

ECOPHYSIOLOGICAL EFFECTS OF HIGH CONCENTRATIONS OF  
IRON AND OTHER HEAVY METALS  
ON ERIOPHORUM ANGUSTIFOLIUM HONCK.  
AND PHRAGMITES AUSTRALIS (CAV.) TRIN EX STEUDEL

By

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To my  
family and the memory of G.W.M

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SUMMARY

Little is known of the effect of high concentrations of heavy metals on the growth of wetland plants, although it is has long been suggested that adaptation to waterlogged anaerobic environments involves the capacity to exclude dissolved iron.

This work investigates the chemical dynamics of selected metal toxins, in particular Fe, in relation to concentration in the substrate and uptake by Eriophorum angustifolium and Phragmites australis taken from mine populations (Parys Mountain, Anglesey and Crymlyn Bog, Swansea) and a non-mine population (Skipwith Common, Yorks) and to provide information on the resistance of these two species to high concentrations of Fe and other heavy metals.

Both field and laboratory work have been used to focus on the above areas. The first part of the study is based on the analysis of plants and soil samples collected from the field and relates heavy metal concentration in plant tissue to concentrations in soil/sediments. The second part of the study is based on laboratory work to determine individual factors influencing heavy metal uptake and resistance in the study species.

Fe, Mn, Cu, Zn and Pb concentrations in plant tissues reflected those in the soil. Seasonal fluctuations in metal concentration in plant tissues were observed but the bulk differences were related to soil heterogeneity between sites.

In culture solutions, Fe uptake in both species increased with increasing Fe supply. E. angustifolium from the Parys site was less sensitive to high Fe concentrations relative to plants from the Skipwith site. Fe-uptake by E. angustifolium, was strongly influenced by pH. Fe-uptake by plants was unaffected by the presence of Fe-plaques on roots.

A relationship between Fe and P is highlighted; enhancing P availability to plants of E. angustifolium in the presence of 100 mg/l Fe by treating half the roots with Fe and the remaining half with 0.1-strength Rorison solution with 1 mg/l Fe, stimulated root oxidation of Fe and the accumulation of Fe-plaque, reducing translocation of Fe to shoots. Plants of the same species with Fe-plaque on the roots accumulated more P than plants without plaque, the bulk of this P was immobile.

E. angustifolium from the Parys Mountain site was found to be more tolerant to Cu but more sensitive to Mn, the reverse was true for plants from the Skipwith site.

The implications of this work in relation to plant growth and resistance to heavy metal-enriched environments are discussed. It is suggested that both species may be constitutionally tolerant to Fe and Mn. Plants from the Parys site may be more resistant to high concentrations of Cu, Zn and Pb.

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## Chapter 1

### General Introduction

#### 1.1 Soil contamination by heavy metals and the evolution of tolerance in plants

The heavy metals, defined by Passow, Rothstein & Clarkson (1961), are those metals that have a density greater than 5 and, as a group, contain about 38 elements, including iron (density 7.9 g/cm<sup>3</sup>, Gilmore 1979), Mn, Cu, Zn and Pb. Their common features in relation to plant growth is that in excessive quantities they are toxic causing, for example root stunting and ultimately death (Bradshaw, McNeilly & Gregory 1965). Local enrichment of heavy metals in soils, either through natural processes (geochemical anomalies) or human activities (exploitation of mineral resources), is usually

associated with increased resistance levels in plants (Antonovics, Bradshaw & Turner 1971). Thus, the presence of a species or a race on metal-contaminated soils suggests that it is tolerant to metal toxicity. Such edaphic adaptation, however, necessitates a specialized physiology resulting in either constitutional tolerance within the species or ecotypic differentiation of the species, into metal-tolerant races which are specifically adapted physiologically (Baker 1981).

According to Bradshaw (1952), the evolution of metal tolerance in plant populations arises as a result of selection in localized areas of heavy metal contamination. Metal-tolerant populations contain individuals which have been selected and which are able to tolerate much higher concentrations of metals than individuals growing in non-contaminated areas (Antonovics et al. 1971). The mechanism of tolerance can be specific such that possession of tolerance to one or two metals does not necessarily confer tolerance to another metal not present at the site in high concentrations (Ernst 1976, Qureshi, Thurman, Hardwick &

Collin 1985).

#### 1.1.1 Strategies for survival in metal-contaminated soils

Levitt (1980) has described the nature and meaning of stress injury and resistance in organisms. In accordance to Levitt's descriptions, heavy metals in the plant environment can operate as 'stress' factors in that they can cause 'strain' by affecting physiological processes in plants. In so doing they can reduce vigour, or totally inhibit plant growth. 'Sensitivity' describes the effects of stress resulting in injury or death of the plant, while 'resistance' refers to the reaction of a plant to heavy metal stress in such a way that it can grow and reproduce successfully. Heavy metal resistance can be achieved in one of two ways: by 'avoidance' and by 'tolerance'. Following Levitt's definitions avoidance is defined as an organism's ability to prevent the uptake of high concentrations of metals. Tolerance is then used to describe plant survival of the

effects of internal stress to excessive levels of metals in plant tissues.

## 1.2 Response of terrestrial plants to toxic metals

There is now assembled a substantial body of information relating to evolutionary, genecological, ecological, physiological and biochemical aspects of heavy metal tolerance in terrestrial plants (Bradshaw et al. 1965, Antonovics, et al. 1971, Ernst 1976, Macnair 1981, Baker and Walker 1989). The emphasis of these studies has been largely comparative, based on experiments on tolerant and non-tolerant clones of grasses such as Agrostis capillaris, Deschampsia cespitosa and Anthoxanthum odoratum, detailed work with Silene vulgaris and interspecific screening experiments in metallophyte floras. These have all provided an insight into tolerance mechanisms (Baker 1987).

A complex picture has emerged where few generalizations

are possible. Yet it is apparent that the mechanisms are largely internal in that metals are rarely not absorbed by plants growing in metalliferous conditions (Baker 1987) and so resistance is achieved by true tolerance (sensu Levitt 1980). What also emerges is that tolerance is manifested by a suite of physiological and biochemical adaptations developed to varying degrees, for different metals in different species and populations (Baker 1987).

### 1.3 Wetland plants and toxic metals

Considerable information exists on the response of terrestrial plants to heavy metal toxins in particular the plant-soil relationships of plants able to colonize metalliferous mine wastes (reviewed by Baker 1987). In contrast, the effect of high concentrations of heavy metals on the growth of wetland plants and their subsequent concentration in plant tissues, as well as the mechanisms by which these plants resist high concentrations of heavy metals,

have received little detailed study.

Much of the data concerning heavy metal uptake in wetland plants has been concerned with the uptake of pollutants in effluent and dredged materials with respect to detoxification ( Folsom, Lee & Bates 1981, Lawson 1985 ). Limited work has centred on heavy metal cycling in wetland ecosystems (Schierup & Larsen 1981, Larsen & Schierup 1981) and little work is available on the between-site variations in plant tissue composition of heavy metals in metal-enriched and contaminated wetlands (Mayor & Gorham 1951, Simmers, Folsom, Lee & Bates 1981, Wheeler, Al-Farraji & Cook 1985). Much less is known about the evolution of heavy metal tolerance and the mechanisms of tolerance in wetland plants. The most comprehensive study reported so far has been for Typha latifolia (Taylor & Crowder 1983a,b,1984).

### 1.3.1 The wetland environment

#### 1.3.1.1 Physico-chemical characteristics of wetland soils

One of the most important characteristics of wetland habitats is the permanently saturated nature of waterlogged soils (Ponnamperuma 1972). Two other factors, however, are of equal importance in identifying waterlogged conditions. They are low oxygen availability and low redox potentials and pH (Ponnamperuma 1972, Gotoh & Patrick 1974, Armstrong 1982). As a direct result of these conditions, there is increased availability of reduced elements, in particular Fe (II) and Mn (II) and reduced forms of sulphur and nitrogen which may also be toxic to plants in high concentrations.

i) Gas exchange/ absence of molecular oxygen

When a soil is submerged, gas exchange between soil and air is drastically reduced, and the rate of oxygen diffusion is no longer sufficient to maintain the supply for aerobic organisms. A new population of anaerobic microorganisms builds up, redox potential declines and chemically reducing conditions predominate (Ponnamperuma 1972).



ii) Oxidation-reduction (redox) potential

When the oxygen supply is limited, a proportion of soil microorganisms make use of electron acceptors other than oxygen for their respiratory oxidations. This results in the conversion of numerous compounds such as Mn (IV), Fe(III) into a state of chemical reduction and is reflected in a lowering of the redox potential (Ponnamperuma 1972).

The redox potential (Eh) of a system is a measure of its tendency to receive or supply electrons and is governed by the nature and proportions of the oxidizing and reducing substances that it contains (Armstrong 1982) For example, a common redox couple in soil is the reversible ferrous:ferric system  $Fe^{2+}(II) \rightleftharpoons Fe^{3+}(III) + e^-$ . In pure solutions, when the ferrous and ferric ions are in equal concentration, this system has an Eh of +771 mV relative to the standard hydrogen electrode of  $Eh = 0.00mV$  ( $1/2H_2 \Rightarrow H^+ + e^-$ ) (Armstrong 1982).

iii) pH and soil chemistry of wetland soils

Soil pH bears an important relationship to a large number of complex inorganic equilibria which may also be under the control of redox conditions and biological activity (Etherington 1982).

Low pH, resulting in large concentrations of  $H^+$ , is also associated with low concentrations of metals such as Ca, Mg and K. Equilibrium processes which are active at specific pH's include the precipitation of phosphorus by Fe and aluminium at low pH. (The significance of this will be made clear later). The biological consequences may be a direct result of the solubility/availability of particular elements, for example, Fe and Mn at different soil pH values, but may also be related to above-threshold limits of toxic elements such as Fe, Mn and Al (Etherington 1982).

### 1.3.2 Adaptation of higher plants to the wetland environment

The wetland environment can be unusually hostile to plant life, yet there is a vast assemblage of species, either

restricted to wetland sites or tolerant to some degree of soil anaerobiosis. The degree to which tolerance is achieved may be dependent on one or more of a number of recognizable features characteristic of plants confined to these conditions. These are: i) the capacity to exclude or tolerate soil-borne toxins ii) the provision of air-space tissue (aerenchyma) iii) the capacity to metabolize anaerobically and to tolerate an accumulation of anaerobic metabolites iv) the capacity to respond successfully to periodic soil flooding (Armstrong 1982).

The first and second points are central to this study and will be amplified below.

#### 1.3.2.1 The exclusion of soil toxins

As mentioned above (1.3.1), waterlogged soils are anaerobic and characterized by low redox potential and sometimes low pH. Under such conditions reduced forms of metals such as Fe (ferrous iron, Fe (II) ) and sometimes Mn

are released into the soil solution from otherwise less soluble forms and become available for plant uptake in concentrations which are potentially toxic. In addition, other compounds which occur frequently under these conditions also pose problems to plants, such as high concentrations of dissolved sulphides and ammonium ions. However, many plant species are able to grow successfully in these conditions without displaying symptoms of toxicity.

Roots of wetland plants such as rice and reed canary grass growing under conditions of inadequate aeration have been shown to oxidize their immediate soil environment (Bartlett 1961). The latter author noted that oxidized Fe deposits were common on the roots of hydrophytes and showed that root-oxidizing activity was specifically correlated with the ability to tolerate waterlogged soil. The presence of these deposits appears to indicate that wetland plants may have the ability to exclude significant amounts of Fe from the roots by re-oxidation processes (Armstrong 1982).

i) Radial oxygen loss and the function of air-spaces

One of the most notable anatomical characteristics of many plants tolerant to oxygen-deficient soils is the presence of air-spaces in the roots (Armstrong 1967, Green & Etherington 1977, Justin & Armstrong 1987). The primary function of air spaces is to provide adequate supply of oxygen to root meristems for growth in poorly aerated soils. In most wetland plants up to 60 % of the plant volume is made up of pore spaces. As a consequence internal diffusive resistance is low and there is efficient movement of oxygen from the organs of oxygen supply (shoots) to the organs of O<sub>2</sub> demand (roots).

Not all wetland plants have aerenchyma or high root porosity, comparisons by Justin and Armstrong (1987) of related genera from different habitats (wetland, intermediate and non-wetland) indicated that preference of wetland habitats in species of Luzula, Lychnis-Silene, Thalictricum and Galium

could not be explained in terms of predisposition to aerenchyma formation. All had low root porosities and lacked

aerenchyma; shallow rooting was their only obvious wetland survival strategy. These authors also distinguished between flood tolerance, which implied the ability to withstand sudden, episodic flooding of the soil, and wetland tolerance, implying ability to survive and compete in permanently flooded soils. Flooding response and tolerance were related to fractional root porosity (gas filled pore volume (porosity) of roots).

The exclusion of soil-borne toxins by oxidation can be related to radial oxygen loss (ROL) from the root causing direct oxidation of the rhizosphere, to enzymic oxidation within the root and its surface and to micro-organism-dependent oxidations adjacent to the root surface (Armstrong 1982). Measurements of radial oxygen loss have shown that the lowest values were to be found in non-wetland plants such as Mercurialis perennis (Martin 1968). Protection by ROL implies the formation of an oxygenated zone around the roots which forms a buffer between the cells of the root and the hostile soil environment. The

pattern of Fe deposition on plant roots appears to support this view. Since soluble Fe and other chemically-reduced products usually abound in waterlogged soils, their presence is reflected in substantial lowering of soil oxidation-reduction potentials. Well ventilated root systems can effect re-oxidation in such soils, but the narrowness of oxidized rhizospheres ensures that, unless root densities are high, re-oxidation is a localized phenomenon (Justin & Armstrong 1987).

### 1.3.3 Response to toxic metals

As mentioned above (1.3), little is known about the uptake and subsequent concentration of heavy metals in toxic concentrations and the mechanisms by which wetland plants resist heavy metal toxins. In addition, limited data exist on the degree of variation between plants in their ability to tolerate high internal concentrations of these metals

(Smirnoff 1981).

i) Fe

Fe is an essential element for all organisms. Its biological usefulness arises from its properties as a transition metal. It can easily undergo changes in oxidation state and form complexes with various organic ligands. Because of this property Fe has a prominent function in electron transfer. For example, it occurs in the cytochromes and ferredoxin. Apart from being complexed in electron transfer proteins it also has other functions. Fe (II) is a cofactor for aconitase and is involved in chlorophyll synthesis (Salisbury & Ross 1978).

The toxicity of Fe, if it is available to plants in large amounts (see Chapter 2 for concentrations which have been shown to be toxic), is a feature that is shared with other micronutrients. Most micronutrients for example Mn, Cu and Zn can be toxic under certain conditions (Antonovics et al. 1971, Foy, Chaney & White 1978, Lepp 1981).



Fe toxicity in waterlogged, reduced soils, is a consequence of increased solubility and mobility of ferrous iron. The toxic effects of high concentrations of Fe are well recognized (Foy et al., 1978), particularly in rice (Tanaka & Navasero 1966b, Tanaka, Loe & Navasero 1966, Tadano 1975, Ottow et al. 1983) and much work has now been reported on the effects of increased availability of reduced Fe (Fe (II)) in relation to waterlogging tolerance (reviewed by Armstrong 1982). However, for the most part, the literature on Fe toxicity is scattered or incomplete. Many examples are simple correlations of high leaf Fe content with visible symptoms, mostly in rice plants and often confounded by the presence of other potential toxicities such as sulphide and Mn (Foy et al., 1978; Woolhouse 1983).

Despite this, several factors have emerged from the literature which have been found to have an important effect on Fe uptake by plants. These are redox potentials (Gotoh & Yamashita 1966, Ponnampereuma 1972), pH (Rediske & Biddulph 1953, Tanaka & Navasero 1966b), availability of Fe (Jones

1971), availability of P ( Tanaka & Navasero 1966b, Waldren, Etherington & Davies 1987) and root oxidation of Fe (see above).

