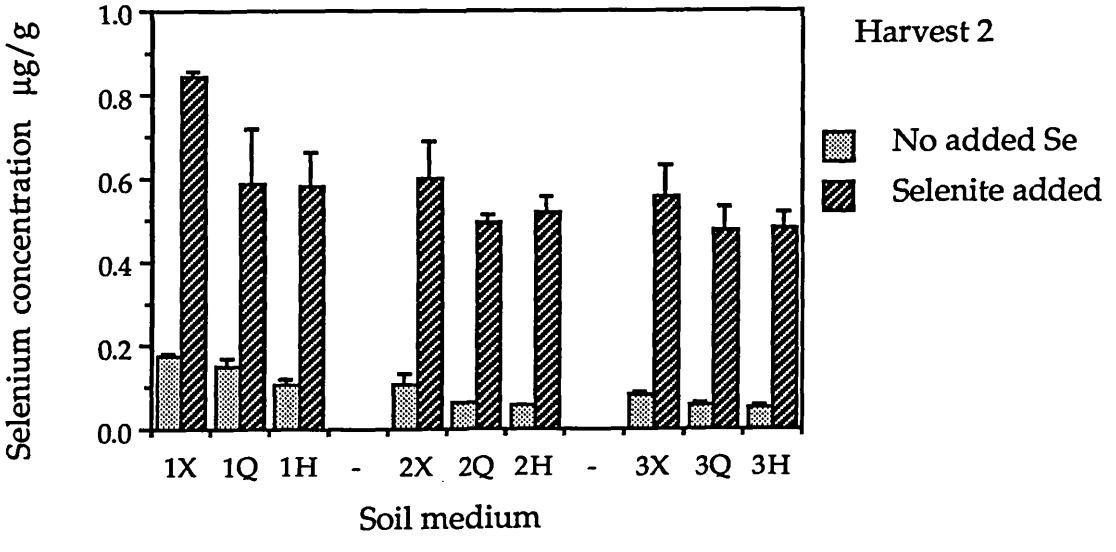


Figure 7.36 **The average selenium concentration (μg/g) of the plants grown in ammended soils with addition of selenite solution and selenate solution**

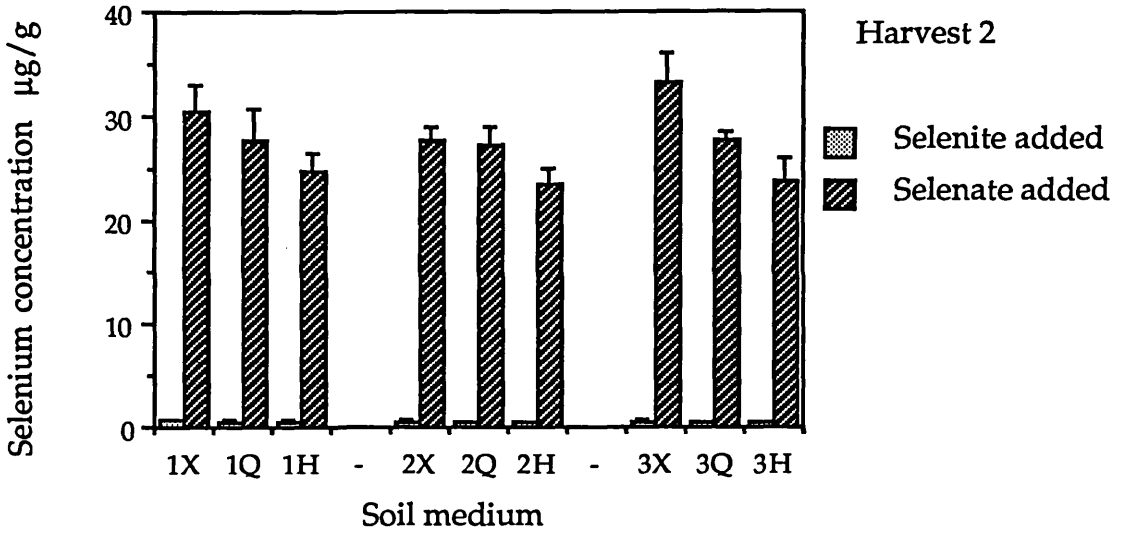


Figure 7.37 **The average selenium uptake (μg/pot) of the plants grown in ammended soils without addition of selenium solution and with addition of selenite solution**

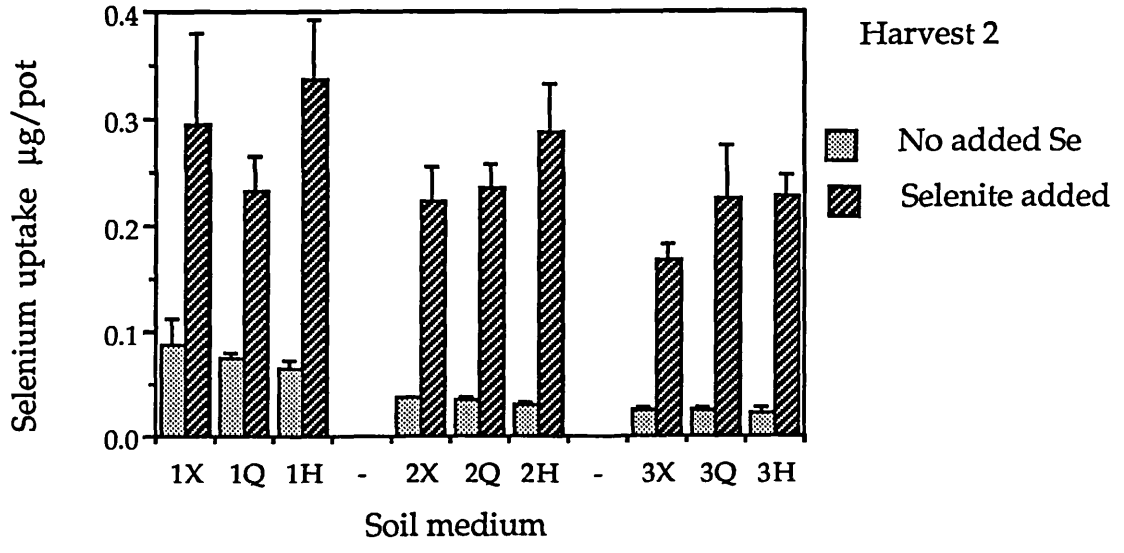
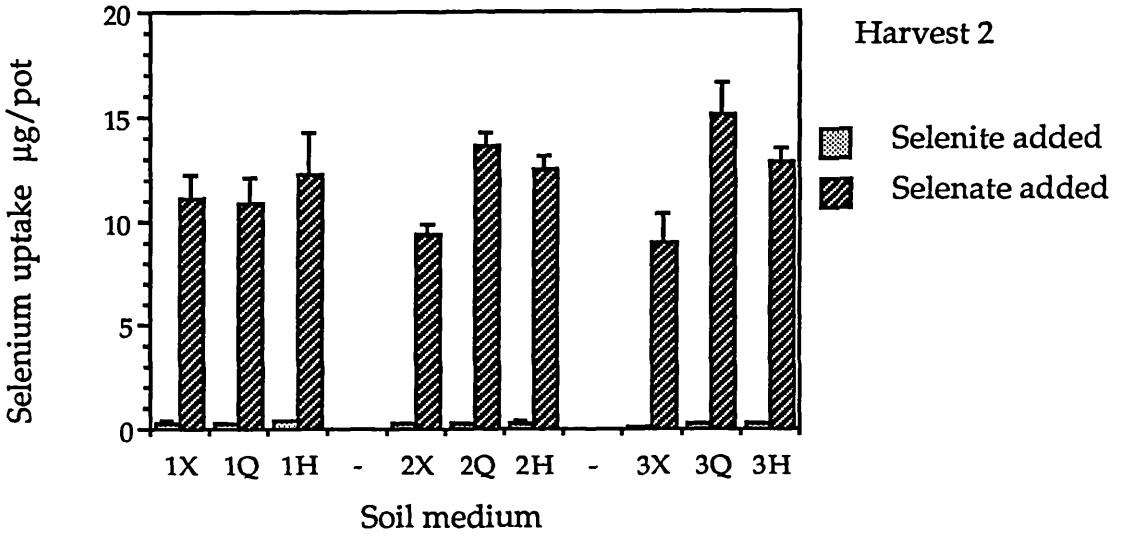


Figure 7.38 **The average selenium uptake (μg/pot) of the plants grown in ammended soils with addition of selenite solution and selenate solution**



CHAPTER 8

CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER WORK

8.1 CONCLUSIONS

In studying problems related to selenium deficiency in livestock, the detection of very low levels of selenium in samples is required, and research often involves analysis of selenium concentrations near to the detection limits of the available methods. This research has compared the relative merits of two analytical methods, ICPAES and spectrofluorimetry, for the analysis of low levels of total selenium in environmental samples, and concluded that the fluorimetric method is more suitable due to the lower detection limits and the better agreement with Certified Reference Materials afforded by this method. A lowering of the system detection limit for selenium analysis by the fluorimetric method to 4.5 ng/g has been achieved during this research, and other minor modifications to the experimental procedures have also been made to the published methods.