Relatively few data exist on the mechanisms involved in the resistance of wetland plants to high concentrations of Fe. However, what is apparent is that oxidation of ferrous iron, which is the plant-available form, may represent a suitable avoidance mechanism (Armstrong & Boatman 1967, Green & Etherington 1977, Foy et al. 1978). Removal of the root in Salix cinerea (waterlogging-tolerant species) causes this species to become more sensitive to much lower concentrations of Fe (Talbot & Etherington 1987), which supports the view that the root is an important site of the mechanism for avoiding potential Fe toxicity.

ii) Mn

Like Fe, Mn is a transition metal and, as such, has some similar properties. It exists primarily in the divalent form and undergoes oxidation during photosynthesis. It is also

important in electron transfer during photosynthesis and the activation of certain enzymes in fatty acid synthesis (Salisbury & Ross 1978).

Increased Mn availability may occur in various metalliferous soils, in particular acidic ones but also in 'normal' soils upon waterlogging (waterlogging promotes the reduction of Mn to the divalent plant-available form (Foy et al. 1978). (The Eh of the solutions of flooded soils high in Mn is usually 100 to 200 mV at pH 6.5 to 7.0. In this region, the stable solid phases are MnO<sub>2</sub> (at Eh greater than 170 mV), Mn<sub>2</sub>O<sub>3</sub> and MnCO<sub>3</sub> (Ponnamperuma, Loy and Tianco 1969). It is not surprising, therefore, that wetland plants usually show an increased resistance to Mn (Verkleij & Schat 1989). In rice, Mn resistance has been attributed to decreased uptake, effected by an increase in oxidizing capacity of the root (Horiguchi 1987). In addition to its ability to oxidize Mn from the divalent (available) form to the tetravalent (unavailable form) (and thereby reduce uptake), the rice plant has high internal tolerance to Mn (Tanaka & Navasero 1966a),

as its shoots accumulate 5 to 10 times as much Mn as those of other grasses such as oats, barley, wheat and ryegrass (Vlamis & Williams 1967).

### iii) Other metals

The tolerance of Typha latifolia to high concentrations of lead, zinc, cadmium, copper and nickel has been demonstrated by McNaughton et al. (1974). A general resistance to heavy metals was implied. This was confirmed again in the same species for Cu and Ni by Taylor and Crowder (1984).

Chiaudani (1969), observed that total Cu concentrations in the sediments of six Italian lakes controlled the accumulation of this element in stems and leaves of Phragmites australis. In contrast, the Cu content of the shoots of plants growing in Cu-enriched and 'normal' lake sediments was similar. This implies that an internal tolerance mechanism is present in this species, which restricts transport of Cu to the shoot.

Recent developments in work on wetland plants and toxic metals suggest the possible restriction of heavy metal uptake by Fe deposits on the roots of certain marsh plants (St-Cyr & Crowder 1987, Otte et al. 1989). Marsh (wetland) plants are often found growing in estuaries and rivers polluted with heavy metals (Simmers et al. 1981). Plants forming deposits of Fe (Fe-plaque) could therefore benefit from absorption and immobilization of the metals by Fe plaque.

#### 1.3.3.1 The mechanism of metal toxicity

The mechanisms of metal toxicity have been studied little. As with all studies of the effect of toxic substances, it is difficult to separate primary and secondary effects. There is no reason to suppose that all toxic metals have the same effect, or even that there is only one primary effect. Some of the direct effects include inhibition of cell elongation by toxic concentrations of Zn (Wainwright & Woolhouse 1975), inhibition of respiration, by toxic concentrations of Cu (Wu, Thurman & Bradshaw 1975) and

inhibition of photosynthetic carbon fixation and nitrogen fixation (in leguminous plants) (Baker & Walker 1989). Indirect effects include the effect on growth performance (biomass yield or growth rate) (Ernst 1976), and on seedling survival (Karataglis 1980d).

The basis of Fe toxicity is probably similar to those described above. Fe toxicity has often been reported to have considerable effects on biomass yield and production in rice (Howeler 1973, Ottow et al. 1983). Symptoms include development of brown spots commencing at the leaf tips, spreading over most of the leaf blade and gradually over most of the aerial part of the plants ultimately leading to death (Tadano 1975). However, symptoms differ significantly with plant age and between varieties. Thus, it is often difficult to identify Fe toxicity by visual symptoms alone. Yet there is no simple relationship between soil Fe concentration and the condition of the plant (Tadano 1975). The susceptibility of rice plants for example, to Fe toxicity is influenced by the physiological status of the plant. Tanaka, Loe and

Navasero (1966) reported that Fe absorption by the roots of rice plants grown at a high Fe concentration was accelerated by excision of the root. Ottow et al. (1983) reported that rice plants deficient in P, K and Ca showed symptoms of Fe toxicity. Implicit in these observations is that healthy plants have mechanisms to protect against the toxic effects of high concentrations of Fe.

#### 1.4 Aims and Approaches

Wetland plants do occur in metal-rich environments, and Phragmites australis a species more characteristic of nutrient- and base-rich conditions (Haslam 1972), was found growing at the disused ochre pits at Mynydd Parys, Anglesey, in association with Eriophorum angustifolium, a species more characteristic of highland regions and lowland bogs (Phillips 1954) at low pH and high concentrations of Fe and other metals present at sites marked by superficial precipitated iron ochre. It has long been

suggested that adaptation to waterlogged anaerobic environments involves the capacity to exclude dissolved Fe (Armstrong 1967, Armstrong and Boatman 1967, Green and Etherington 1977, Good and Patrick 1987). Adaptation to the wetland environment may confer resistance to potentially toxic concentrations of heavy metals such as Fe and Mn. It is probable that the success of these two species under the above conditions is related to the ability to exclude Fe.

#### 1.4.1 Aims

This project has two main aims, summarized:

i) to investigate the chemical dynamics of selected metal toxins, in particular Fe, in relation to concentration in the substrate and uptake by Eriophorum angustifolium and Phragmites australis.

ii) to provide information on the resistance of these two species to high concentrations of Fe and other heavy metals.

The work was also believed to have a more general application with regard to plant tolerance of anoxic



conditions.

#### 1.4.2 Approaches

Both field and laboratory work have been used to focus on the areas outlined above. The first part of the study is based on analysis of plant and soil samples collected from the field to follow heavy metal uptake in Eriophorum and Phragmites, and relates concentration in plant tissues to concentration in the soil/sediment. In order to obtain a complete picture of the dynamics of selected heavy metals in the environment, a study of seasonal cycling of heavy metals in plants and soils was undertaken in two metal-rich sites and a 'normal' wetland site. The second part of the study is based on laboratory work to determine under controlled conditions some of the factors influencing metal uptake and resistance in these two species.

## CHAPTER 2

SOIL/SEDIMENT HEAVY METAL CONCENTRATIONS AND CORRESPONDING  
METAL CONCENTRATIONS IN PLANT TISSUES FROM A CONTAMINATED AND  
UNCONTAMINATED WETLAND: A PILOT STUDY.

### 2.1 Introduction

Wetland plants have been considered as model organisms for investigating ionic exchange and the mechanism of nutrient absorption (Dykyjova 1979). Correlations between water and soil/sediment chemistry and the amount of mineral ions, such as N, P, K and Ca accumulated by aquatic plants are well documented (Dykyjova and Hradecka 1976). In general, plants growing in nutrient-rich habitats accumulate more nutrients than plants occurring in nutrient-poor ones. The mineral nutrient status of plants is further affected by a variety of other factors, such as geographical location, climatological factors, chemistry and accumulation of

sediments and different types of pollution (Dykyjova 1979). It is also highly dependent on the kind of organ analyzed, the variability between different populations of infraspecific forms, ecotypes or clones (Waisel and Rechav 1971, Dykyjova & Hradecka 1976) and ecological differentiation due to different chemical habitats. Thus, interpretation of the analysis of the same species from different habitats must therefore consider the specific conditions of the environment. Much of the work mentioned above has focused on nutrient dynamics in wetlands uncontaminated with heavy metals. Similar factors are likely to influence metal uptake in contaminated wetlands.

i) Comparison between sediment heavy metal concentrations and corresponding metal concentrations in plant tissues in contaminated and uncontaminated wetland sites.

Examples of sediment metal concentrations and corresponding concentrations in plant tissues in contaminated wetlands and uncontaminated wetlands are

sparse. Available data are given in tables 2.1-2.6. Total sediment concentrations of Fe, Mn, Cu, Zn, Ca and Mg with corresponding metal concentrations in tissues of Typha latifolia growing in Cu and Ni contaminated wetlands were analyzed by Taylor & Crowder (1983a) (Table 2.1). Sediment total Cu and Ni concentrations reached values potentially toxic to plant life. Shoot Cu concentrations were considered to be high, while concentrations of Fe, Mn, Zn, Ca and Mg were considered typical of sediments overlying rocks of Precambrian origin.

Total and extractable metal concentrations in five of the most contaminated freshwater wetlands in the United States are shown in Tables 2.2 & 2.3 (Folsom, Lee & Bates 1981). Total metal concentrations are within the same range reported by the above authors, but total Cu is lower than the concentration considered to be toxic by Taylor & Crowder (1983a). Cu concentrations in tubers of Carex esculentus (Folsom et al. 1981) are lower than in equivalent tissues of T. latifolia and probably do not represent excessive

Table 2.3 Extractable (organic extractant, DTPA) heavy metal concentrations (ug/g) in reduced (flooded) sediments from five of the most contaminated sites in North America with distance increasing 1 - 3. (Modified from Folsom et al. 1981.)

Site		Metal Concentration (ug/g)				
		Fe	Mn	Cu	Zn	Pb
Detroit River	1	910	131.4	<0.025	48	80
	2	921	13.1	13.0	277	181
	3	394	34.3	9.0	2	5
Michigan City Harbour	1	867	29.1	18.0	180	-
	2	1013	53.0	0.04	180	43
	3	1420	37.4	<0.005	5	45
Indiana Harbour	1	1044	48.0	<0.005	206	42
	2	1194	63.0	<0.005	48	37
	3	1363	12.0	<0.005	11	6
Milwaukee Harbour	1	1087	53.0	0.79	145	262
	2	883	81.0	11.0	21	8
	3	832	63.0	8.0	15	9
Minominee River	1	850	147.0	<0.005	5	6
	2	587	71.0	4.0	5	19
	3	1153	429.0	<0.005	6	5

Table 2.4 Concentrations (ug/g) of heavy metals in tuber tissue of Cyperus esculentus grown in freshwater sediments under reduced conditions from five of the most contaminated sites in North America with distance increasing 1 - 3. (Modified from Folsom et al. 1981.)

Site		Metal Concentration (ug/g)				
		Fe	Mn	Cu	Zn	Pb
Detroit River	1	1943	44	6	47	5
	2	1610	17	7	40	3
	3	1577	26	13	70	3
Michigan City Harbour	1	1073	22	5	54	2
	2	1155	17	5	55	3
	3	1704	28	5	55	4
Indiana Harbour	1	2086	47	5	50	4
	2	3587	49	6	55	9
	3	9120	64	7	151	33
Milwaukee Harbour	1	1108	20	9	46	6
	2	1586	20	7	42	1
	3	1232	12	7	40	1
Minominee River	1	598	19	5	35	1
	2	2273	51	7	44	7
	3	215	21	3	7	2

concentrations (table 2.4); the same applies to Pb concentrations.

Most of the literature on heavy metal accumulation by Phragmites is concerned with trace metals essential for plant nutrition (Table 2.6), especially Fe, Mn, Cu and Zn, of which only Cu and Zn are commonly cited as being toxic in high concentrations. No data for corresponding soil/sediment metal concentrations have been found. Data for Fe and Mn concentrations in Phragmites in uncontaminated wetlands (Table 2.6) show values that are generally lower than those given by Taylor & Crowder (1983a) and Folsom et al. (1981). Concentrations of Fe from both contaminated and uncontaminated sites suggest that they can be expected to be high for wetlands by virtue of the greater availability of Fe. Cu and Zn content of reed tissues in 'normal' wetlands are similar to those of T. latifolia found in Cu and Ni contaminated wetlands.

ii) Concentrations of heavy metals in plant organs

Table 2.5 Shoot uptake (concentration x aboveground yield) (ug) of heavy metals by Cyperus esculentus grown in freshwater sediments under reduced conditions from five of the most contaminated sites in North America with distance increasing 1 - 3. (Modified from Folsom et al. 1981.)

Site		Metal Concentration (ug)				
		Fe	Mn	Cu	Zn	Pb
Detroit River	1	200	1300	28	552	97
	2	7200	4600	211	10939	539
	3	200	4200	11	953	6
Michigan City Harbour	1	3700	54100	172	6669	71
	2	6200	65700	404	12450	242
	3	7700	28900	280	5949	122
Indiana Harbour	1	1000	26900	42	1384	26
	2	2500	51800	19	4324	63
	3	7500	29200	220	7127	92
Milwaukee Harbour	1	6900	52200	470	6470	139
	2	1300	30300	135	4545	39
	3	1300	23100	114	4089	48
Minominee River	1	1000	27000	311	1146	43
	2	1200	10600	132	2676	44
	3	100	1500	16	1	2

Table 2.6 Concentrations (ug/g) of heavy metals recorded in different organs of Phragmites australis.

Organ	Metal Concentration (µg/g)				
	Fe	Mn	Cu	Zn	Pb
Rhizome	300 a 370 - 640 e	37 a	3.7 a	14.0 a 20.7 b	2.5 b
Roots	1390 a 2730-14390 d	253 a	35.5-193.5 a 21 b	37.0 a 112.0 b	14.0 b
Stems	120 a 110 - 160 d	59 a	0.4 - 2.2 f 1.0 a	17.0 a 14.0 b	0.3 - 3.5 1.0 b
Leaves	136 a 100 - 360 c 140 - 330 d 230 e	166 a <50 - 660 b 230 e	11.8- 26.5 f 3.0 b	22.0 a 20.0 b	3.0 b

Sources:

a Kovacs, Precsenyi and Podani (1978)

b Kovacs, Precsenyi and Podani (1978) (1 year later)

c Allan and Pearsall (1963) - maximum and minimum for 12 sites

d Ho (1981)

e Mayer and Gorham (1951)

f Kufel (1978)

Taylor & Crowder (1983a) observed that, despite high concentrations of Cu and Ni in contaminated wetlands surrounding Cu smelters and on Ni tailings, these metals were largely excluded from above-ground tissues of Typha latifolia L. Concentrations of iron in tissues of this species were unusually low and may have reflected induced iron deficiency. Generally, patterns of uptake of Cu, Ni, Mn and Mg were similar; increasing significantly from leaf bases to mid-sections to leaf tips, with the exception of Ca; where an inverse relationship was true. Roots showed higher concentrations than rhizomes and aerial parts of the plant contained the lowest concentrations. Zn, Mg and Ca accumulated in all tissues of T. latifolia. Concentrations of Fe and Mn in all tissues and Cu and Ni concentrations in below-ground and reproductive organs reflected those in the soil.

In Phragmites, much interest has centred on the uptake from pollutants in effluent and dredged materials with respect to detoxification; this is reviewed by Van der



Werff, Simmers & Kay (1987). Allen & Pearsall (1963) have shown that N, P, K and Mg are retranslocated, whilst Na, Ca, Fe and Mn are retained in the senescing leaves of Phragmites. Due to its abundance, relatively small changes in Ca concentrations can have significant effects upon that of other plant nutrients and of potentially toxic metals (Rorison & Robinson 1984); hence its inclusion in analyses of metal uptake (Wilkins 1957, Robson & Loneragan 1970, Baker 1978b). Various workers have found that pH and Ca concentration differ greatly in the surface water of reed stands. Allen & Pearsall (1963) observed values between 3 and 270 mg/l Ca. Total and extractable soil Ca concentrations differ enormously, 9600 ug/g (dry weight) total Ca being recorded by Bayley & O'Neill (1972) and 39500 ug/g by Kaul (1984). Ammonium acetate extractable fractions have been reported to differ between 66000 and 429000 ug/g Ca under waterlogged conditions. Tissue concentrations of Ca in Phragmites also vary from site to site and depend on such factors as degree of eutrophication, soil structure, pH and physiological age

of tissues.

In general, roots, leaves and stems of Phragmites have greater tissue Mg concentrations than rhizomes. Soil concentrations of Mg are recognized as following similar fluctuations as Ca. Ulehova, Husak & Dvorak (1973) observed values between 4 and 25mg/l in surface water. Recorded values of total Mg concentrations in wetland soils range from 48.1 ug/g (Ho 1981) to 7900 ug/g dry weight (Bayley & O'Neill 1972) who also recorded extractable Mg values between 360 and 1540 ug/g. Localization of Mg in Phragmites appears to follow similar patterns as described for Ca (Dykyova 1979, Allen & Pearsall 1963).

High concentrations of heavy metals, such as Zn, Cu and Pb, in tissues of Phragmites seem to reflect both relative availability of metals in sediments to the plant (Larsen & Schierup 1981), and the degree of contamination of that sediment (Chiaudani 1969). Plants growing in contaminated wetlands generally have greater concentrations of heavy metals in tissues in comparison with plants growing in less

contaminated areas, as would be expected (Chiaudani 1969, Schierup & Larsen 1981).

As previously inferred, little is known of the habitat characteristics of Phragmites concerning Fe and Mn status. Data do, however, exist regarding the concentrations of these metals in different organs of Phragmites. Rhizomes were reported to have between 370 and 640 ug/g dry weight, roots 273 - 14390 ug/g, stems 110 - 160 ug/g and leaves 140 - 330 ug/g Fe (Ho 1981); this aspect is reviewed by Van der Werff et al. (1987). Kovacs, Precsenyi and Podani (1978) reported similar findings to Ho (1981): Fe concentrations were greater in the leaves than in stems but were substantially lower than those in roots.

Phragmites can withstand environmental extremes (Kufel and Kufel, 1980), including the presence of toxic contaminants. Very little, however, is known about the uptake of contaminants by reedbeds, the physiological effects of contaminants on the growth and development of reeds or the potential for contaminant entry into the food chain via reed

beds.

The literature on heavy metal accumulation by reeds is concerned primarily with trace metals essential for plant nutrition. Stems and leaves tend to have the lowest concentrations of Cu and Zn, (Kovacs et al. 1978; Larsen and Schierup, 1981). Accumulations of Cu, Pb and Zn generally were greatest in the roots and/ or rhizomes and decreased in the above-ground plant parts (Van der Werff et al. 1987).

Little work has been done on the the performance or micronutrient status of Eriophorum in potentially metal-toxic environments. Reports on the uptake of Ca and Mn in shoots and roots of Eriophorum, Molinia caerulea (L.) Moench and Rumex acetosa L. by Nazrul-Islam (1976), suggested that the most Mn-resistant species, Eriophorum, contained the lowest concentrations of Mn, both in the shoots and the roots, susceptibility to Mn being evident when more Mn was translocated to shoots (as in the case of R. acetosa). Existing work on the nutrient dynamics of the species has centred on plant N, P, Ca and Mg

concentrations or responses to soil applications of these nutrients. Ca and Mg have been reported to accumulate in tissues of Eriophorum (Chapin, Van Cleve and Tieszen, 1975).

Detailed work has been completed on Cyperus esculentus L., another member of the Cyperaceae. Folsom, Lee and Bates, (1981) showed that C. esculentus concentrated as much as 151 ug/g (dried tissue) Zn, 8 ug/g Cu, 67 ug/g Fe and approximately 2 ug/g Pb from dredged soil/sediment material taken from heavy metal polluted sites in the United States. Similar values were recorded in the same species sampled from 'natural' stands: 150 ug/g Zn, 13ug/g Cu, 9120 ug/g Fe, 64 ug/g Mn and 32 ug/g Pb were recorded for tuber tissues (Folsom et al. (1981). The data suggest that C. esculentus contains similar concentrations of these metals irrespective of site.

Review of the current literature available on the relationships between heavy metal concentrations in tissues of Eriophorum and Phragmites, and soil heavy metal concentrations suggests a need for detailed study of

the dynamics of heavy metals in soil/sediments. The present study aims (1) to provide information on the uptake of heavy metals by Eriophorum and Phragmites in relation to metal concentration in soil/sediments. It includes an iron-rich site suspected to contain high concentrations of other heavy metals including Cu, Zn and Pb (Parys Mountain, Anglesey, section 2.1.2.1) and a comparable site with low iron and 'normal' micronutrient levels (Skipwith Common, Yorkshire, section 2.1.2.3). (2) to examine the differences in metal content of plant parts to determine the extent of internal regulation of potentially toxic concentrations of heavy metals. (3) this investigation was to examine particular areas of interest for later study of the seasonal dynamics of some metals in wetlands with regard to uptake by plants and their concentrations in the substrate. (4) to suggest possible mechanisms by which these species resist high concentrations of toxic metals. The observations from this study were used as the basis for future investigations.

2.1.1 Ecology of Eriophorum angustifolium and Phragmites australis

2.1.1.1 Eriophorum angustifolium (Cottongrass, family Cyperaceae)

i) Habitat

This species is abundant in all highland regions and lowland bogs, mostly on ombrotrophic mire, or moorland associated with moist rather than waterlogged conditions, or in shallow moorland pools. It is also extremely widespread in poor fens deficient in Fe (Perring & Walters 1962) However, isolated cases have been reported for mine spoil (Grime, Hodgeson & Hunt 1988). It has been recorded on eroded peatland areas with much bare soil being able to colonize disturbed areas (Phillips 1954).

ii) Substrate

Eriophorum angustifolium occurs in a variety of soils

from acid peat to calcareous mineral soils with a CaCO<sub>3</sub> content of 60% (Phillips 1954). pH values of 3.5-7 and 5-7 have been recorded for soil and peat in which the species appeared healthy (Phillips 1954, Shaw 1989). Pearsall (1938) showed that it can tolerate a wide range of pH, oxidation-reduction potential, nitrate, base deficiency and ferrous Fe. The growth of Eriophorum is enhanced by the presence of Ca in culture solution (15-20 mg/l at pH 4.5, Pearsall & Wray 1927) . The above authors also found the effect of Ca was increased by an increase in Na and K, or a rise in pH. A rise in temperature raises the Ca requirement in this species (Pearsall & Wray 1927). Extractable Ca concentrations between 224-3307 mg/l (ammonium acetate extraction) have been measured for peat soils supporting Eriophorum (Shaw 1989).

### iii) Performance in various habitats

Eriophorum grows in a variety of habitats but may be eliminated through competition for nutrients and shading (Phillips 1954). In environments deficient in mineral



nutrients, stunted shoots lacking the channelled leaf blade are produced, and the species is often eliminated (Phillips 1954). Pearsall and Wray (1927) demonstrated that pH above 7 or excess Ca may be deleterious to the growth of the species. A high base ratio (Na/Ca, K/Ca) partly compensates for Ca deficiency.

#### iv) Growth habit and morphology

Eriophorum is a perennial, semi-robust helophyte. Extensive unbranched rhizomes without buds end in swollen stocks which bear buds and foliage leaves (Phillips 1954). The plant has a shallow root system (Figure 2.1).

#### v) Phenology

New growth by Eriophorum begins in early spring, usually in March and April. New shoots and rhizomes are produced continuously during the succeeding summer and early autumn, up until October. The leaves persist throughout the growing season but begin to die back in late autumn, changing from green to red and then they wither to brown, the leaf tips changing colour before the blades. Leaves of

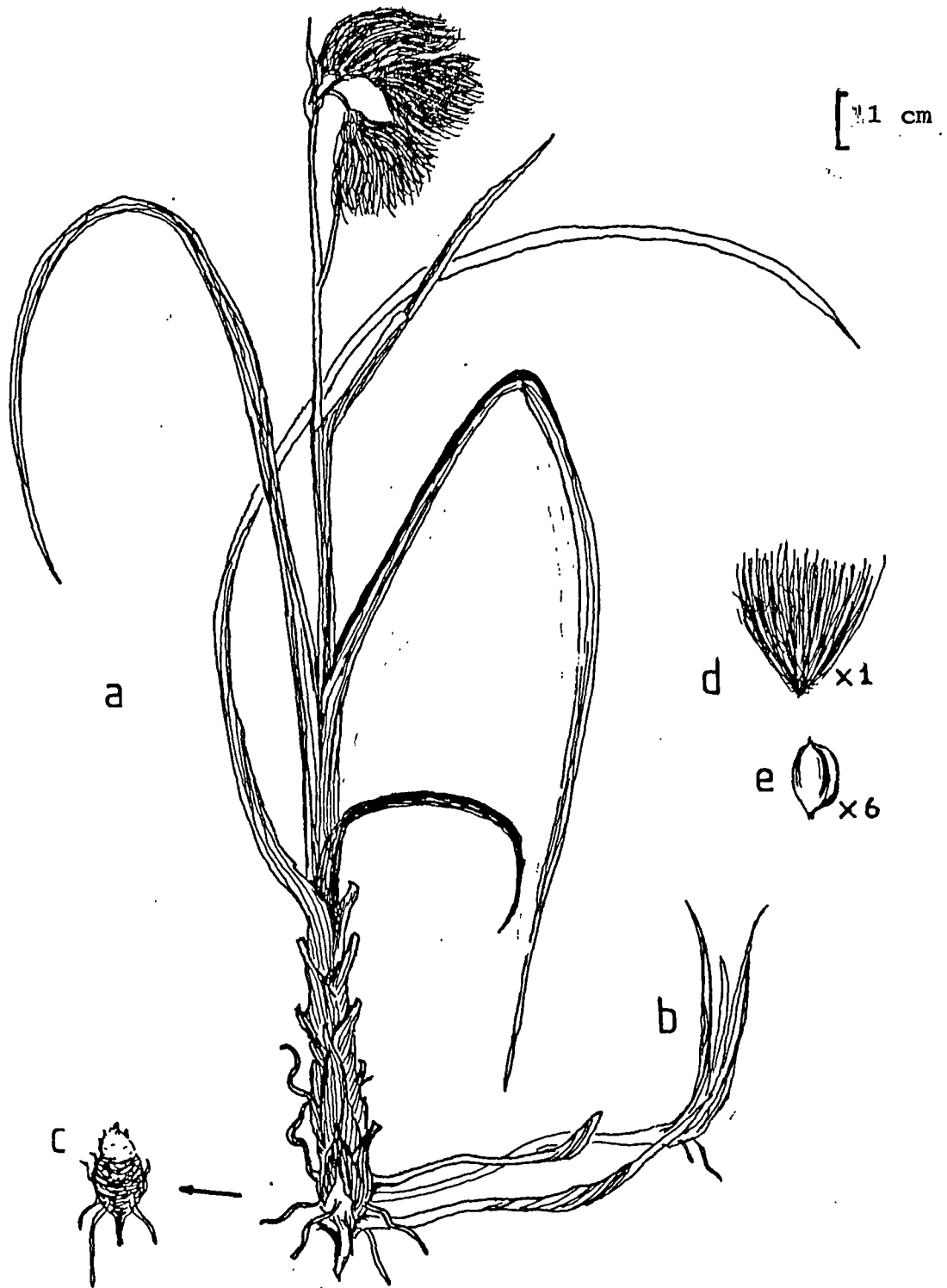


Figure 2.1

Plant of *Eriophorum angustifolium* (a), showing (b) daughter plant (offset), (c) shoot base, (d) nut (seed) and bristles and (e) nut.

Eriophorum may remain partly green throughout the winter.

The species regenerates by mainly vegetative means through the extension of rhizomes, forming large patches. Establishment from seed is infrequent (Grime et al. 1988). Flowering begins with elongation of the spikes in March or early April. Fruiting peaks in mid summer but varies with locality.

vi) Floral biology and propagation

The seeds are wind dispersed after growth of the perianth hairs to form long silky tassles. Fruits may be germinated in the laboratory in peat or on wet filter paper in petri dishes (Phillips 1954). Percentage germination is variable. Some fruits are able to germinate two seasons after collection, but the effect of aging is noticeable in the decline of viability. Experiments by Phillips (1954) indicated the existence of a dormant period at the beginning of October, some time after the ripening of the fruit. Percentage germination decreased more rapidly in fruits exposed to nocturnal fall in temperature than those kept in a

closed laboratory. During the dormant period the effect of low temperature is to break dormancy; temperatures above freezing have the greatest effect. One week at 4°C was found to be very effective; longer exposure was found to decrease the subsequent percentage germination (Phillips 1954). The conditions necessary for the establishment of Eriophorum seedlings are: little competition, either a neutral pH or relatively high Ca status (Pearsall and Wray 1927), and sufficient moisture during the early stages of germination, but no standing water (Phillips 1954).

vii) Population differentiation

Ecotypes of Eriophorum are very variable in length of rhizome and aerial shoots, leaf form, spikelet number and length of perianth bristles. Several varieties have been described but the criteria used to distinguish these have been shown to depend on characters which vary with the environment (Phillips 1954).

2.1.1.2 Phragmites australis (Common reed, family Gramineae)

Phragmites is a cosmopolitan species occurring in freshwater, brackish, and in some cases also marine littoral communities almost all over the world (for detailed accounts see Haslam 1972, Dykyjova and Hradecka 1976).

i) Habitat

The plant is able to withstand extremes of environmental conditions. It is usually found in low-lying areas, intermittently or permanently flooded with shallow still water where, under the right conditions, it may flourish and become the dominant species (Van der Werff, Simmers and Kay 1987). This species may be found in marshes, fens, around lakes, alongside rivers, estuaries and (rarely) in ombrotrophic bogs.

ii) Substrate

Stands of Phragmites are found in a wide range of soil types, ranging from silt and clay to gravel and peat (Van der Toorn 1972). Organic matter content may vary between 1-97%

(Haslam 1972). It can survive without contact with soil and may form floating mats. Plants can be found growing in a wide range of soil/sediment pH between 3.6 and 8.6, but more robust stands appear within the range 5.5 to 7.5 (Haslam 1972).

### iii) Performance in various habitats

Soil and water chemistry are very important for the growth of Phragmites as stands may be tall and dense in nutrient-rich habitats (Gorham & Pearsall 1956, Bjork 1967, Dykyjova and Hradecka 1976), and very sparse in nutrient-poor ones (Haslam 1965). Thus Phragmites tends to grow well in mesotrophic and eutrophic habitats. Nutrient uptake is mainly from the upper 0.5 m of the soil, by the branched horizontal roots of the upper rhizomes (Haslam 1973).

A number of substrate characteristics have been found to limit productivity, in particular, availability of P and N (Haslam 1965, Bjork 1967, Van der Toorn 1972).