This study has investigated the interrelationships of the physical and chemical soil factors which influence the uptake of selenium into plants. Many of these factors have been previously identified as influencing soil and herbage selenium concentration at high levels of soil selenium concentration. However, in Britain large areas of grazing land lie in areas of the country which have low to marginal soil selenium levels, and selenium deficiency problems in livestock have occurred in some areas. Total soil selenium concentration has been shown to be an unreliable indicator of herbage selenium concentration, and information on other soil and plant factors affecting plant selenium uptake at relatively low levels of soil selenium was required.

The influence that the various factors studied in this research have on the herbage selenium concentration at low levels of soil selenium is discussed below,

and is also compared with the results obtained from other research on soils containing high or toxic levels of selenium. Some consideration has also been given to the interdependence of these soil factors in the discussion.

The selenium concentration of the soil parent material greatly influences the total soil selenium concentration, and this factor appears to override other soil factors, especially where the parent material has a relatively high selenium concentration. Parent materials which have a lower selenium concentration may produce soils with variable selenium concentrations depending on the factors influencing the soil formation. The soil at Site 10 formed from marine black shale has a relatively high selenium concentration compared with all the other sites studied. The results from Site 10 have appeared as a separate group in many of the comparisons of soil selenium with other soil factors. This demonstrates that varying soil factors such as pH and organic matter content have little effect on the total selenium content of this soil, which is actually very close to that of the underlying parent material. In contrast, the sites studied in North Wales have a wide range of total selenium concentrations although all the sites overlay the same relatively uniform parent material. At these sites the selenium concentration of the parent material is much lower, and other soil factors have a relatively greater influence on the selenium concentration of the soils during their development.

A positive correlation (r=0.405) has been found in this research between soil seleium concentrations and the selenium concentration of the herbage. This is a much better relationship than has been found by previous studies in this country (MAFF, 1983). So although other soil factors have considerable influence on the concentration of selenium in soils and herbage, the total soil selenium concentration is still an important factor in assessing the uptake of selenium into herbage.

A strong negative correlation was obtained between soil pH and total soil selenium concentration. It was concluded that this inverse relationship was due to the presence of high levels of organic material in some of the soils studied. Organic peat soils are invariably acidic and the sites studied here have also contained slightly elevated levels of selenium due to the association of selenium

with organic material in soils. Generally, the pH of the soil does not affect the total selenium concentration of the soil, although the speciation of the soil selenium depends on the pH and redox conditions of the soil. However if leaching conditions are prevalent in the soil, alkaline pH's may favour the formation of soluble selenate ions which would then be leached from the surface soils.

The speciation of selenium in the soil is heavily dependent upon the soil pH, although soil moisture conditions can also be important. The uptake of selenium by plants is thought to be dependent upon the species of selenium present in the soil and their relative solubility. Consequently plant uptake of selenium is affected by the soil pH. In normal mineral soils, the plant uptake of selenium from soil increases in alkaline conditions, with plant selenium concentrations showing a positive correlation with pH, especially above pH 7-8. This relationship has been found by many workers at high and low levels of total soil selenium (eg. Geering et al., 1968, Gissel-Nielsen, 1971b, Hamdy and Gissel-Nielsen, 1976b). The results from this research show a slight negative correlation beween the plant selenium concentrations and the soil pH, which is in conflict with most other results. However, in 1981, Paasikallio also found a negative correlation between plant uptake of ^{75}Se and soil pH when the plants (barley) were grown in organic peat soils. In soils with a lower organic matter content and a higher iron concentration, the expected positive relationship was found.

From this it can be concluded that, in the presence of iron, the solubility of selenium in the soil is principally governed by iron oxide - selenite adsorption complexes. These have been shown to be less stable above pH 7-8, when they begin to break down and release more selenite into the soil solution (Allaway et al., 1967, Howard, 1972). In alkaline conditions, the oxidation of selenite ions to selenate ions is favoured and the presence of the readily soluble selenate ions in the soil will also increase the availability of the soil selenium to plants.

But, in very organic soils, the iron concentration is low and the results from this research suggest that a separate selenium adsorption process occurs in organic soils of low iron concentration. Due to the acidic pH prevalent in peat soils, the selenium may be assumed to be in the form of selenite ions or as more reduced

selenium species. The organic material in the soil may be acting as a weak exchange site for selenite ions, with the selenite being released in more acid conditions. This is one possible explanation of the inverse relationship between plant selenium concentrations and soil pH found in soils of high organic matter content. In soils with a higher iron concentration, the ferric oxide-selenite adsorption complexes presumably bind the selenite more strongly than the exchange sites of the organic material and these adsorption complexes would become the dominant factor affecting the solubility of selenium in the soil.

Since the sites studied in this research included both organic peat soils and mineral brown earths, a mixture of both processes is seen in the results, producing only a slight negative correlation between plant selenium concentrations and soil pH. Unfortunately there were insufficient sites studied to separate the results for peat soils and mineral soils, in order to investigate further the possibility of the organic matter/selenite adsorption mechanism which is suggested for the peat soils.

The organic matter content for the sites studied showed a strong positive correlation with selenium concentration in the soils. This increase in the total selenium concentration of the soil with organic matter content is due to the accumulation of selenium in organic material. The selenium is found as sulphur analogues in plant proteins and amino acids. The increase of selenium in the soil produced by an increase in soil organic matter is especially noticeable in conditions of low overall selenium concentration. The accumulation of organic material can then provide a larger relative increase in the soil selenium concentration. Only a small non-significant positive correlation was found between soil organic matter content and herbage selenium concentrations. So although the soil organic matter content can significantly increase the soil selenium concentration, it would appear that the presence of soil organic matter does not have any appreciable effect on the percentage uptake of soil selenium by the herbage.

Selenium concentrations in both soil and herbage showed no relationship with the total iron concentrations in the soil. However a measure of the pyrophosphate extractable iron content of the soils was shown to have a positive correlation with the soil selenium concentrations. This relationship between

pyrophosphate extractable iron and soil selenium concentrations had been found previously in a study of selenium concentrations in soil profiles (Smith, 1983). A slight, non-significant positive correlation was found between the pyrophosphate iron content and the selenium concentrations in the herbage. Although this is only a slight relationship, the multiple regression analysis found that the pyrophosphate extractable iron concentration was an important factor in describing the variance of the herbage selenium concentrations at the sites studied.

Soil selenium concentrations were found to be influenced by the particle size distribution in the soil. Soils with a high sand fraction were low in selenium, this is partly due to the low selenium concentration of many sandstone parent materials (Thornton et al., 1983) and partly due to leaching of selenium from very sandy soils. The soil selenium concentration showed positive correlations with the silt fraction of the soil. Whether there is a direct association between soil selenium and the silt particles, or whether this correlation is reflecting the antagonistic relationship between the silt and sand particle size fractions in the soil, is not known. For the clay size fraction, a significant positive correlation was only found between the subsoil clay fraction and the soil selenium concentration. This may be due to the larger clay fraction present in the subsoil at many sites, and to the association between soil selenium and clay minerals that has been noticed by other workers. Neal and Sposito (1987a,b, 1989) have found that clay mineral complexation with selenite ions, as well as the expected iron oxide complexation, is an important process in the adsorption of selenium by alluvial soils from the San Joaquin Valley, California.

No significant correlations were found between herbage selenium concentration and any of the particle size fractions. This suggests that the particle size distribution of the soil mineral fraction does not affect the availability of soil selenium to the plants.

A measurement of the cation exchange capacity was made on the soils studied, and this was found to show a strong positive correlation with the soil selenium concentrations. However, since the cation exchange capacity is strongly influenced by the presence of soil organic matter, it was concluded that this relationship was explained by the relationship between soil selenium

concentrations and soil organic matter content. There was no relationship found between soil cation exchange capacity and herbage selenium concentrations.

Despite many suggestions by other workers (Hurd-Karrer, 1938, Ravikovitch and Margolin, 1959, Fleming, 1980) that the presence of sulphur in the soil inhibits the uptake of selenium by plants, no relationship between soil sulphur concentration and selenium concentration in the herbage was found in this research. The inhibition effect of soil sulphur on plant selenium uptake has only been noticed in situations of high soil selenium concentration. The possible competitive uptake by plants between sulphur and selenium may not be noticable in conditions of low to normal soil selenium concentration.