#### a) Contaminated wetlands

It is known that Phragmites is able to withstand a

wide range of different habitats and its presence on heavy metal-polluted dredged material has been documented by Van der Werff et al. (1987). However, most of the literature on heavy metal accumulation by Phragmites is concerned with trace metals essential for plant nutrition (Van der Werff et al 1987).

iv) Growth habit and morphology

The morphology of Phragmites is variable. Normally in a sparse stand a subsidiary bud near the base of the previous year's growth develops (see Figure 2.2), in late summer into a horizontal rhizome growing in a similar direction to its predecessor (Haslam 1969, 1972). After extending for about 1 m, the apex of the rhizome turns upwards and remains dormant over the winter, and grows into an aerial shoot the following spring. Thereafter, each progressive subsidiary shoot derived from the previous season's growth becomes smaller. Any lateral bud may develop into a horizontal or vertical rhizome, or an aerial stem. Lateral buds on horizontal rhizomes may form horizontal or

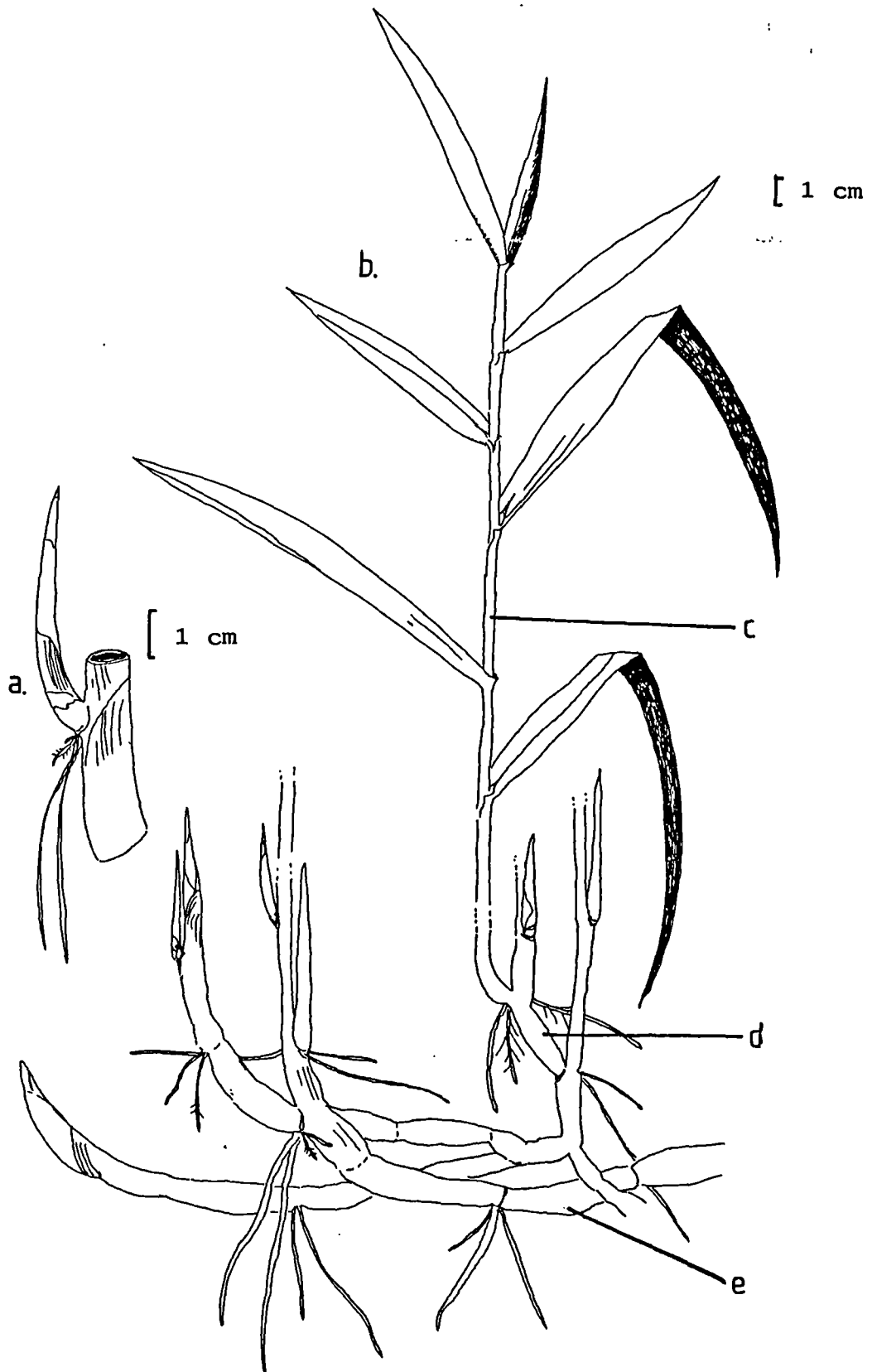


Figure 2.2

Plant of Phragmites australis showing (a) section of plant used for propagation (rooted subsidiary shoot with section of rhizome), (b) vegetative shoot, (c) culm (stem), (d) part of vertical rhizome and (e) horizontal rhizome



vertical rhizomes or, on cut portions at the soil surface, aerial shoots (Haslam 1972).

v) Phenology

In Britain, horizontal rhizomes of Phragmites start growing in late summer and terminal buds are near the surface in mid-November. Spring emergence is determined primarily by internal factors, but is usually during late March to late April. The period of rapid emergence lasts 1-3 months (April-June), depending on conditions, and a few shoots may continue to arise until mid-September, when frosts stop further surface growth (Haslam 1972). Maximum productivity in a Phragmites stand in Britain occurs from June-September. Flowering is in late August to early September and fruits ripen in November. Summer (July and August) is the period for rapid rhizome growth, while the most active root growth occurs in Spring (April-May) and Autumn (September) (Fiala 1976). Accumulation of reserve material takes place in both new and old rhizomes (Fiala 1976).

vi) Floral biology and propagation

In Britain, seed production by Phragmites is poor due to the onset of early winters (Haslam 1972). Viability of the numerously produced seed is very variable. Field germination is often very poor and seedlings may be killed by winter and spring frosts (Haslam 1972). Commercial propagation is either by seed, or by rhizome portions (Lawson 1985), with or without roots, planted before the spring emergence (preferable to the use of seed in Britain due to the frequently low germination rate) (Haslam 1972).

vii) Population differentiation

Many morphological differences previously considered genetic in origin have proved to be due to phenotypic plasticity when transplants are continued over two years (Haslam 1972). Ecotypic differentiation has been recorded in this species under saline conditions (Waisel and Rechav 1971). Ecotypic differentiation in plant species inhabiting more than one habitat type (or biotope (Ejork 1967) defined as "the totality of the environmental conditions" under which

an individual exists) is a common phenomenon. Ecotype are formed by response of the genotype to selective pressures exerted by the environment (Waisel and Rechav 1971). Ecotypes of Phragmites have also been discussed by Van der Toorn (1972) Clones from different habitats were found to differ in for example, seed production, shoot dimensions, shoot density, panicle frequency and flowering time (Bjork 1967).

## 2.1.2 Site descriptions

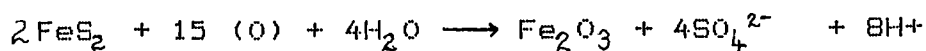
2.1.2.1 Parys Mountain, Anglesey (Mynydd Parys, Great open cast, Parys Mountain, SSSI, Anglesey (grid reference SH 390244).

The is a disused .opencast copper mine which was the site of considerable mining activity from 1750 to 1850 and Anglesey's greatest mineral industry, dominating the world markets for copper (Rowlands 1981). It is one of the most famous mineralized sites in Wales. Geologically, the form of the lead-zinc mineralization is unique in Britain (Anon. 1987)

being the only Kuroko-style deposit in the UK. To understand the conditions to which the vegetation is exposed, an appreciation of the geology and chemical nature of the site is necessary.

i(a) Geology and geochemistry

The bedrock is mostly of shales, fine-grain sedimentary rocks interspersed with material of volcanic origin which became folded to form a syncline after the Caledonian Orogeny (Curtis 1986). The material of mining interest was felsite (volcanic in origin), rich in feldspathic minerals, such as (listed according to abundance), iron pyrites (FeS<sub>2</sub>) and the rarer sulphides, such as chalcopyrite (CuFeS<sub>2</sub>) for which the mine was most important. Galena (PbS) and blende (ZnS) were also found in the deposits. As only the chalcopyrite was of economic importance, the spoil tips were largely composed of iron pyrites. FeS<sub>2</sub> is thermodynamically unstable on exposure to air and the following chemical reaction takes place:

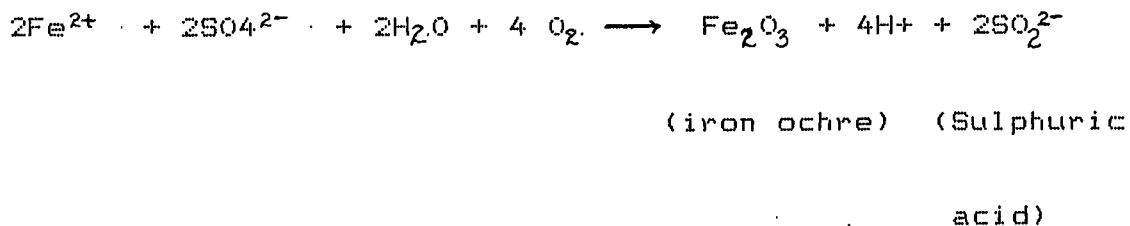


This reaction results in the formation of insoluble iron oxides (FeO) and sulphuric acid (H<sub>2</sub>SO<sub>4</sub>). The latter would also react with other minerals, thus increasing the quantity of dissolved metals (eg Zn, Pb)

As part of the extraction process involved in removing Cu from minerals, 'ochre pits' were constructed. These were generally small scale operations designed to remove any remaining Cu from the Cu-rich drainage water that drained from the mining area. These small ponds were connected so that they drained into each other. Rain water seeping through weathered material and discarded ore would drain and collect in these ponds to which scrap metal (Fe) added. The technique relied on the chemical reduction of Cu in the presence of Fe:



These ponds were then drained and the precipitated Cu removed. However, the Fe liberated into solution was further oxidized to form iron ochre, the characteristic red-brown precipitates found deposited in the settling ponds, the reaction was represented by the equation:



ii) Subsite information

In the pilot study, three of the four Parys Mountain subsites were included in the analyses (Chapter 2). In Chapter 3, all four subsites were included in the investigation of the seasonal dynamics in heavy metal status of plants and soils.

(a) Subsite 1. Represents a mixed stand of Eriophorum angustifolium and Phragmites australis on the south edge of the ochre pit shown in Figure 2.3. This stand was characterized by depauperate plants of Eriophorum and Phragmites.

(b) Subsite 2. Represents a mixed stand of Eriophorum and Phragmites on the North edge of the ochre pit and characterized by vigorous growth of Eriophorum and Phragmites. Soil/sediments were waterlogged throughout the year.

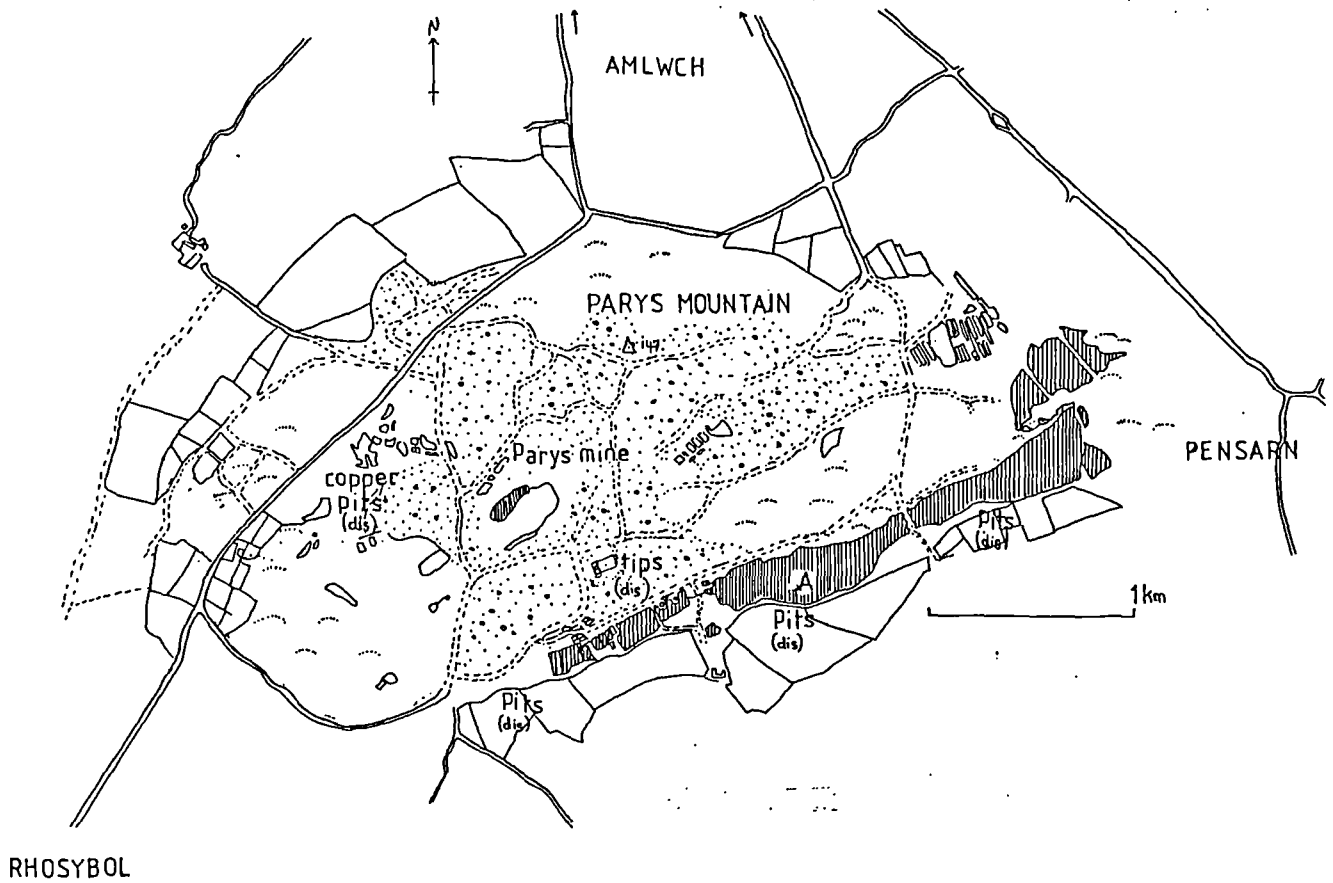


Figure 2.3

Map of Parys Mountain, Anglesey showing location of ochre pits (disused) and sampling area (A) from which soil/sediment and plant samples were taken. Shaded area represents open water.

(c) Subsite 3. This subsite was located on the north side of the ochre pit and characterized by a pure stand of vigorous plants of Eriophorum.

(d) Subsite 4. A pure stand of Phragmites with vigorous growth close to pasture land on the south side of the pit. Soil conditions were drier than the other subsites.

2.1.2.2 Crymlyn Bog, Swansea, S. Wales (Grid reference SS 295269).

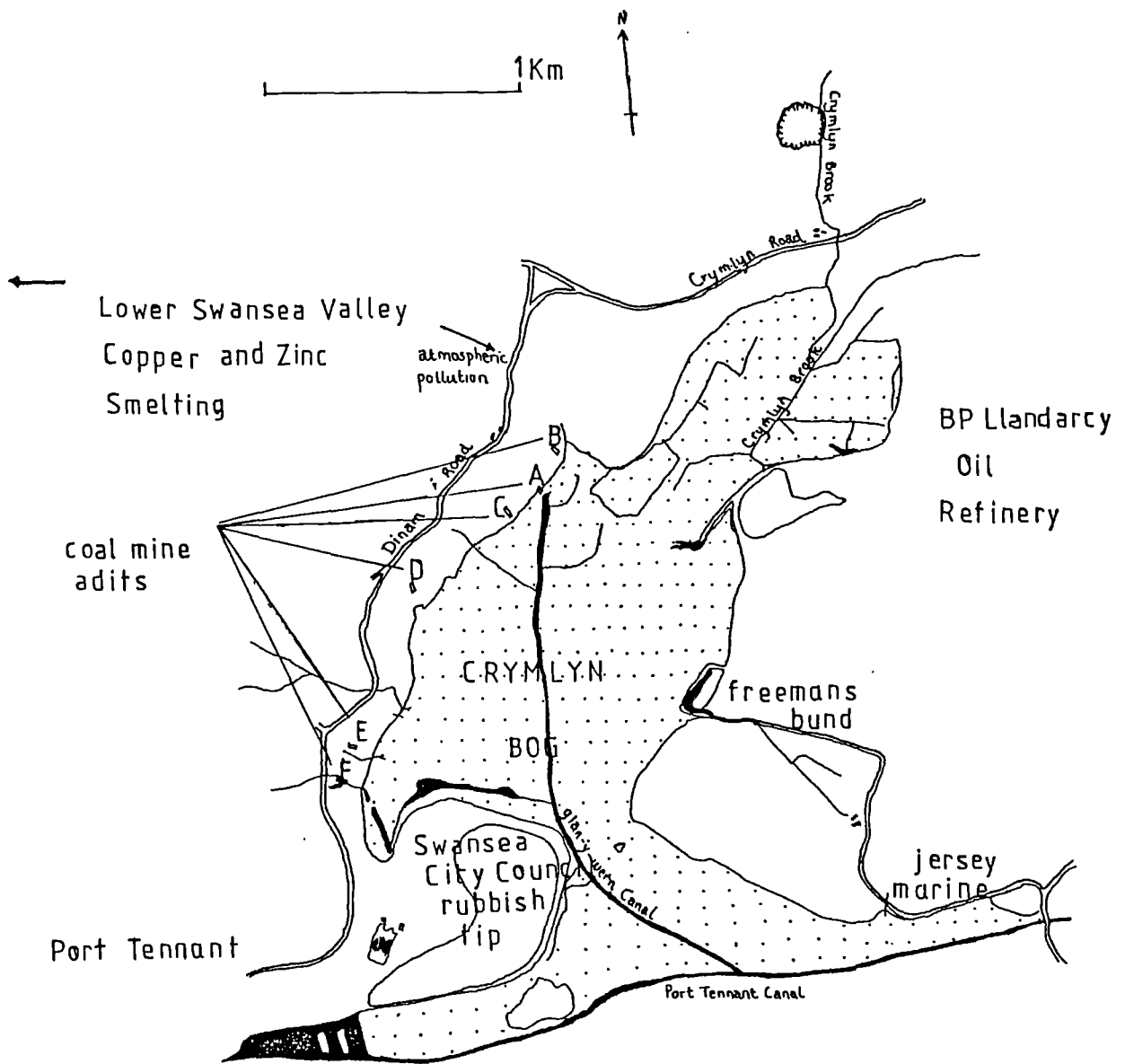
Crymlyn Bog is located to the east of Swansea between the Rivers Tawe and Neath. It is separated from the sea to the south by the Crymlyn Burrows, an area of sand dunes. Coal was formerly extracted from several adits located on the western side of the mire, these mines are now disused but springs and discharges thought to derive from the mines introduce iron-rich ground water to the mire margin. The mire has also been influenced by atmospheric pollution from the Lower Swansea valley copper and zinc smelting industry to the west, refinery discharge from the Llandarcy Oil Refinery



to the east, discharge from the Swansea City Council rubbish tip and the Burrows Chemical Works tip to the south (Headley 1989). The area of study is located along the west edge, near the top end of the disused Glan-y-wern Canal and close to a disused coal mine adit (see Figure 2.4).

a) Geology and geochemistry

The stratigraphy of the mire comprises successive layers of peat overlying estuarine silt. The surrounding landscape comprises glacial drift over Pennant measures. The latter include the Grovesend, Swansea and Hughs beds of Carboniferous origin which comprise the Upper Coal Measures. These layers originally occurred as horizontal sheets, but as a result of repeated regional oscillations and compression during the Caledonian orogeny they were bent into synclines and anticlines (George 1970). The Pennant Measures comprise rhythmic sequences of mudstone, siltstone, grits, fine clays and coals. The mudstones commonly contain pyrite. The Upper Coal Measures consist predominantly of thick feldspathic, micaceous sandstones and grits (Pennant sandstones). The



**Figure 2.4**

Map of Crymlyn Bog, Swansea showing sampling area (A) from which soil/sediment and plant samples were taken. Area (A) is also the site of one of six coal mine adits.

Grovesend beds comprise argillaceous shales, shales and sandy shales, while massive sandstones are absent from the Swansea beds. Coal is extensively worked only in the former beds and reach their greatest thickness below Swansea and Gorseinon (George 1970).

The chemistry of the ground water from the S. Wales coal measures varies widely from good to poor quality drinking water. Mean chloride ion concentrations are 20 mg/l with Fe contents around 0.2 mg/l (Headley 1989). Water supplies from the Upper Coal Measures are generally rich in calcium bicarbonate at the edges of the coal field. Water supplies derived from the Upper Coal Measures tend to be sulphate-rich with increasing depth and water derived from Middle and Lower Coal Measures have been found to be sodium-rich. Water pumped from old mine workings have been found to be acidic, rich in Fe and sulphate with dissolved solids reaching 10,000 mg/l with the possible presence of sulphuric acid (Headley 1989).

#### b) Soils and drainage

Below the mire, the stratigraphy comprises comparative

horizontal layers of submerged peat (George 1970). These strata, formerly known as Submerged Forest series display characteristic features of ancient marsh conditions. For much of post-glacial times similar conditions appear widespread along the S. Wales coastline, many of the bays displaying evidence of ancient marsh conditions in the present lacustrine, estuarine and terrestrial sediments. The terrestrial sediments are richly humic soils that have compacted to form peat. Their main mass is composed of branches, twigs and leaves of oak, hazel, alder and birch. The peat beds are interbedded with silts, muds, clays and gravels, which bears evidence of submergence caused by eustasis in isostatic changes in land-sea level. The sand dunes of the coast impede drainage and behind them are stretches of flat alluvial marshland running parallel with the coast, such as found between Swansea and Porthcawl (George 1970).

ii) Subsite information

Two subsites were included: (a) a mixed stand of

Eriophorum and Phragmites close to a spring and (b) a pure stand of vigorous Phragmites. Soil/Sediments were waterlogged throughout the year.

2.1.2.3 Skipwith Common, SSSI. Yorkshire (grid reference SE 671381).

This nature reserve belonging to the Yorkshire Wildlife Trust, was chosen as a control site as it had no known history of mining activity (Figure 2.5) The nature reserve is an extensive tract of heathland lying at a height of 9m above sea level, on a spur of glacial sands forming the watershed between the lower Derwent and the Ouse valleys, in the Vale of York. It is an extremely varied area containing large tracts of wet heath merging into poor-fen swamp communities. Poor-fen communities have developed in areas that may have originated as peat-cuttings in former valley mire (Thompson, Smith & Jefferson 1987).

a) Geology

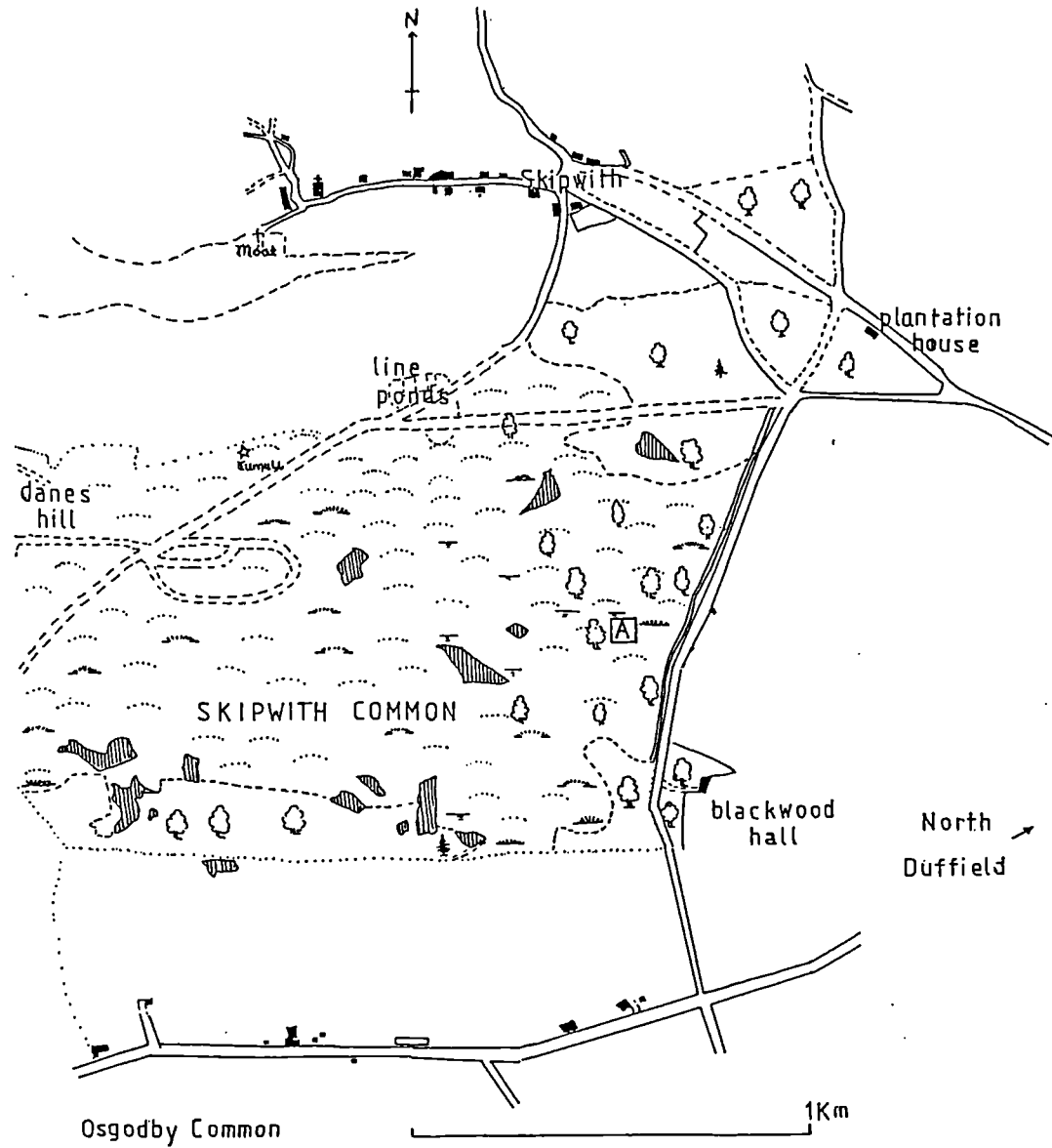


Figure 2.5

Map of Skipwith Common, Yorkshire showing sampling area (A) from which soil/sediment and plant samples were taken. □ Denotes area covered by survey. Shaded areas represent open water bodies.

The Vale of York was produced over many millenia by the wearing away of soft Triassic or New Red Sandstone series of strata. During the last Ice age, an ice wall partially blocked the vale, converting the basin into a great inland lake, that became partially blocked with materials brought down by the rivers from the surrounding hills, thus forming a wide alluvial plain with islets of trias rising out of it. A bed of brown clay of unknown thickness is thought to extend below the Common. Older geological survey sheets name these deposits "Warp and Lacustrine clay" while more recent sheets refer to "Silt and Clay" or the "25 Foot-Drift". Above the clay is a bed of sand of variable thickness, which due to the impermeable nature of the clay below is waterlogged where it is in contact with the clay forming quicksand. Beneath the sand and laminated clays are 250 metres of Bunter Sandstone, forming the solid geology of the area. Coal seams are known to reach below the Common (Thompson et al. 1987).

#### b) Soils

Most of the area around the nature reserve is

characterized by soil type 821a, Everingham series, described as aeolian sand- a deep stoneless permeable fine sandy soil with the ground water controlled by ditches (Thompson et al. 1987) The soil type on the reserve is classified as type 552a, Kexby series; aeolian sand forming a deep stoneless fine sandy soil affected by ground water<sup>(Thompson et al. 1987)</sup> Where ground water is almost permanent, peat collects and becomes incorporated with the sand.

c) Drainage and hydrology

Like other lowland heaths in the Vale of York, the Skipwith heaths have formed where a saucer-like depression in the glacial clay contains a layer of lacustrine sand (Thompson et al. 1987). The clay impedes drainage, with the result that the sand is frequently waterlogged or submerged. On the nature reserve the level of water fluctuates around 9.0m, so that there are considerable areas of standing water, even throughout the driest summers. The ponds in which the poor-fen communities have developed may have resulted from peat cutting activities, mostly in the 18th and 19th



centuries: they are all very shallow with less than a metre-depth of water.

ii) Subsite information

No subsites were used as no obvious heterogeneity was identified in the soils and vegetation.

## 2.2 Methods

### 2.2.1 Sites

Preliminary to following changes in elemental composition of Phragmites australis and Eriophorum angustifolium in relation to seasonal chemistry in iron-rich wetlands, a pilot investigation was made to establish the variation of metal ions in soils and tissues at two of the sites:

1) Parys Mountain (section 2.1.2.1). Based on the heterogeneity of the vegetation, the site was divided into subsites to include two mixed stands of Phragmites and Eriophorum and a pure stand of Eriophorum (Parys sub-sites 1, 2 and 3).

2) Skipwith Common, Yorkshire (section 2.1.2.3). No obvious heterogeneity in the vegetation and soils was identified and only one site was sampled.

### 2.2.2 Preparation of plant material for determination of metals

Five entire plants of each species were collected at each sub-site along with samples of upper (0 - 25 cm depth) and lower (>25 cm) sediments. Direct observation of the soil-sediment at Parys Mountain indicated an obvious two-fold layering of sediments and therefore the possibility of heterogeneity in the metal status of the substrate. For direct comparison soils were also collected at two depths (<25 cm and >25 cm) from Skipwith Common. All plant and soil material was collected in November 1985.

Plants and soil material were stored at 5° C prior to washing the plant material and preparation of soils. Analyses were completed within 14 days for plant material and 48 days for soils. (Soils were sealed in black plastic bags and only the central section was used for the analysis to reduce the risk of using soils whose chemical status may have changed through prolonged storage).

Plant material was washed thoroughly (5 tap water rinses

and 2 distilled water rinses) and divided into the following sections:

a) Phragmites i) subsidiary shoots ii) dead shoots  
iii) shoot bases iv) rhizome v) roots.

b) Eriophorum i) leaf tips ii) green leaves iii) dead leaves iv) shoot base v) roots (laterals).

It must be emphasized that to reduce external contamination of Fe (which was potentially a serious problem), thorough washing of the plants formed the major part of the preparation of material for this analysis.

Individual plants were collected of the same approximate size and age in Eriophorum. Due to the nature of the growth habitat of Phragmites (section 2.1.1.2) an individual plant was categorised as a section of rhizome of approximately the same diameter attached to last season's primary, with a single subsidiary shoot. The latter was independent of the rest of the plant in-as-much as it bore independent roots (see Figure 2.2). These were selected at random from the site to be sampled. After washing, the

samples were dried in an oven at 50°C for 48 hours.

### 2.2.3 Digestion of plant material

Due to the large number of plant samples and the wide range of metals to be analyzed, dry ashing and subsequent acid digestion was considered the most efficient method.

Weighed samples (between 0.5 and 1.0 g) were ashed in 25 ml Pyrex beakers in a muffle furnace at 475° c overnight. The ashed material was then taken up in 2 ml of 2 M hydrochloric acid (Analytical reagent(A.R) grade) and warmed for 5 minutes on a hot plate and finally made up to 10 ml with distilled water. Digests of both species produced a residue of siliceous material, which had to be filtered or centrifuged prior to analysis by Flame Atomic Absorption Spectrophotometry. Centrifugation appeared to be the best method as freshly diluted samples could be centrifuged in the tubes used to store the analyte (Samples were centrifuged in a bench centrifuge at 3000 rpm for 5 minutes). Acidified samples (in 10% acid) had an indefinite storage life provided

that each sample was adequately sealed. However, all samples of this nature were analysed within 48 days of digestion.

#### 2.2.4 Preparation of soil samples

pH values were determined in de-ionized water/soil slurry (2 (water):1 (soil)). Readings were taken while stirring the slurry following a 15 minute equilibration period.

Following this procedure the soil was divided equally into two halves and homogenized samples were taken for total and extractable analysis of Fe, Mn, Cu, Zn, Pb, Ca, and Mg.

##### 2.2.4.1 Total soil extractions

For the total extraction, sediments were air-dried for 48 hours and then dried for a further 2 days at 48° C. After this period, the samples were ground lightly with a pestle and mortar and sieved ( 2mm-mesh sieve). 1g of oven-dried soil was then pre-digested in 10 ml of concentrated nitric acid ( $\text{HNO}_3$ , A.R grade) overnight and then

placed in a water bath at 60°C for one hour. The digests were agitated for the first 15 minutes to reduce frothing, particularly from the samples derived from the more organic soils from Skipwith Common. The samples were made up to 100 ml volume with distilled water and finally, filtered by gravity through Whatman no.2 filter paper.

#### 2.2.4.2 Extractable soil fraction

Five gram samples of fresh sediment were extracted with 50 ml of acetic acid (2% v/v, pH 4), sealed in 60 ml centrifuge tubes and put on an end - over- end shaker for one hour then filtered through Whatman no. 2 filter paper.

#### 2.2.5 Elemental analysis.

All determinations of elemental concentrations were made by Flame Atomic Absorption Spectroscopy (Perkin-Elmer 3030). As a routine procedure, lanthanum chloride ( $\text{LaCl}_3$ ), was added at 0.1% to samples in which Ca was determined, to eliminate interference from P.

All soil concentrations of Fe, Cu, Mn, Zn, Pb, Ca, and Mg were calculated on a ug metal/g dry weight or fresh weight basis. Metal concentrations in plant tissues were expressed as ug metal/g dry weight.