All these soil factors considered above were used for multiple regression analysis, in order to produce an equation for the variation in herbage selenium concentrations found in this research. The most important factors determining the herbage selenium concentration identified by this multiple regression analysis were soil selenium concentration, pyrophosphate extractable iron concentration, soil organic matter content, soil moisture content and soil pH. Of these, the soil moisture content is dependent upon the organic matter content and possibly the clay content of the soil and perhaps should not have been included as an independent variable in the analysis. The equation produced accounted for 36.6% of the variation in herbage selenium concentration.

A limited selenium speciation study of extracted soil solutions was undertaken in this research, examining mainly the total selenium in the soil solutions and the selenite ion concentration in the same solutions. The amount of total selenium found in the soil solutions broadly reflected the selenium concentration of the soil itself. The percentage of the total selenium found as the selenite ion in the soil solutions, increased in soils with a lower pH. It would be expected that more acid conditions would favour the formation of selenite ions in the soil. The lowest percentage of selenite in the soil solution was found in the alkaline soil at Site 12, Romney Marsh.

One important relationship noticed from these speciation results, was that the herbage selenium concentration showed a stronger correlation with the selenite ion in the soil solution than with the total selenium concentration in the

soil solution. This may suggest that the selenite ion in the soil solution is more readily available for plant uptake than other forms of selenium present in the soil solution. No measurement of the selenate ion concentration or other species of selenium was made on these soil solutions and therefore no estimate could be made on the relative availability of these species to plants could be made. Generally it has been considered that the selenate ion would be most readily taken up from solution by plants, with the plant absorption mechanism for selenate ions being similar to that for sulphate ions (Shrift and Ulrich, 1969, Ferrari and Renosto, 1972, Asher, Butler and Peterson, 1977, Gissel-Nielsen, 1979).

Seasonal variation in pasture herbage selenium concentration has been detected in this research. No significant seasonal variation was found in the soil selenium content or in other soil parameters. Some of the herbage seasonal variation in selenium concentration was attributed to soil contamination of the collected herbage, despite washing prior to analysis. A range of 0.4%- 24.8% of the herbage selenium concentration was estimated to be due to this soil contamination and the percentage was generally greater in winter and early spring due to the pasture conditions at these times of year. Sites on rough grazing peat moorland produced much less soil contamination of herbage than farm fields due to the unbroken vegetation cover on the moorland soils. Soil ingestion from contaminated herbage has been shown to be an important source of some trace elements for grazing animals (Russell, 1987). However, the selenium status of sheep has not been shown to be affected by the ingestion of soil, perhaps because only selenium deficient animals respond to selenium supplementation in any form (Brebner, 1986).

A significant seasonal variation was found in herbage selenium concentrations which could not be explained by the variations in the soil contamination of herbage. The selenium concentration of herbage, corrected to remove the contribution from soil contamination, was found to be lowest in summer and autumn when the herbage growth rate was greatest, and higher in winter with the summer concentrations around 1/3 of the winter concentrations. Consequently the sampling time for any field based study of selenium is very important and research which only involves summer sampling may

underestimate the average annual selenium concentration of the herbage. This variation in herbage selenium concentration through the seasons is assumed to be a dilution effect, with the rate of uptake of selenium not keeping up with the growth rates of the plants during the summer months.

Such dilution effects have important consequences for pasture management with regards to selenium deficiency. High levels of nitrogen fertilisation, although allowing greater livestock productivity will be expected to aggrevate selenium deficiency since the selenium concentration in the pasture herbage will be reduced. The seasonal variation of selenium in herbage also means that the lowest selenium concentration in the grazing forage occurs at the time of year when the livestock have their lowest selenium status (Russell, 1987) and are also growing fastest and may be expected to have their greatest demand for selenium. Concentrate feeds, most with added selenium, are frequently provided in winter when forage is in short supply, however selenium supplementation may actually be most necessary in spring and summer when the herbage selenium concentrations fall.

Since the work in the 1930's (see Rosenfeld and Beath, 1964) it has been recognised that different plant species accumulate selenium from soil at widely varying rates. Plants growing on soils of high selenium concentrations can accumulate hundreds or even thousands of μg/g Se. Plants growing on soils of relatively low selenium concentrations can also show large variations in selenium content between species. Clovers have been shown to accumulate lower selenium concentrations than grasses when grown on many types of soils (Davies and Watkinson, 1966). Bisbjerg and Gissel-Nielsen (1969) found the following decrease in plant selenium concentrations on low selenium Danish soils: crucifers > ryegrass > legumes > cereals. The limited study in this research has shown considerable differences between the plant species growing at several of the field sites. The selenium concentration of the soils at the field sites ranged from (0.125 - 1.363 μg/g Se). At Site 10, where the soil selenium concentration was 1.363 μg/g, the following decrease in plant selenium concentrations was found: buttercup > perennial ryegass > clover. There was a relatively high proportion of buttercup in the herbage at this site and this has presumably increased the overall

concentration of selenium in the herbage. However the selenium levels found in the soil and herbage at this site, although relatively high, are not toxic and the selenium concentrations found in the buttercup plants would not cause any problems to the grazing livestock. On the Welsh moorland sites (Sites 5-7) with a range of soil selenium concentrations of 0.323 - 0.755 μg/g Se the following decrease in plant selenium concentrations was found: lichens > mosses > grasses > sedges > heather.

In general the relative species differences in plant selenium accumulation found in this research is similar to that found previously by other workers. Similar patterns in the relative uptake by different plant species appear to occur in plants grown both on soils of a relatively high selenium concentration and on soils of low to marginal selenium concentration.

Of special interest is that the moss and lichen species growing on the moorland sites have a higher selenium concentation than the grass and other species growing at these sites. On unimproved upland grazing pasture such species may provide a large percentage of the available forage especially in winter and early spring before the grass species begin to grow rapidly. The proportional contribution of moss and lichen species to the diet of sheep is not certain, however if it is significant, the availability of such plants in the forage may provide an important source of selenium to the grazing livestock. In association with this point, Site 3 in North Wales was reseeded permanent pasture on improved moorland soil and the herbage collected from this site had the lowest average selenium concentration of all the sites studied (0.059 μg/g Se). The soil selenium concentration at this site was not particulaly low (0.434 μg/g Se), and is higher than the brown earth soils sampled at the other sites in the area (Sites 1-4 & 8), but slightly lower than the peat moorland sites nearby (Sites 5, 6 & 7). However the percentage uptake of selenium by plants from soil is very low at Site 3 (13.6%) suggesting that the soil conditions and sward composition associated with this improved upland pasture may inhibit the uptake of soil selenium by the herbage and possibly produce selenium deficiency in the grazing livestock.

The first greenhouse experiment was carried out to investigate the effect of sulphur additions on the uptake of selenium by pasture species. No sulphur interaction on the uptake of low levels of added selenite or selenate was found in this experiment. However the diffferent plant species used in this experiment showed different rates of uptake of selenium. Generally the clover species were found to have lower selenium concentrations than the grass species for any particular treatment, although the selenium uptake of the grass species was often greatly enhanced due to the increase in growth noticed with nitrogen fertilisation.

A dilution effect producing lower selenium concentrations in plants was also noticed in the initial greenhouse experiment when nitrogen fertilisation was used. The increase in growth rate caused by the addition of nitrogen fertilisers to plants was not matched by the uptake of selenium into the plants even when sodium selenate was added to the experimental plants. The total uptake of selenium per pot was increased by the use of nitrogen fertilisers but the actual concentration of selenium in the herbage was lower than that in pots with no added fertiliser.

In this experiment the plants were grown in vermiculite, and in contrast to plants grown in soil in other experiments, added selenite was taken up by all plant species to a greater extent than added selenate. This is presumed to be due to the vermiculite growth medium and the fact that the iron added in the nutrient solution was in the form of iron EDTA.

The subsequent greenhouse experiment investigated the effect of incorporation of organic matter (peat) into soils of various native selenium concentrations. Solutions of selenite and selenate were also added to these soils and the uptake of selenium in *Lolium perenne* was monitored. The incorporation of organic matter into soils increased the selenium concentration of soils low in selenium, and also increased the growth rate of the grass plants. The uptake of selenate by the plants was greater than that of selenite, presumably because the selenite ion added to the soils was rendered insoluble by adsorption onto iron oxides. In general, the uptake of both native and applied selenium increased slightly in the soils ammended with organic matter. Part of this response may be due to the increase in plant growth noticed with organic matter

incorporation. The concentration of selenium in plants was not substantially altered by the addition of organic material to the soil. This situation is rather different from the effect that nitrogen fertilisation has on selenium concentration in plants, where an increase in growth produces a strong dilution of the selenium concentration in the plants.

8.2 RECOMMENDATIONS FOR FURTHER WORK

One of the major limitations for research related to selenium deficiency is the accurate detection of the low levels of selenium present in the samples. Further improvements to the existing analytical methods in order to produce lower detection limits would be invaluable, and the possible developments of new methods should also be considered. The use of GC-Mass Spectrometry may provide improvements over many existing methods.