#### 2.2.6 Analysis of data

The data were analyzed using the GENSTAT statistical package (Alvey, Galway and Lane 1982). Full analyses of variance were executed for all data sets. Data were transformed using natural logarithms since the variances were related to the means. For ease of interpretation the values on the y-axis have been back-transformed.

Unless otherwise stated significance was tested at the 5% level ( $p < 0.05$ ).



## 2.3 Results

### 2.3.1 Soil characteristics of Parys Mountain subsites 1,2,3 and Skipwith Common 2.3.1.1 Sediment and soil pH

Surface (<25 cm depth) and subsurface sediments (>25 cm depth) were found to differ in pH. The top 25 cm were more acidic than the lower sediments at all the subsites sampled from Parys Mountain. The surface sediment was the most consistent, averaging pH 2.7 (mean of 5 replicates), the subsurface sediments were more variable ranging between pH 3.3-4.7. There was no variation in pH at the same depths measured at Skipwith Common. (The mean pH value was 5.3).

### 2.3.1.2 Soil analysis

Total (Nitric acid digest) metal concentrations in the sediments from Parys Mountain differed with depth- the lower sediments were more metalliferous- and with site (Figures 2.6-2.8). For Skipwith common (Figure 2.9),

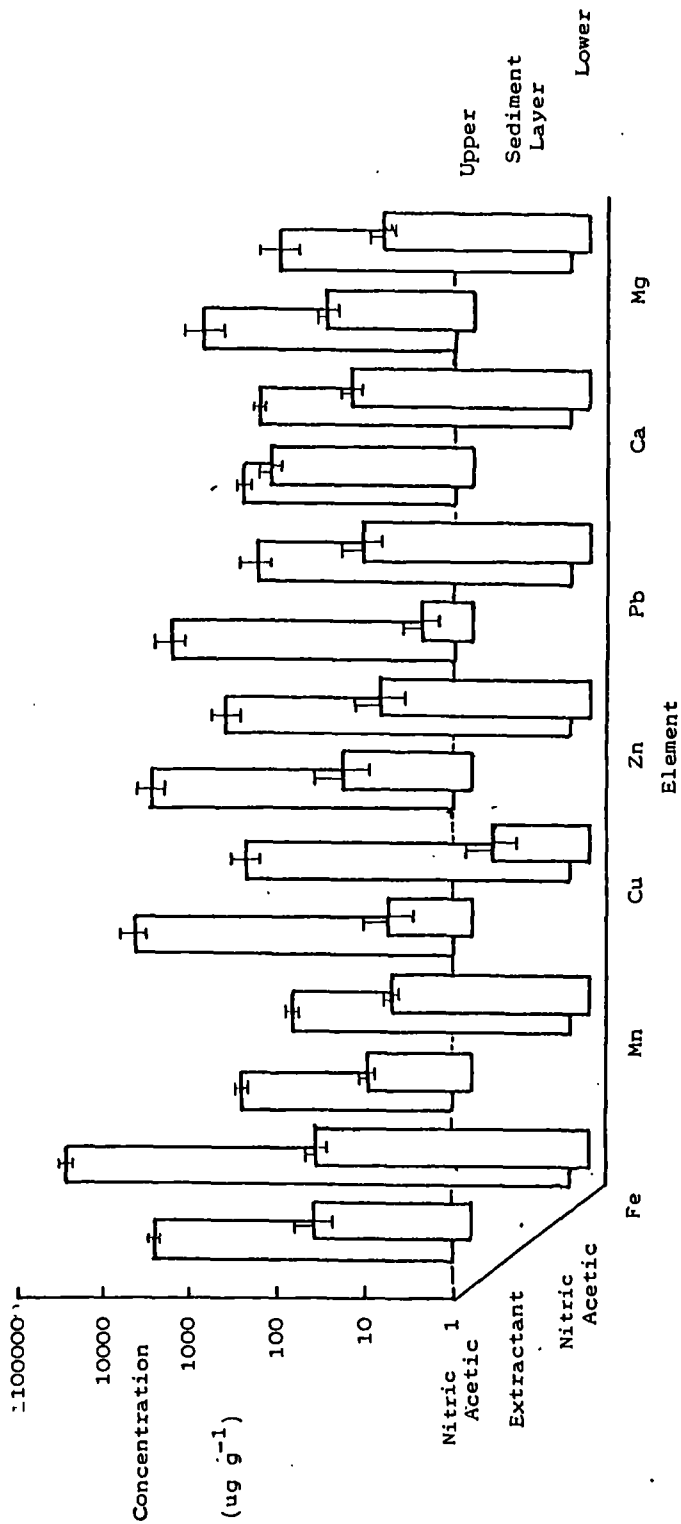


Figure 2.6

Total (Nitric acid) and Extractable (Acetic acid) metal concentrations ( $\mu\text{g/g}$ ) of upper (<25 cm) and lower (>25 cm) soil/sediments sampled in November 1985 from Parys Mountain subsite 1. (Vertical bars represent Least Significant Differences). ( $p < 0.05$ ).

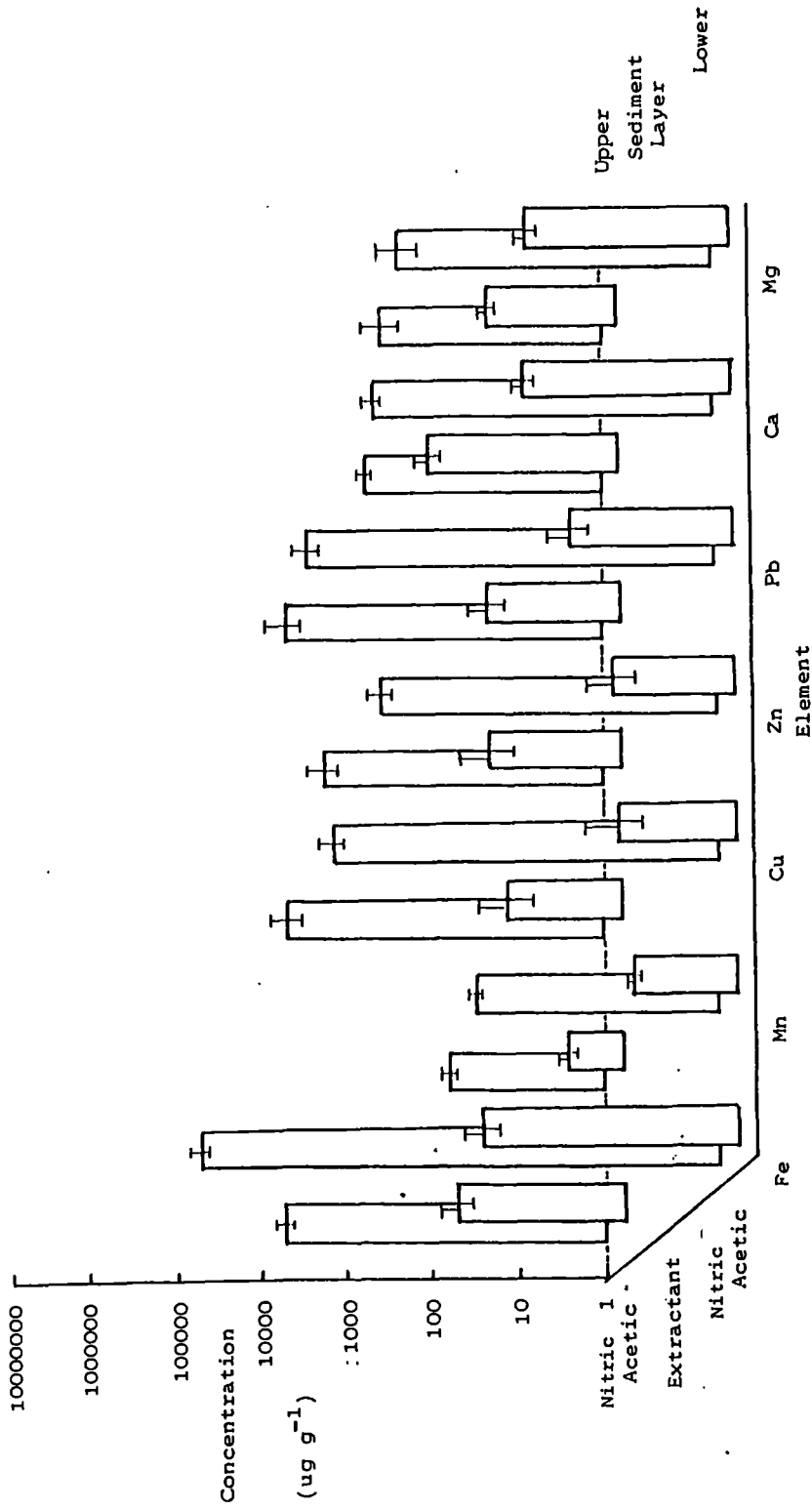


Figure 2.7

Total (Nitric acid) and Extractable (Acetic acid) metal concentrations (ug/g) of upper (<25 cm) and lower (>25 cm) soil/sediments sampled in November 1985 from Parys Mountain subsite 2. (Vertical bars represent Least Significant Differences). ( $p < 0.05$ )

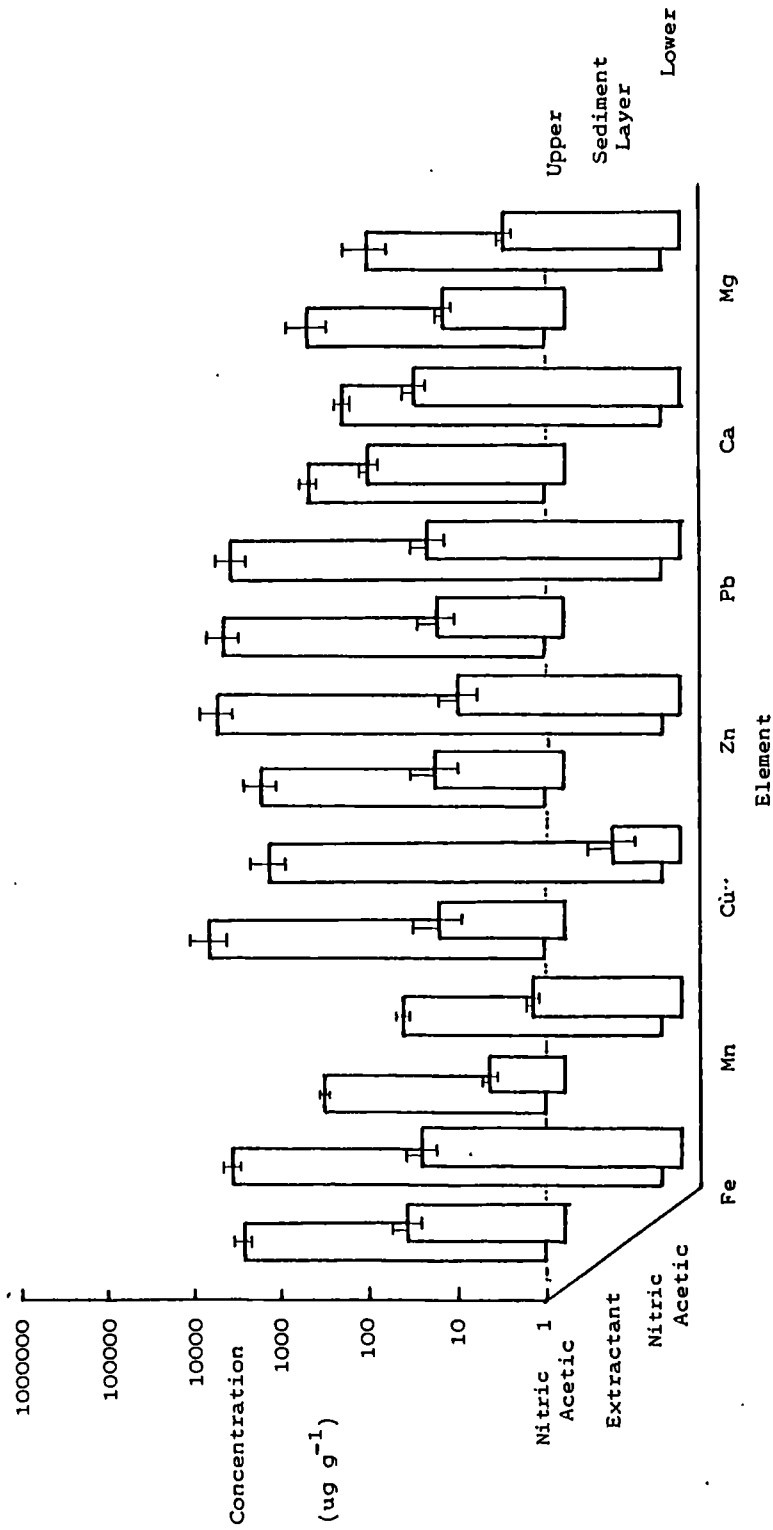


Figure 2.8

Total (Nitric acid) and Extractable (Acetic acid) metal concentrations ( $\mu\text{g/g}$ ) of upper (<25 cm) and lower (>25 cm) soil/sediments sampled in November 1985 from Parys Mountain subsite 3. (Vertical bars represent Least Significant Differences). ( $p < 0.05$ )

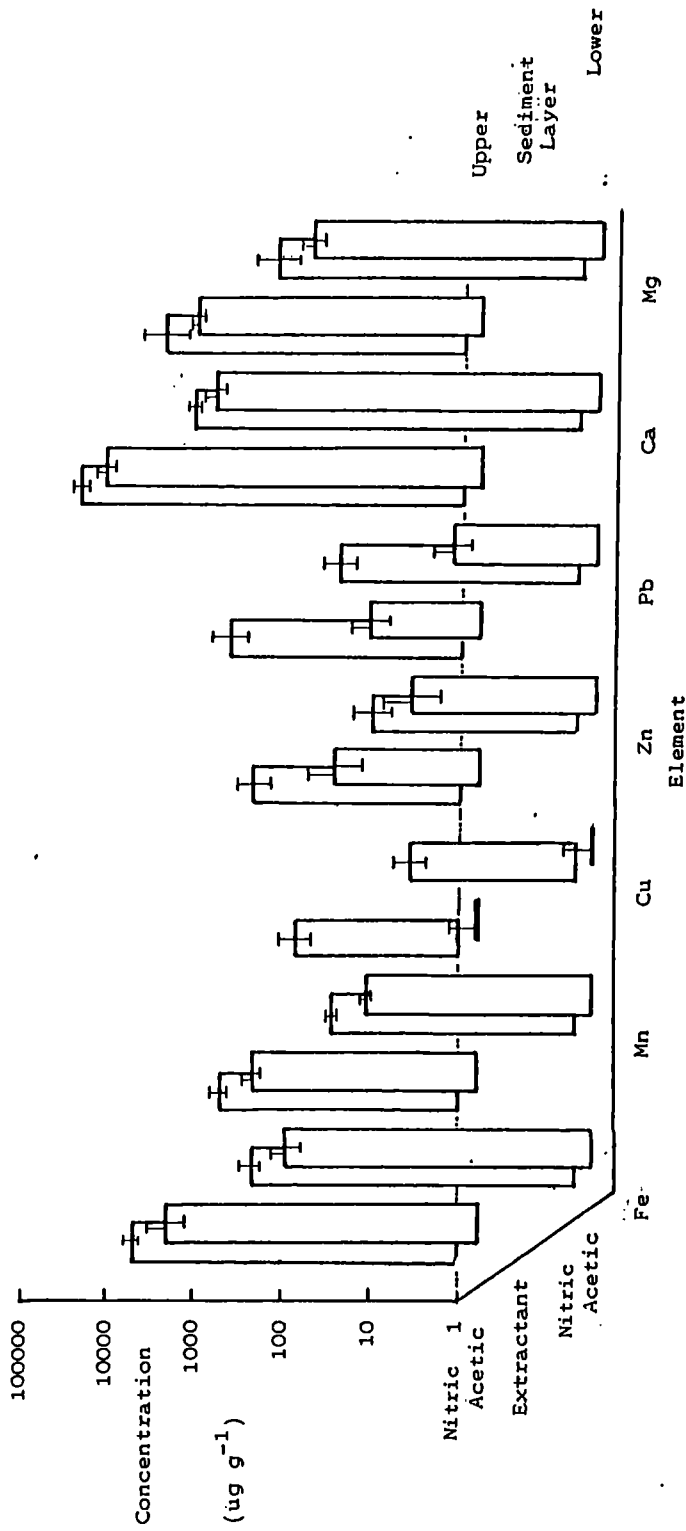


Figure 2.9

Total (Nitric acid) and Extractable (Acetic acid) metal concentrations ( $\mu\text{g/g}$ ) of upper (<25 cm) and lower (>25 cm) soil/sediments sampled in November 1985 from Skipwith Common. (Vertical bars represent Least Significant Differences). ( $p < 0.05$ )

however, total metal concentration for the depths sampled were constant. Extractable metal concentrations for sites and subsites were a fraction of the total element values present in the sediments and soils. (Between 25% and 50% of total metal concentration). There was a significant ( $p < 0.05$ ) increase in the concentration of exchangeable metals with depth at Parys (Figures 2.6-2.8). Metal analysis of sediments confirmed that Parys was Fe-rich and that a degree of heterogeneity was present at the subsites and in the soil/sediments. Despite high concentrations of total Fe at Parys, extractable values were lower than at Skipwith (more of the total was extractable). Both sites were particularly Mn enriched. Mn concentrations of the Parys subsurface sediments showed a greater degree of homogeneity than values observed for the remaining elements. With the exception of Fe and Mn, Skipwith soil/sediments had lower total and extractable Cu, Zn and Pb concentrations (Figure 2.9).