Further studies on the speciation of selenium in soils and soil solutions would provide more information on the precise chemical pathways of selenium in soils and its uptake by plants. It would be preferable to analyse soil solutions from freshly collected soils, rather than those extracted from previously dried soils and equlibrated to field moisture content in the laboratory. Quantitative speciation in the soil itself could perhaps be acheived by a sequential heating process, measuring the quantities of selenium volatilised at each successive temperature.

Better quantitative identification of the organic selenium compounds present in the soil is required, as very little is known about the relative amounts of organic selenium compounds in soils. The availability to plants of selenium in organic combinations has also been questioned, and this availability could perhaps be effectively studied by ^{75}Se tracer experiments.

Radioactive tracers (^{75}Se) could also be used in further plant uptake experiments at low levels of selenium. This approach has been used by many workers in the past but more information is still required on the mechanism of selenium uptake by plants, and on the equilibrium systems of the various selenium species in the soil which are important for plant uptake. The use of tracers could also provide more information on the fate of selenium added to soils as an amelioration technique for selenium deficiency problems.

Lag and Steinnes (1978) suggested that a contribution to the soil selenium may be made from the atmosphere, brought to the soil by precipitation. This theory needs further substantiation to determine whether selenium is being added to the soil system from rainwater. Preconcentration techniques will be required for

any such study, since the concentration of selenium in rainwater found in this study was below the detection limit of many analytical methods

This research has identified the major physical and chemical soil factors influencing selenium uptake by plants and some statistical treatment of the results has been attempted. However, the sites studied were carefully chosen and therefore do not provide a statistically representative sample. There would be scope for a statistical study of many randomly selected field sites, analysing samples only for those factors shown, in this study, to be of greatest importance to plant selenium uptake. This would hopefully provide sufficient information for a full multiple-regression analysis to further quantify the factors affecting selenium uptake by plants from soil at low levels of soil selenium concentration.

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Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	0.215	0.128	0.069	0.056	0.151	0.037	0.033	0.059
2	0.149	0.079	0.093	—	—	—	—	—
3	—	0.098	0.059	0.066	—	0.031	0.050	0.054
4	0.145	0.128	0.055	0.047	0.124	0.031	0.032	0.093
5	—	0.274	0.169	0.188	0.319	0.220	0.115	0.199
6	—	0.276	0.099	0.162	0.290	0.140	0.071	0.126
7	—	0.223	0.070	0.097	0.264	0.103	0.065	0.085
8	—	—	—	—	0.220	0.067	0.046	0.090
9	—	0.195	0.072	0.057	0.205	0.213	0.028	0.072
10	0.650	0.346	0.096	0.136	0.419	0.730	0.185	0.16
11	0.259	0.109	0.088	0.093	0.304	0.564	0.075	0.096
12	0.163	0.094	0.072	0.096	0.082	0.412	0.053	0.08
13	—	—	—	—	0.171	0.087	0.085	—
14	—	—	—	—	0.139	0.122	0.107	—
15	—	—	—	—	0.124	0.065	0.092	—
16	—	—	—	—	0.245	0.147	0.231	—

Table A 1 Selenium concentration in herbage (μg/g) (spectrofluorimetry)

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	0.120	—	0.018	0.024	0.072	0.012	0.012	0.024
2	0.054	0.024	0.042	—	—	—	—	—
3	—	0.030	0.018	0.030	—	0.012	0.030	0.012
4	0.060	0.042	0.024	0.012	0.054	0.012	0.012	0.036
5	—	0.210	0.096	0.108	0.204	0.120	0.084	—
6	—	0.150	0.054	0.096	0.114	0.072	0.024	—
7	—	0.102	0.024	0.060	0.150	0.054	0.030	—
8	—	—	—	—	0.114	—	0.018	0.024
9	—	0.030	—	0.030	0.096	0.012	0.012	0.018
10	0.360	0.240	0.054	0.084	0.282	0.060	0.096	0.096
11	0.168	0.060	0.060	0.054	0.114	0.042	0.024	0.042
12	0.090	0.042	0.036	0.054	0.036	0.192	0.012	0.018
13	—	—	—	—	0.060	0.060	0.030	—
14	—	—	—	—	0.036	0.078	0.054	—
15	—	—	—	—	0.024	0.042	0.048	—
16	—	—	—	—	0.090	0.120	0.012	—

Table A 2 Selenium concentration in herbage (μg/g) (ICPAES)

Sampling Date

Site No.	1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	0.348	0.336	0.321	0.288	0.342	0.378	0.326	0.294
2	0.192	0.162	0.195	—	—	—	—	—
3	0.462	0.429	0.438	0.432	0.420	0.498	0.414	0.378
4	0.219	0.204	0.213	0.183	0.198	0.180	0.216	0.189
5	0.348	0.318	0.321	0.297	0.318	0.312	0.288	0.378
6	0.528	0.636	0.804	0.777	0.672	0.708	0.852	0.756
7	0.708	0.837	0.711	0.789	0.780	0.672	0.669	0.870
8	—	—	—	—	0.132	0.120	0.126	0.120
9	0.189	0.186	0.141	0.114	0.066	0.114	0.114	0.150
10	1.353	1.436	1.386	1.410	1.326	1.344	1.320	1.329
11	0.363	0.330	0.315	0.342	0.348	0.330	0.316	0.294
12	0.096	0.228	0.138	0.111	0.108	0.114	0.100	0.108
13	0.216	—	0.153	0.165	0.216	0.216	0.216	0.186
14	0.153	—	0.141	0.159	0.228	0.210	0.186	0.174
15	—	—	0.156	0.147	0.180	0.198	0.156	0.174
16	—	—	0.138	0.126	0.180	0.192	0.138	0.168

Table A 3 Selenium concentration in topsoil (μg/g)

Sampling Date

Site No.	1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	—	—	—	—	—	—	—	—
2	—	0.180	—	—	—	—	—	—
3	—	0.396	—	—	0.426	0.462	0.366	0.414
4	—	0.216	—	—	0.174	0.204	0.180	0.177
5	—	0.420	—	—	0.396	0.390	0.378	0.432
6	—	0.375	—	—	0.444	0.444	0.474	0.414
7	—	0.405	—	—	0.468	0.396	0.417	0.456
8	—	—	—	—	0.132	0.108	0.114	0.408
9	—	0.114	—	—	0.036	0.144	0.108	0.162
10	—	1.455	—	—	1.320	1.428	1.290	1.422
11	—	0.318	—	—	0.330	0.342	0.258	0.282
12	—	0.108	—	—	0.120	0.108	0.106	0.108
13	—	—	—	—	0.228	0.216	0.180	0.198
14	—	—	—	—	0.228	0.228	0.186	0.222
15	—	—	—	—	0.198	0.180	0.162	0.222
16	—	—	—	—	0.180	0.174	0.150	0.162

Table A 4 Selenium concentration in subsoil (μg/g)

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	30.0	—	7.5	6.0	22.5	4.5	3.0	10.5
2	10.5	7.5	12.0	—	—	—	—	—
3	—	9.0	6.0	7.5	—	3.0	4.5	12.0
4	—	13.5	6.0	4.5	19.5	3.0	3.0	33.0
5	—	7.5	4.5	6.0	15.0	6.0	3.0	—
6	—	7.5	3.0	4.5	6.0	3.0	3.0	—
7	—	4.5	3.0	3.0	7.5	3.0	3.0	—
8	—	—	—	—	25.5	7.5	4.5	18.0
9	—	9.0	—	3.0	43.5	3.0	3.0	7.5
10	55.5	36.0	4.5	10.5	40.5	3.0	3.0	9.0
11	55.5	24.0	9.0	10.5	69.0	4.5	3.75	16.5
12	19.5	30.0	9.0	22.5	18.0	6.0	6.0	21.0
13	—	—	—	—	6.0	3.0	4.5	—
14	—	—	—	—	6.0	3.0	3.0	—
15	—	—	—	—	6.0	3.0	3.0	—
16	—	—	—	—	7.5	3.0	3.0	—

Table A5 Titanium concentration in herbage (μg/g)

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	540	467	564	487	475	569	421	787
2	696	560	694	—	—	—	—	—
3	691	622	691	598	492	761	595	882
4	532	503	576	458	403	562	382	718
5	547	684	786	629	505	659	545	700
6	436	434	290	264	306	511	193	355
7	313	269	425	230	185	284	242	234
8	—	—	—	—	394	374	341	697
9	727	676	743	757	638	866	968	870
10	680	512	707	737	518	562	570	810
11	892	882	992	743	752	1040	852	864
12	733	643	700	581	654	745	722	750
13	472	—	683	523	332	497	574	517
14	536	—	74.4	695	353	566	544	529
15	—	—	10.8	526	394	459	494	440
16	—	—	641	482	329	374	450	456

Table A6 Titanium concentration in topsoil (μg/g)

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	43.1	43.3	41.2	42.8	42.8	42.9	41.8	47.5
2	53.5	54.6	54.5	—	—	—	—	—
3	25.1	25.0	23.5	24.3	24.0	24.7	25.2	24.8
4	44.4	45.9	42.7	46.4	45.7	45.1	45.6	45.8
5	10.1	13.2	7.02	10.6	8.40	9.42	9.42	9.42
6	3.36	2.34	1.26	1.44	2.82	2.10	1.62	2.34
7	1.68	4.32	6.42	1.80	1.56	1.44	2.76	1.44
8	—	—	—	—	47.7	48.7	45.6	48.3
9	20.1	21.3	20.1	21.3	22.9	18.3	17.8	18.8
10	65.6	64.1	64.0	63.5	62.2	65.4	64.0	65.1
11	30.2	30.8	29.1	29.9	28.4	31.3	30.1	30.8
12	30.0	32.2	31.6	31.1	30.4	30.5	31.0	30.4
13	7.50	—	7.20	7.02	5.46	4.74	5.28	5.46
14	7.74	—	4.68	7.08	5.88	5.22	5.40	5.94
15	—	—	7.14	6.84	4.86	4.08	4.74	4.80
16	—	—	6.84	6.72	4.80	4.08	4.08	4.20

Table A7 Lithium concentration in topsoil (μg/g)

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	1420	1310	1620	1370	1500	1660	1430	1670
2	479	516	566	—	—	—	—	—
3	1130	1180	1430	1140	1240	1090	1050	1160
4	1450	1440	1520	1450	1450	1490	1480	1440
5	516	484	498	424	497	531	512	496
6	1160	1430	1590	1570	1220	1490	1500	1670
7	1530	1360	1380	1640	1660	1630	1490	1660
8	—	—	—	—	586	565	574	538
9	933	898	892	959	917	870	815	838
10	1700	1700	1700	1670	1650	1750	1610	1770
11	1230	1210	1220	1210	1350	1230	1250	1210
12	709	796	910	736	727	778	725	681
13	828	—	779	697	1030	996	1020	971
14	7650	—	129	796	1060	1060	1040	1020
15	—	—	44	802	1000	974	1000	986
16	—	—	743	697	1000	1010	1000	1050

Table A8 Phosphorous concentration in topsoil (μg/g)

Sampling Date

Site No.	1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	—	—	—	—	—	—	—	—
2	—	69300	—	—	—	—	—	—
3	—	61400	—	—	53400	64100	63200	59400
4	—	70300	—	—	68800	71400	69700	67600
5	—	67800	—	—	64500	64700	65600	63700
6	—	54300	—	—	48600	46200	46800	41400
7	—	45900	—	—	40200	45700	46800	44800
8	—	—	—	—	52900	69800	68100	64900
9	—	53800	—	—	54700	47600	47700	48100
10	—	67600	—	—	66800	69500	67500	65800
11	—	39900	—	—	38900	40000	40900	40300
12	—	45600	—	—	44700	44200	44400	42600
13	—	—	—	—	10500	10200	9870	9770
14	—	—	—	—	10900	10500	10500	10400
15	—	—	—	—	9640	8650	9170	9260
16	—	—	—	—	8970	8180	8470	8200

Table A9 Aluminium concentration in subsoil (μg/g)

Sampling Date

Site No.	1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	4430	—	491	421	2480	205	85	1040
2	1080	720	1400	—	—	—	—	—
3	—	738	361	571	—	111	259	890
4	1530	1490	336	338	2390	111	309	—
5	—	316	211	250	1150	196	158	4440
6	—	428	198	243	326	104	63	—
7	—	226	185	249	395	169	156	—
8	—	—	—	—	3520	738	379	—
9	—	430	—	118	3010	58	58	2280
10	5400	3100	111	528	3290	76	84	253
11	2860	806	229	393	3740	155	113	328
12	964	1530	260	1240	704	175	198	560
13	—	—	—	—	180	86	148	916
14	—	—	—	—	146	108	201	—
15	—	—	—	—	150	80	186	—
16	—	—	—	—	200	124	159	—

Table A10 Aluminium concentration in herbage (μg/g)

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	2.93	—	0.45	0.38	1.80	0.15	0.15	0.75
2	0.98	0.60	1.28	—	—	—	—	—
3	—	0.15	0.30	0.38	—	0.15	0.15	0.38
4	1.05	0.98	0.38	0.30	1.88	0.15	0.15	3.45
5	—	0.15	0.30	0.30	0.90	0.23	0.15	—
6	—	0.15	0.38	0.15	0.23	0.15	0.15	—
7	—	0.15	0.23	0.15	0.23	0.15	0.15	—
8	—	—	—	—	3.08	1.58	0.60	2.18
9	—	0.30	—	0.23	1.58	0.15	0.15	0.23
10	5.47	3.30	0.38	0.68	3.30	0.15	0.15	0.38
11	2.25	0.83	0.38	0.38	3.15	0.15	0.15	0.38
12	0.75	1.50	0.53	1.28	0.68	0.38	0.38	0.83
13	—	—	—	—	0.23	0.15	0.15	—
14	—	—	—	—	0.38	0.15	0.15	—
15	—	—	—	—	0.23	0.15	0.15	—
16	—	—	—	—	0.30	0.15	0.15	—

Table A11 Lithium concentration in herbage (µg/g)

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	1710	—	1850	2590	1610	2630	2220	3090
2	2460	1470	1030	—	—	—	—	—
3	—	3150	3280	2000	—	2980	4460	3930
4	2110	1540	1440	1520	1320	1620	3110	1540
5	—	268	261	226	606	189	229	—
6	—	431	373	539	180	278	543	—
7	—	840	340	618	238	516	371	—
8	—	—	—	—	728	1610	1440	1550
9	—	1280	—	2880	1230	1750	2320	2370
10	955	974	1600	2550	571	1990	3140	4020
11	1680	1800	2800	1320	760	1350	949	1690
12	2010	1300	823	1480	1640	1160	646	3200
13	—	—	—	—	640	74	129	—
14	—	—	—	—	666	44	101	—
15	—	—	—	—	535	23	54	—
16	—	—	—	—	748	41	64	—

Table A12 Sodium concentration in herbage (µg/g)

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	7850	—	10100	15300	13500	13300	13000	15300
2	14400	14100	11800	—	—	—	—	—
3	—	10400	13000	15800	—	13100	14400	13500
4	12700	13600	12200	16600	9630	14800	12600	13100
5	—	4950	8040	4570	5500	4780	7320	—
6	—	7430	10100	7560	3420	9130	11100	—
7	—	9100	10900	10500	5200	11400	11800	—
8	—	—	—	—	8640	11700	14100	13000
9	—	20600	—	22300	8920	12800	14800	15500
10	6320	10000	13800	13200	6530	12700	13500	12600
11	10000	11700	13800	17200	10000	14700	16400	17300
12	10000	10500	14100	15400	13200	13500	14200	13900
13	—	—	—	—	16300	14000	10400	—
14	—	—	—	—	15100	14300	10000	—
15	—	—	—	—	16100	11600	11800	—
16	—	—	—	—	14800	14100	11300	—

Table A13 Potassium concentration in herbage (μg/g)

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	4620	—	8250	6390	5840	7010	7110	5750
2	3160	5910	7240	—	—	—	—	—
3	—	7390	7810	6260	—	5910	6500	5340
4	303	6320	6770	3970	5530	5370	5960	3930
5	—	2120	2130	2070	2610	1350	1710	—
6	—	2760	1440	1440	1330	1070	1290	—
7	—	2080	1310	1660	1910	1330	1330	—
8	—	—	—	—	4690	7290	6080	5870
9	—	7440	—	7230	4930	5830	5830	6140
10	4190	7220	8450	6410	5270	5930	7420	7320
11	3770	6440	8270	7830	4050	6460	5940	4300
12	8160	12100	7780	7120	8370	9470	7490	6380
13	—	—	—	—	4550	6500	13100	—
14	—	—	—	—	46530	3640	8580	—
15	—	—	—	—	4380	5420	11200	—
16	—	—	—	—	4550	3920	12600	—

Table A14 Calcium concentration in herbage (μg/g)