Extractable Zn and Pb concentrations emphasized the

complexity of the Parys soil/sediments, where minimum and maximum values were recorded in neighbouring subsites (Figures 2.6-2.9). A distinction could be made between Cu-rich and low Cu sites; total Cu concentrations in the Parys sediments were approximately forty times greater than the Skipwith totals,

It was thought that Skipwith would be the more Ca enriched site and total and extractable metal analysis confirmed this (Figure 2.9). Both total and extractable Mg concentrations followed the patterns outlined for Ca.

### 2.3.2 Chemical analysis of plant material

#### 2.3.2.1 Phragmites australis

Above and below ground Fe concentrations differed in Phragmites (Figure 2.10i). Root samples consistently accumulated the highest Fe concentrations throughout all the sites sampled. Root Fe concentrations reflected soil Fe

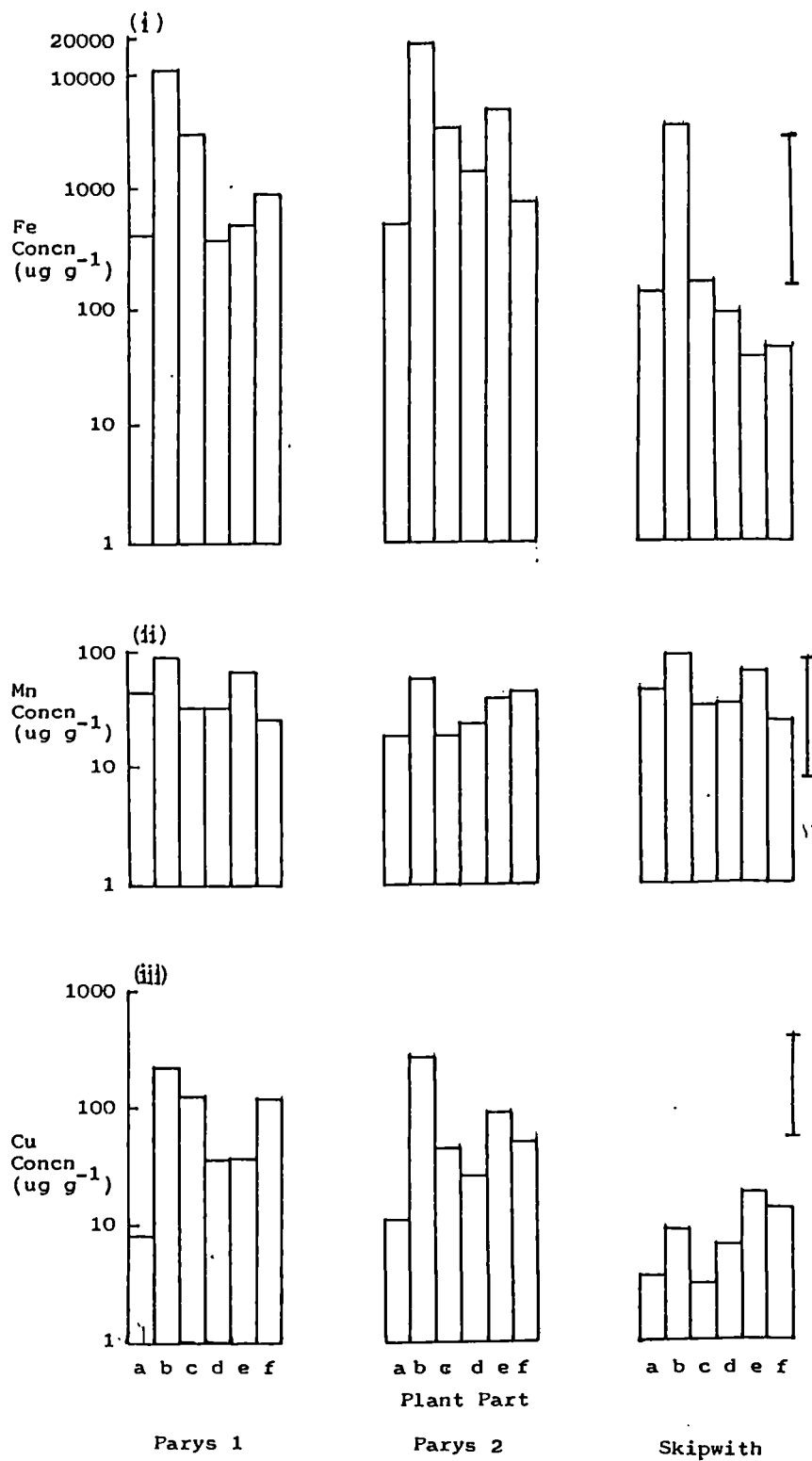


Figure 2.10

Iron (i), manganese (ii) and copper (iii) concentrations (ug/g) of (a) rhizomes, (b) roots, (c) dead leaves, (d) shoots (e) shoot bases and (f) dead shoots of Phragmites australis sampled in November 1985 from Parys Mountain (subsites 1 and 2) and Skipwith Common. (Vertical bars represent Least Significant Differences). ( $p < 0.05$ ).



concentrations. Tissues which were not metabolically active (dead leaves and shoot bases) concentrated more Fe than active tissues (Living leaves and rhizomes). Despite differences in the elemental concentrations of the two sites, the patterns, of Fe localization were the same for the two populations.

As with Fe, roots contained greater concentrations per gram dry weight of Cu, Mn, Zn and Pb than the remaining tissues (Figures 2.10ii-2.11ii). Localization of Mn and Cu (Figures 2.10ii, 2.10iii) in the remaining organs was similar to Fe, in that the lowest tissue concentrations were found in the shoot.

In general, metal concentrations in rhizome tissues were low despite contact with the soil/sediments, suggesting that cleaning of the plants has been adequate. Low rhizome metal concentrations indicate a lack of transport of metals into this organ.

Shoot Zn (Figure 2.11i) and Pb (Figure 2.11ii) concentrations were not significantly different between

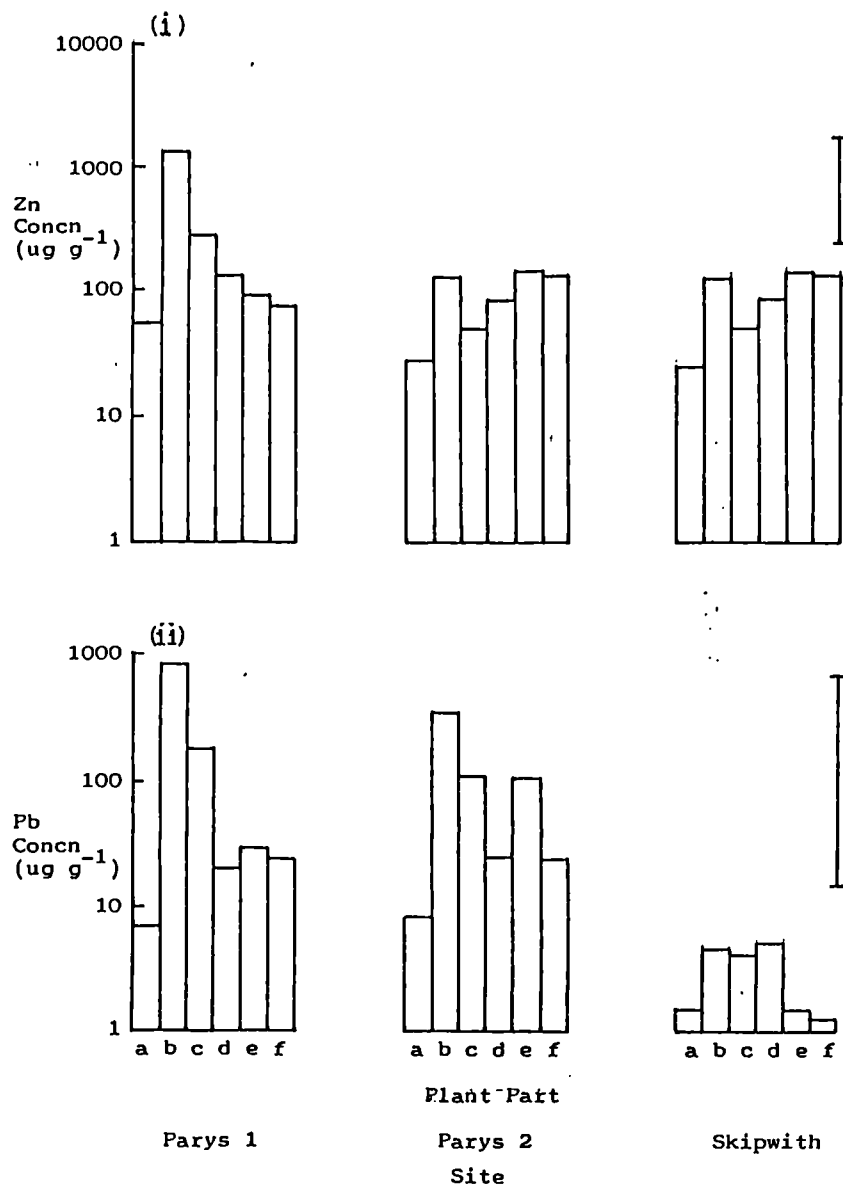


Figure 2.11

Zinc (i) and lead (ii) concentrations (ug/g) of (a) rhizomes, (b) roots, (c) dead leaves, (d) shoots (e) shoot bases and (f) dead shoots of *Phragmites australis* sampled in November 1985 from Parys Mountain (subsites 1 and 2) and Skipwith Common. (Vertical bars represent Least Significant Differences). ( $p < 0.05$ ).

populations despite differences in root Zn and Pb concentrations.

For Ca, there was a significant relationship between site and plant part ( $p < 0.001$ ) (Figure 2.12i). Tissues sampled from Skipwith had higher Ca concentrations than those sampled from Parys. Generally, roots and dead leaves localized more Ca than rhizomes and living leaves. Mg was concentrated in the root and the subsidiary shoot with marginally lower values being observed for the remaining tissues. Total plant Mg did not as a whole, differ between sites (Figure 2.12 ii).

#### 2.3.2.2 Eriophorum angustifolium

Similar trends were observed in Eriophorum as for Phragmites. The concentration of metals was not distributed evenly throughout the plant but tended to be localized in specific tissues. In addition, there was a highly significant difference between mean total element

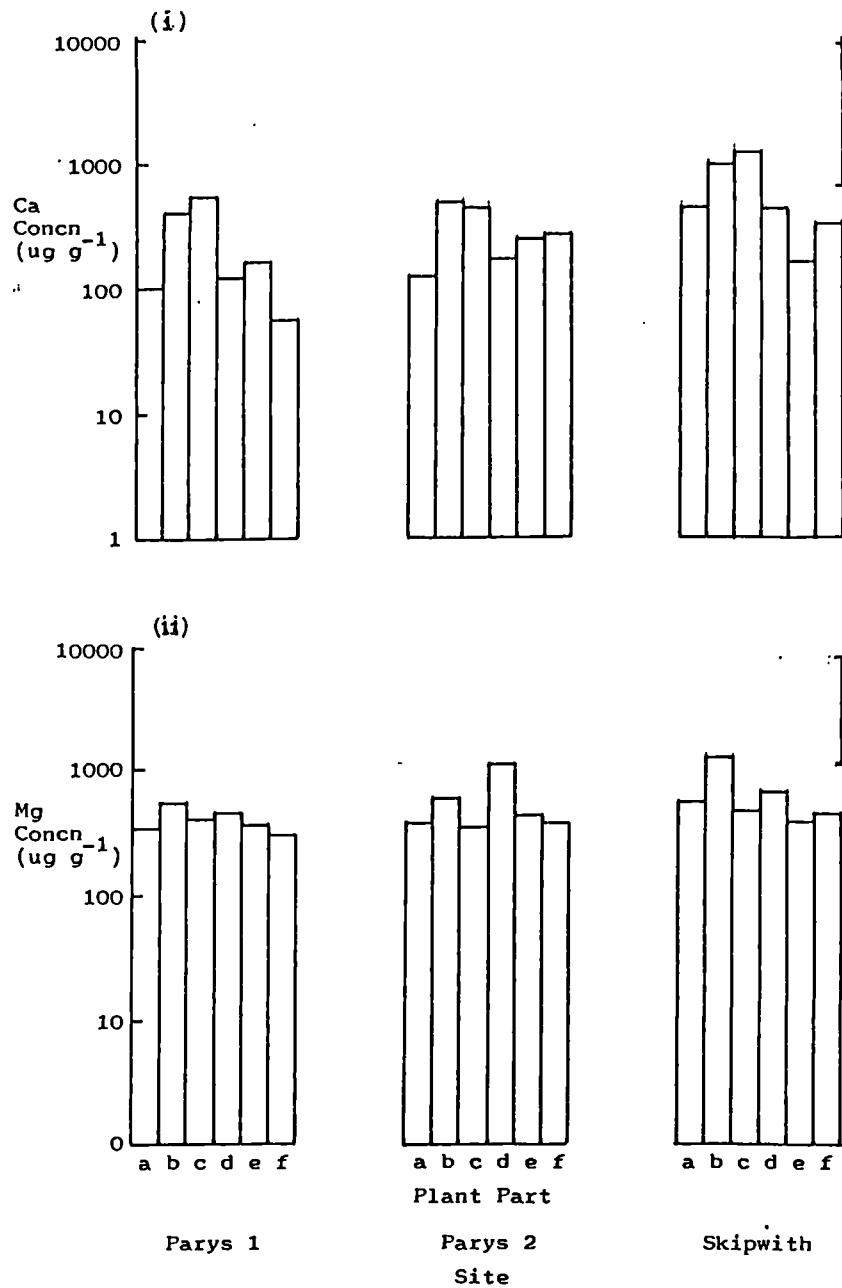


Figure 2.12

Calcium (i) and Magnesium (ii) concentrations (ug/g) of (a) rhizomes, (b) roots, (c) dead leaves, (d) shoots (e) shoot bases and (f) dead shoots of Phragmites australis sampled in November 1985 from Parys Mountain (subsites 1 and 2) and Skipwith Common. (Vertical bars represent Least Significant Differences). ( $p < 0.05$ ).

content of whole plants from different sites.

Fe localization in tissues of both populations were similar. Roots and dead leaves concentrated more of the metal. Parys subsite 1 plants had significantly lower total Fe than did plants from the remaining subsites and Skipwith Common, but these plants were visibly smaller and more depauperate (Figure 2.13i).

Plants sampled from Skipwith concentrated more Mn (Figure 2.13ii). Significant differences were observed in the localization of Mn in different organs and green leaves (living leaves) had the highest concentrations. Lower values were found in the remaining tissues.

There was a significant interaction between site and the distribution of Cu in this plant. Cu was concentrated in dead leaves, roots and shoot bases at higher extractable soil concentrations. At lower extractable Cu concentrations Cu was localized in shoot bases and roots only (Figure 2.14i).