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	6.25	—	1.50	1.75	4.50	0.75	0.50	1.75
2	1.75	1.50	2.75	—	—	—	—	—
3	—	1.25	1.25	1.50	—	0.50	0.75	1.75
4	2.50	2.50	1.50	1.50	3.75	0.75	1.00	6.25
5	—	0.75	1.25	1.00	2.50	1.00	4.00	—
6	—	1.50	1.00	1.00	1.00	1.50	1.50	—
7	—	1.00	1.75	1.00	1.00	0.75	0.75	—
8	—	—	—	—	5.50	1.50	1.00	3.50
9	—	1.50	—	1.25	6.25	0.50	0.50	1.00
10	9.00	6.00	0.75	1.50	6.00	0.75	0.50	0.88
11	5.25	2.00	1.50	1.25	7.50	1.25	0.63	1.25
12	1.75	3.00	2.00	2.50	1.75	0.75	0.50	1.75
13	—	—	—	—	1.25	0.50	0.50	—
14	—	—	—	—	1.00	0.75	2.00	—
15	—	—	—	—	1.00	1.00	0.75	—
16	—	—	—	—	1.25	1.00	0.75	—

Table A15 Chromium concentration in herbage (µg/g)

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	6.25	—	3.75	2.50	5.00	2.50	2.50	2.50
2	6.25	2.50	2.50	—	—	—	—	—
3	—	2.50	2.50	2.50	—	2.50	2.50	2.50
4	3.75	2.50	5.00	2.50	6.25	2.50	2.50	3.75
5	—	18.8	8.75	10.0	13.8	10.0	6.25	—
6	—	18.8	2.50	11.3	13.8	5.00	2.50	—
7	—	12.5	2.50	6.25	12.5	2.50	3.13	—
8	—	—	—	—	10.0	2.50	2.50	3.75
9	—	2.50	—	2.50	5.00	2.50	2.50	2.50
10	31.3	21.3	2.50	2.50	26.3	2.50	2.50	3.13
11	37.5	11.3	2.50	5.00	67.5	2.50	2.50	7.50
12	6.25	3.75	3.75	2.50	3.75	2.50	2.50	2.50
13	—	—	—	—	3.75	2.50	2.50	—
14	—	—	—	—	2.50	2.50	2.50	—
15	—	—	—	—	2.50	2.50	2.50	—
16	—	—	—	—	3.75	2.50	2.50	—

Table A16 Lead concentration in herbage (µg/g)

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	0.75	—	3.75	1.75	1.75	2.50	2.50	2.00
2	2.25	1.75	3.00	—	—	—	—	—
3	—	1.50	2.25	2.25	—	2.00	2.00	2.00
4	1.25	1.50	2.75	1.00	1.75	1.75	2.00	1.00
5	—	0.50	1.25	1.00	1.00	0.75	0.50	—
6	—	1.25	1.25	0.50	1.00	1.00	1.20	—
7	—	1.00	1.00	1.00	1.00	0.75	1.50	—
8	—	—	—	—	1.25	1.75	3.25	3.00
9	—	2.25	—	3.50	1.25	0.75	1.25	2.00
10	1.25	2.75	3.00	2.75	2.00	3.00	4.75	3.00
11	1.00	2.75	3.25	1.50	1.00	2.00	1.88	2.00
12	2.25	2.75	3.00	2.50	2.25	4.00	4.00	2.50
13	—	—	—	—	2.50	2.75	3.50	—
14	—	—	—	—	1.50	2.50	4.25	—
15	—	—	—	—	1.50	2.00	3.50	—
16	—	—	—	—	2.25	3.25	6.50	—

Table A17 Molybdenum concentration in herbage (µg/g)

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	13.5	—	44.0	12.9	10.3	11.1	9.75	12.5
2	13.8	13.5	30.3	—	—	—	—	—
3	—	11.3	30.6	11.1	—	10.5	12.0	11.0
4	13.8	21.5	16.8	13.1	12.3	14.6	11.0	17.6
5	—	13.1	10.1	6.38	6.63	6.88	9.25	—
6	—	16.1	9.88	8.50	7.63	9.38	7.38	—
7	—	13.8	9.88	8.88	7.75	7.38	7.69	—
8	—	—	—	—	14.1	10.3	9.00	13.1
9	—	17.4	—	9.63	8.63	5.75	7.75	10.9
10	22.8	20.1	13.6	9.63	15.1	10.5	17.5	11.4
11	17.3	14.8	32.0	13.0	15.8	12.4	11.1	14.3
12	11.1	10.0	26.1	12.1	10.6	9.88	12.1	13.0
13	—	—	—	—	8.75	7.50	9.00	—
14	—	—	—	—	9.13	8.88	10.4	—
15	—	—	—	—	8.75	7.63	9.88	—
16	—	—	—	—	9.75	10.1	9.00	—

Table A18 Copper concentration in herbage (µg/g)

Sampling Date

Site No.	1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	2.50	—	28.8	1.75	2.75	0.50	1.00	1.25
2	2.50	2.00	5.75	—	—	—	—	—
3	—	0.75	4.25	1.25	—	0.50	1.75	0.50
4	3.00	2.75	10.8	2.0	3.50	2.00	2.50	3.75
5	—	1.75	2.50	1.75	2.25	1.25	3.00	—
6	—	1.75	1.50	1.50	1.50	1.50	1.50	—
7	—	1.75	1.50	1.50	1.25	1.00	1.50	—
8	—	—	—	—	11.0	4.00	4.25	6.75
9	—	6.50	—	1.00	3.25	0.50	1.00	0.50
10	8.50	5.75	2.75	3.50	6.00	2.50	5.75	2.63
11	4.75	2.00	18.8	2.25	7.00	2.25	1.88	2.06
12	2.50	1.75	2.75	2.50	1.50	1.25	1.50	1.25
13	—	—	—	—	0.50	0.50	0.50	—
14	—	—	—	—	0.50	0.50	2.00	—
15	—	—	—	—	0.50	0.50	0.50	—
16	—	—	—	—	0.50	1.00	0.50	—

Table A19 Nickel concentration in herbage (µg/g)

Sampling Date

Site No.	1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	1880	—	1700	1930	1730	1860	2040	2010
2	1990	2080	1910	—	—	—	—	—
3	—	1650	1720	1640	—	1660	1800	1700
4	1880	2160	2000	2000	2200	2250	2710	2540
5	—	593	910	800	883	553	885	—
6	—	1020	1280	1180	995	1000	1340	—
7	—	1410	1240	1550	1450	1390	1320	—
8	—	—	—	—	1800	1930	1880	2250
9	—	1690	—	2120	1790	1550	1630	1930
10	1300	1800	1970	1960	1340	1790	2920	2400
11	1660	1950	2490	1740	1570	1980	2060	1920
12	1800	1530	1700	1810	1440	1700	1730	1850
13	—	—	—	—	1040	1340	1500	—
14	—	—	—	—	1120	1530	1860	—
15	—	—	—	—	1110	1350	2030	—
16	—	—	—	—	1110	1170	1910	—

Table A20 Magnesium concentration in herbage (µg/g)

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	8.25	—	1.25	1.00	5.25	0.38	0.25	2.00
2	1.88	1.25	2.25	—	—	—	—	—
3	—	1.50	0.75	1.38	—	0.25	0.50	2.00
4	2.75	2.63	0.75	0.75	4.38	0.25	0.50	6.88
5	—	3.13	1.25	1.63	3.75	1.63	0.88	—
6	—	4.13	0.63	1.88	3.00	1.00	0.38	—
7	—	2.13	0.38	1.00	2.63	0.88	0.50	—
8	—	—	—	—	5.38	1.00	0.50	2.88
9	—	1.13	—	0.63	7.25	0.25	0.25	0.63
10	14.9	8.88	0.38	1.63	10.25	0.25	0.25	0.94
11	6.00	1.75	0.75	1.13	8.00	0.38	0.38	1.13
12	3.16	3.88	0.75	3.25	2.38	0.50	0.50	2.25
13	—	—	—	—	1.13	0.25	0.38	—
14	—	—	—	—	0.75	0.25	0.38	—
15	—	—	—	—	0.88	0.25	0.25	—
16	—	—	—	—	1.13	0.25	0.38	—

Table A21 Vanadium concentration in herbage (μg/g)

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	14.5	—	17.1	13.2	12.8	10.3	11.6	9.07
2	10.7	13.4	17.0	—	—	—	—	—
3	—	8.00	8.92	7.50	—	5.47	7.73	7.35
4	12.1	15.8	16.7	9.52	14.8	11.4	13.3	12.1
5	—	8.90	5.62	6.90	7.50	4.05	4.88	—
6	—	9.82	4.73	6.90	8.85	3.83	4.35	—
7	—	9.75	4.95	8.77	13.2	5.70	5.51	—
8	—	—	—	—	19.4	22.7	18.2	17.3
9	—	11.3	—	8.70	11.1	6.38	8.25	7.57
10	11.9	12.2	8.85	7.65	10.1	4.95	7.05	8.25
11	8.85	8.55	10.4	6.00	8.40	6.97	7.39	6.07
12	25.2	29.0	22.1	22.5	22.7	22.7	19.6	21.1
13	—	—	—	—	7.65	11.1	30.2	—
14	—	—	—	—	8.55	7.42	27.2	—
15	—	—	—	—	8.25	9.90	27.8	—
16	—	—	—	—	8.02	7.13	33.4	—

Table A22 Strontium in field herbage (μg/g)

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	—	—	—	—	—	—	—	—
2	—	5.49	—	—	—	—	—	—
3	—	6.25	—	—	6.15	6.15	6.23	6.19
4	—	5.27	—	—	5.51	5.28	5.35	5.33
5	—	4.34	—	—	4.26	4.35	4.07	4.38
6	—	4.13	—	—	4.03	4.02	4.08	4.00
7	—	3.98	—	—	4.09	4.11	4.08	4.14
8	—	—	—	—	5.58	5.50	5.46	5.45
9	—	5.74	—	—	6.08	5.85	5.88	6.16
10	—	5.71	—	—	5.88	5.76	5.82	5.95
11	—	6.06	—	—	5.22	5.94	6.28	—
12	—	7.99	—	—	8.16	8.10	8.07	8.21
13	—	—	—	—	7.48	6.95	7.21	7.43
14	—	—	—	—	7.37	7.33	7.38	7.61
15	—	—	—	—	7.04	6.88	7.04	7.07
16	—	—	—	—	7.35	7.25	7.40	7.59

Table A23 pH (DIW) in subsoil

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	—	—	—	—	—	—	—	—
2	—	4.69	—	—	—	—	—	—
3	—	5.49	—	—	5.43	5.46	5.56	5.55
4	—	4.63	—	—	4.64	4.64	4.68	4.70
5	—	3.62	—	—	3.47	3.62	3.44	3.61
6	—	3.52	—	—	3.37	3.40	3.43	3.35
7	—	3.41	—	—	3.38	3.48	3.51	3.47
8	—	—	—	—	4.86	4.84	4.76	4.82
9	—	5.24	—	—	5.27	5.22	5.21	5.33
10	—	5.09	—	—	5.20	5.07	5.14	5.18
11	—	5.48	—	—	4.47	5.33	5.69	—
12	—	7.54	—	—	7.60	7.59	7.62	7.68
13	—	—	—	—	6.72	6.26	6.35	6.50
14	—	—	—	—	6.56	6.46	6.60	6.77
15	—	—	—	—	6.25	6.13	6.28	6.16
16	—	—	—	—	6.52	6.41	6.57	6.80

Table A24 pH ($CaCl_2$) subsoil

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	5.21	5.34	5.67	5.65	5.32	5.60	—	5.71
2	4.82	4.78	5.14	—	—	—	—	—
3	5.39	5.58	5.42	5.51	5.42	5.51	5.50	5.54
4	4.60	4.57	4.61	4.51	4.63	4.62	4.64	4.64
5	3.33	3.41	3.26	3.39	3.26	3.35	3.26	3.39
6	3.51	3.37	3.45	3.63	3.25	3.38	3.37	3.41
7	3.35	3.47	3.54	3.99	3.33	3.63	3.49	3.51
8	—	—	—	—	4.95	4.89	4.80	4.92
9	5.45	5.51	5.35	5.32	5.46	5.23	5.22	5.34
10	5.18	5.22	5.01	5.10	5.21	5.06	5.04	5.15
11	5.24	5.37	5.18	5.25	4.80	5.21	—	—
12	7.51	7.48	7.32	7.51	7.49	7.40	7.52	7.68
13	6.88	—	5.69	5.22	6.51	6.31	6.32	6.39
14	4.34	—	5.05	7.07	6.73	6.38	6.50	6.49
15	—	—	5.09	4.51	6.02	6.04	6.04	6.19
16	—	—	4.05	4.08	6.55	6.48	6.32	6.64

Table A25 pH ($CaCl_2$) in topsoil

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	2620	—	2930	3330	3830	3220	3440	3560
2	2590	2950	2760	—	—	—	—	—
3	—	3070	2520	3080	—	3100	3790	2700
4	2520	3640	3080	3630	3030	4600	3323	3710
5	—	1250	1750	1220	973	1300	1623	—
6	—	1740	1860	1470	1260	1570	1680	—
7	—	2310	1750	1830	1810	1870	1810	—
8	—	—	—	—	2600	3080	2800	3330
9	—	3220	—	3220	3370	2690	3090	4100
10	2390	3340	2810	3110	2780	2840	5500	4670
11	3240	3810	4760	4340	3360	4410	4120	4240
12	4710	3650	4830	4370	3880	4420	3750	3470
13	—	—	—	—	3790	3450	3040	—
14	—	—	—	—	4000	2370	2800	—
15	—	—	—	—	4180	2880	2990	—
16	—	—	—	—	4110	2770	3050	—

Table A26 Sulphur concentration in herbage (µg/g)

Sampling Date

Site No.	1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	930	760	920	820	910	1060	940	660
2	830	790	920	—	—	—	—	—
3	600	610	580	690	660	640	550	570
4	540	540	690	740	640	630	570	575
5	930	670	860	760	810	850	850	840
6	2060	2730	3390	3450	2560	3070	2870	3450
7	3230	2670	2800	3230	3320	3330	2920	3270
8	—	—	—	—	560	540	510	470
9	450	480	480	490	420	480	440	430
10	880	850	910	990	960	910	890	880
11	1000	830	930	960	950	990	913	856
12	1060	990	1190	1120	1000	1190	1020	840
13	180	—	180	160	170	190	160	150
14	180	—	170	160	190	210	190	140
15	—	—	190	180	150	160	170	140
16	—	—	180	150	170	130	100	130

Table A27 Sulphur concentration in topsoil (µg/g)

Sampling Date

Site No.	1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	—	—	—	—	—	—	—	—
2	—	860	—	—	—	—	—	—
3	—	500	—	—	540	390	460	480
4	—	470	—	—	580	540	530	450
5	—	490	—	—	600	560	510	500
6	—	1160	—	—	1260	1360	1330	1600
7	—	1240	—	—	1640	1420	1340	1260
8	—	350	—	—	560	550	530	480
9	—	580	—	—	380	400	330	320
10	—	590	—	—	700	670	580	600
11	—	850	—	—	860	900	790	772
12	—	—	—	—	930	850	840	780
13	—	—	—	—	190	140	140	150
14	—	—	—	—	240	110	160	160
15	—	—	—	—	180	140	160	150
16	—		—	—	140	180	110	110

Table A28 Sulphur concentration in subsoil (µg/g)

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	13.7	12.6	18.0	14.4	14.01	15.8	16.0	9.70
2	12.9	6.77	7.00	—	—	—	—	—
3	11.4	10.9	12.5	12.3	11.6	11.3	9.05	10.1
4	11.6	7.82	10.6	10.9	7.87	8.20	6.62	8.83
5	66.9	20.7	29.0	20.7	20.4	22.1	30.8	35.0
6	86.7	69.4	80.2	85.6	73.9	51.1	82.8	71.7
7	70.5	75.2	66.8	81.0	82.6	71.9	67.0	83.2
8	—	—	—	—	6.75	5.36	6.22	7.42
9	8.42	7.63	7.76	8.02	7.32	7.23	6.80	7.39
10	14.5	13.9	14.7	14.1	14.5	13.3	17.1	14.3
11	13.6	10.5	11.9	10.9	12.2	10.9	10.6	10.9
12	7.27	9.33	11.4	7.93	5.39	9.63	7.67	6.09
13	2.37	—	2.63	2.68	2.44	2.61	2.61	2.50
14	2.34	—	2.35	2.53	2.84	2.99	2.84	2.26
15	—	—	2.49	2.86	2.27	2.79	2.39	2.57
16	—	—	2.18	2.44	1.92	2.12	3.60	1.67

Table A29 Organic matter content of topsoil (%)

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	—	—	—	—	—	—	—	—
2	—	6.61	—	—	—	—	—	—
3	—	9.11	—	—	9.32	7.45	9.01	8.47
4	—	7.24	—	—	6.33	7.64	6.89	7.41
5	—	9.88	—	—	14.4	10.1	16.8	12.0
6	—	21.6	—	—	31.9	25.4	25.5	33.0
7	—	44.4	—	—	33.9	33.4	27.9	24.8
8	—	—	—	—	5.36	4.95	5.52	5.51
9	—	5.63	—	—	4.84	6.36	5.76	5.09
10	—	10.0	—	—	12.0	11.0	11.2	10.4
11	—	8.39	—	—	8.96	9.18	9.19	7.64
12	—	7.42	—	—	9.08	6.27	7.33	7.18
13	—	—	—	—	2.63	2.65	2.54	2.48
14	—	—	—	—	2.82	3.11	2.79	2.55
15	—	—	—	—	2.26	2.99	2.67	2.69
16	—	—	—	—	1.92	2.17	1.97	1.88

Table A30 Organic matter content of subsoil (%)

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	82.7	76.9	54.0	77.1	76.1	75.2	70.4	64.2
2	86.9	93.1	92.9	—	—	—	—	—
3	85.5	85.7	83.3	84.4	86.7	78.5	89.3	87.9
4	76.2	80.5	73.2	75.7	82.4	74.9	76.9	72.5
5	33.1	79.3	71.0	79.3	79.6	77.9	69.2	65.0
6	13.3	30.7	19.8	14.4	26.0	48.9	17.2	28.3
7	29.5	24.8	33.2	19.0	17.4	28.1	33.1	16.8
8	—	—	—	—	83.8	91.1	89.7	88.2
9	91.1	90.9	89.1	88.6	87.8	87.2	91.6	89.5
10	85.2	85.6	87.9	85.4	84.1	86.5	82.4	85.7
11	86.3	89.1	87.5	88.8	87.6	88.9	89.0	88.9
12	92.7	90.7	88.6	92.1	94.6	90.4	92.6	93.9
13	87.1	—	94.9	96.5	96.6	95.7	97.3	95.5
14	96.4	—	89.4	95.1	95.3	95.4	95.4	94.2
15	—	—	95.2	96.2	95.2	87.6	94.3	94.5
16	—	—	94.9	94.2	96.5	93.2	95.1	96.1

Table A31 Mineral fraction in the topsoil (%)

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	2800	—	384	341	1690	194	119	752
2	723	475	937	—	—	—	—	—
3	—	546	261	441	—	121	203	626
4	1090	1090	357	301	1810	167	255	3390
5	—	315	199	236	876	207	166	—
6	—	386	164	219	269	129	83	—
7	—	263	192	231	360	173	160	—
8	—	—	—	—	3650	678	348	590
9	—	376	—	126	2190	83	88	240
10	2500	1370	117	302	1550	78	116	226
11	2320	663	231	362	3010	182	158	467
12	765	1160	243	931	554	168	179	713
13	—	—	—	—	343	120	142	—
14	—	—	—	—	298	76	185	—
15	—	—	—	—	294	103	123	—
16	—	—	—	—	415	111	140	—

Table A32 Iron concentration in herbage (µg/g)

Sampling Date

Site No.	1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	37440	39880	36880	38800	38790	39130	38960	48760
2	32650	33390	32760	—	—	—	—	—
3	39430	40060	38480	38720	38400	40100	39450	41690
4	40300	41600	40050	41180	41400	41720	41840	41510
5	22170	28830	18210	25180	16880	21960	19840	22740
6	9850	11340	11130	8510	10220	8400	12210	9500
7	7350	13740	16740	10210	10170	9430	12030	11410
8	—	—	—	—	39410	39870	38540	39880
9	32100	36510	35660	35590	36050	33430	32530	32280
10	36420	41270	35860	37040	37590	38170	36520	37410
11	29820	31850	30140	30610	30130	32750	31470	31650
12	26350	27130	26660	26240	26420	26980	26880	25780
13	37150	—	36350	35950	37270	37280	36720	37030
14	30110	—	34251	34630	42220	42700	41530	39300
15	—	—	33194	35710	35360	35680	36680	36220
16	—	—	33170	33390	30910	33050	33300	32760

Table A33 Iron concentration in topsoil (μg/g)

Sampling Date

Site No.	1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	—	—	—	—	—	—	—	—
2	—	3370	—	—	—	—	—	—
3	—	40480	—	—	40710	42050	40750	40450
4	—	42450	—	—	42070	43260	42610	40820
5	—	40020	—	—	37830	38910	40070	37660
6	—	18200	—	—	15570	15980	16140	11780
7	—	11640	—	—	11170	16420	16480	19230
8	—	—	—	—	39450	40560	39380	37460
9	—	37660	—	—	38120	32680	33180	32800
10	—	44600	—	—	42130	45730	44380	43420
11	—	31910	—	—	30910	33000	32590	31910
12	—	28090	—	—	27600	27600	27560	26910
13	—	—	—	—	38740	37010	36790	35510
14	—	—	—	—	40640	41400	40280	46830
15	—	—	—	—	34010	35560	36030	41700
16	—	—	—	—	32480	30420	32060	31390

Table A34 Iron concentration in subsoil (μg/g)

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	0.680	0.686	0.662	0.614	—	—	—	0.721
2	0.179	0.122	0.142	—	—	—	—	—
3	0.805	0.801	0.875	0.848	—	—	0.812	0.904
4	0.486	0.503	0.559	0.578	—	—	0.535	0.556
5	0.693	0.840	0.687	0.666	—	—	0.601	0.849
6	0.503	0.774	0.867	0.660	—	—	0.909	0.644
7	—	0.736	0.841	0.740	—	—	0.691	0.826
8	—	0.335	—	—	—	—	0.354	0.410
9	0.350	0.411	0.342	0.356	—	—	0.342	0.344
10	0.401	0.356	0.465	0.465	—	—	0.453	0.448
11	0.373	0.074	0.375	0.425	—	—	—	—
12	0.054	—	0.060	0.069	—	—	0.071	0.061
13	0.042	—	0.062	—	—	—	0.056	0.049
14	0.094	—	0.077	—	—	—	0.056	0.047
15	—	—	0.085	—	—	—	0.053	0.055
16	—		0.098	—	—	—	0.054	0.055

Table A35 Topsoil pyrophosphate extractable iron content (%)

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	—	—	—	—	—	—	—	—
2	—	0.142	—	—	—	—	—	—
3	—	0.861	—	—	—	—	0.841	0.948
4	—	0.477	—	—	—	—	0.517	0.531
5	—	1.259	—	—	—	—	1.203	1.218
6	—	0.591	—	—	—	—	0.678	0.597
7	—	0.370	—	—	—	—	0.592	0.768
8	—	—	—	—	—	—	0.328	0.336
9	—	0.325	—	—	—	—	0.340	0.354
10	—	0.381	—	—	—	—	0.404	0.424
11	—	0.310	—	—	—	—	0.322	—
12	—	0.070	—	—	—	—	0.069	0.062
13	—	—	—	—	—	—	0.066	0.051
14	—	—	—	—	—	—	0.057	0.051
15	—	—	—	—	—	—	0.066	0.059
16	—	—	—	—	—	—	0.053	0.053

Table A36 Subsoil pyrophosphate extractable iron content (%)

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	9.13	33.1	31.9	32.4	36.5	37.2	—	34.2
2	3.91	15.6	17.2	—	—	—	—	—
3	31.6	30.5	30.3	27.8	31.6	27.9	24.4	31.2
4	24.3	23.8	23.6	25.8	24.4	24.4	21.7	25.7
5	64.4	48.9	61.8	44.7	53.3	54.6	57.6	60.7
6	84.3	96.4	110	103	87.8	85.9	109	106
7	94.9	96.4	91.9	109	118	109	97.0	107
8	—	—	—	—	12.0	10.4	12.7	13.8
9	19.6	20.0	19.5	21.5	19.3	18.9	19.1	20.8
10	39.7	36.2	38.9	39.1	35.7	37.4	39.0	40.9
11	28.3	26.9	26.9	24.3	26.3	27.5	—	—
12	24.1	25.3	28.5	24.6	25.3	25.0	23.4	24.6
13	10.4	—	9.48	10.1	10.1	9.30	9.22	9.13
14	9.70	—	9.39	10.3	10.1	9.65	10.5	10.2
15	—	—	8.61	9.57	8.43	8.52	8.69	9.04
16	—	—	8.61	9.30	7.83	7.04	8.43	8.26

Table A37 Cation exchange capacity of topsoil (me/100g)

Site No.	Sampling Date 1 Jan 86	2 May 86	3 Jul 86	4 Oct 86	5 Feb 87	6 May 87	7 Jul 87	8 Oct 87
1	—	—	—	—	—	—	—	—
2	—	15.7	—	—	—	—	—	—
3	—	26.7	—	—	28.2	24.8	28.1	29.7
4	—	23.4	—	—	24.9	23.0	26.5	24.3
5	—	32.9	—	—	37.9	33.9	43.0	42.6
6	—	55.0	—	—	55.9	52.8	65.2	60.6
7	—	64.4	—	—	80.4	66.3	69.0	59.9
8	—	—	—	—	12.4	8.35	12.5	11.5
9	—	16.7	—	—	18.3	17.7	19.5	18.7
10	—	29.2	—	—	35.0	32.5	36.7	35.9
11	—	23.7	—	—	22.4	23.7	26.0	—
12	—	25.2	—	—	25.4	23.0	24.1	25.6
13	—	—	—	—	9.30	9.39	9.91	9.48
14	—	—	—	—	10.1	9.39	11.2	12.0
15	—	—	—	—	8.96	8.87	10.7	9.39
16	—	—	—	—	8.43	7.57	8.69	8.00

Table A38 Cation exchange capacity of subsoil (me/100g)