There was little difference in the localization of Zn between populations (Figure 2.14ii). Zn concentrations in

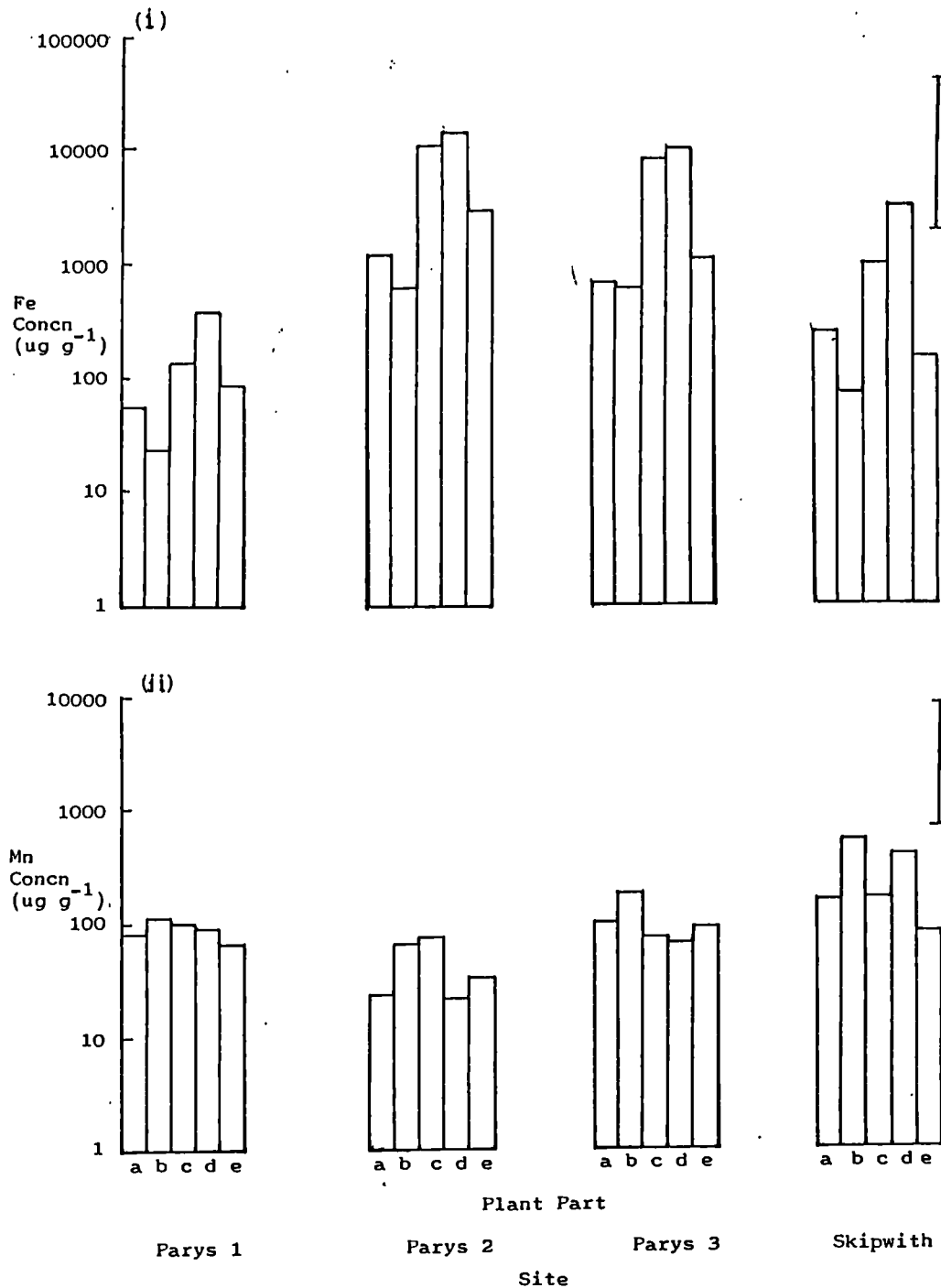


Figure 2.13

Iron (i) and manganese (ii) concentrations ( $\mu\text{g/g}$ ) of (a) leaf tips, (b) living leaves, (c) dead leaves, (d) roots and (e) shoot bases of *Eriophorum angustifolium* sampled in November 1985 from Parys Mountain (subsites 1, 2 and 3) and Skipwith Common. (Vertical bars represent Least Significant Differences). ( $p < 0.05$ ).

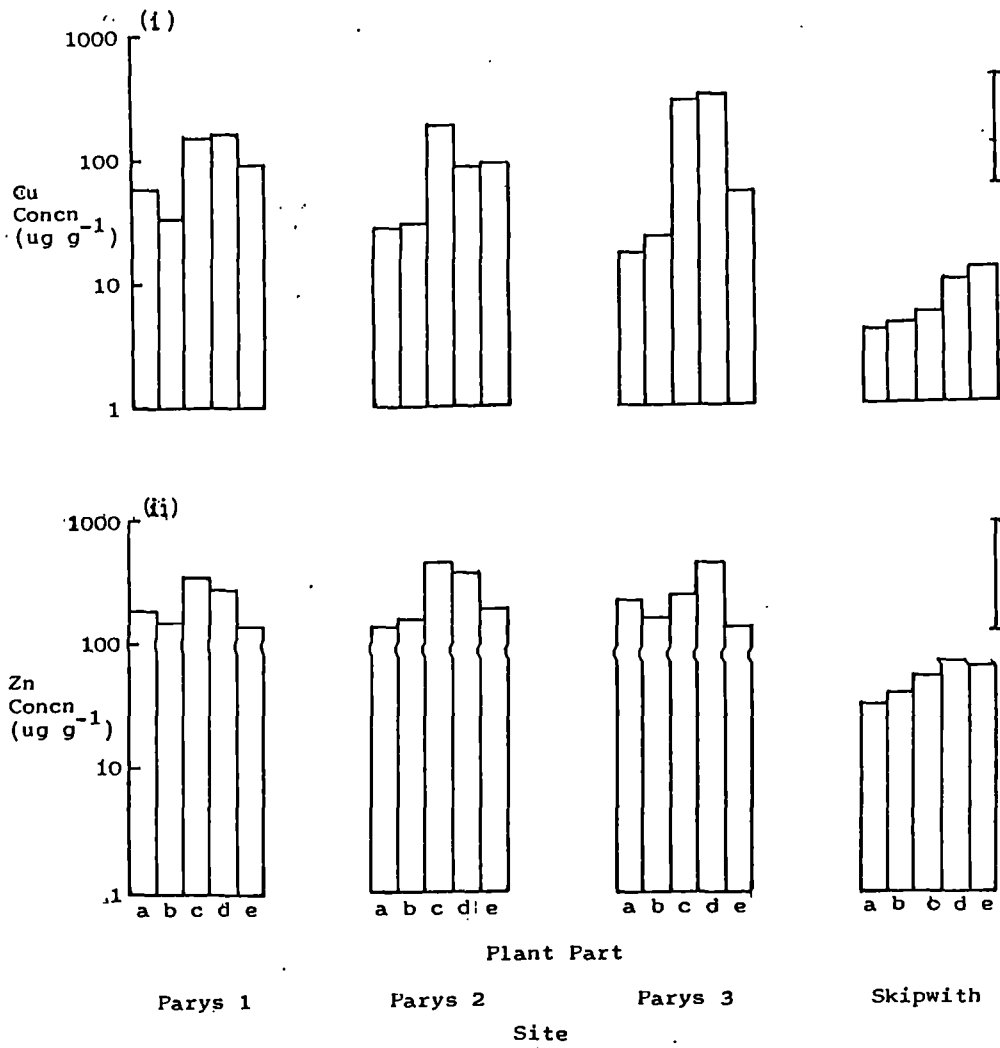


Figure 2.14

Copper (i) and zinc (ii) concentrations ( $\mu\text{g/g}$ ) of (a) leaf tips, (b) living leaves, (c) dead leaves, (d) roots and (e) shoot bases of Eriophorum angustifolium sampled in November 1985 from Parys Mountain (subsites 1, 2 and 3) and Skipwith Common. (Vertical bars represent Least Significant Differences). ( $p < 0.05$ ).

the plants were dependent on extractability in the soil.

Pb content of plant tissues was dependent on extractability of Pb for uptake by the plant (Figure 2.15i). Plants from Skipwith Common had lower concentrations of Pb in tissues and this was localized in all organs except shoot bases. In the more Pb enriched Parys subsites, Pb was not concentrated in metabolically active tissues (ie live leaf) but in dead leaves, roots and shoot bases. At subsite 1 leaf tips were found to concentrate Pb.

There was no significant difference in Ca concentrations (Figure 2.15ii) between populations despite differences in soil Ca extractability. Ca was found in all tissues in similar proportions. Mg concentrations in tissues of E. angustifolium were difficult to interpret owing to the high degree of variability between values. In general, plants from Skipwith had higher Mg contents than those for Parys Mountain (Figure 2.15iii).



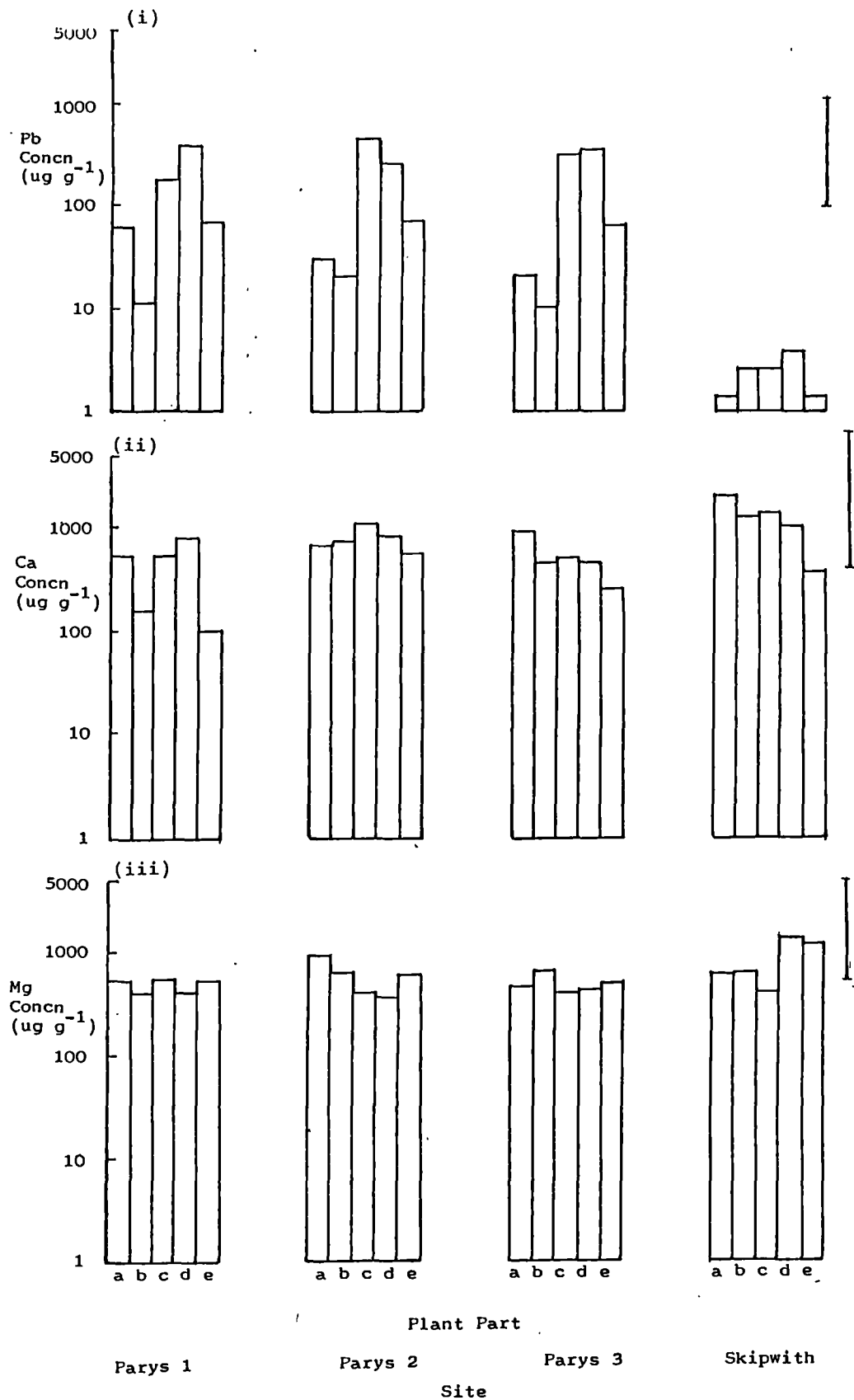


Figure 2.15

Lead (i), calcium (ii) and magnesium (iii) concentrations (ug/g) of (a) leaf tips, (b) living leaves, (c) dead leaves, (d) roots and (e) shoot bases of Eriophorum angustifolium sampled in November 1985 from Parys Mountain (subsites 1, 2 and 3) and Skipwith Common. (Vertical bars represent Least Significant Differences). ( $p < 0.05$ )

## 2.4 Discussion

### 2.4.1 Soil metal status

Chemical analysis of the soil/sediment samples revealed a considerable heterogeneity of metal status in the substratum of the Parys Mountain sites. Field observations at the site indicated some heterogeneity in the growth and distribution of Phragmites australis and Eriophorum angustifolium and so it was considered necessary to include sub-sites. Lack of variation metal content of the upper sediment appeared to be due to its alluvial origins, the sediment probably having been washed down into the stream from the higher settling ponds and being deposited evenly across the surface of the larger settling pond in which the study took place. The bulk of roots of both species were found in the lower sediments. It is likely, therefore, that the metal status of the plants would have been predominantly influenced by the substratum, yet despite the apparent heterogeneity of the sediments in

this layer metal concentrations in plant tissues of both species were remarkably consistent.

Although useful, total metal extractions of the sediment gave no clear indication of what was available for uptake by plants. However, it was obvious that although each site was potentially rich in certain metals these were probably in a form that were not available for absorption by plant roots; for example, the presence of large deposits of iron ochre on the surface of the settling ponds.

One of the most notable findings of this investigation was the high proportion of both Fe and Mn present at both the non-mine (Skipwith) and the mine (Parys) sites. Total and extractable Fe and Mn were within the range reported by Folsom et al. (1981) for heavy metal contaminated freshwater sediments (see Tables 2.2 & 2.3), but total Mn concentrations were higher than those observed by Taylor & Crowder (1983a), in wetlands surrounding a copper smelter. In contrast to the findings of the former authors, extractable Fe and Mn concentrations were more variable (between approximately,

55-3000 ug/g Fe and 4-1000 ug/g Mn, for both sites).

High concentrations of reduced forms of Fe and Mn are a common characteristic of reduced waterlogged environments (see Chapter 1). Falling redox potential may allow Fe (II) and Mn (II) to be produced, sometimes rising to toxic concentrations of several hundred mg/l (Etherington 1982). Sediment Fe concentrations in both sites were often within the range considered to be toxic to growth of rice plants. Normal growth has been shown to be unaffected by concentrations of 16 mg/l Fe in clay soil (recalculated from Tanaka & Navasero 1966b), while toxicity symptoms were observed at concentrations exceeding 875 mg/l Fe in acid soils. Mn concentrations in the same soils were not toxic at 0.2-22.4 mg/l.

Extractable Mn in the soil/sediments in this study exceeded concentrations shown to be toxic to plants in solution culture. Because of difficulties inherent in chemically simulating the action of plant roots in absorbing mineral nutrients from the soil, it is not possible to make

direct comparisons between availability of metals in natural soils and solution cultures. However, Martin (1968) reported that concentrations in excess of 30 mg/l Mn (recalculated from original data) caused toxicity in Carex sylvatica Hudson and Deschampsia caespitosa L. Beauv. and 20 mg/l Mn caused stunting in Eriophorum (Nazrul -Islam 1976).

Thus, availability of Fe and Mn in the sediments of both sites appears to vary between possible toxic concentrations and non toxic concentrations according to location in the wetland.

Total Cu concentrations exceeded those reported by Taylor & Crowder (1983a). According to these authors 3738 ug/g total Cu was potentially toxic to plant growth. Although total Cu concentrations exceeded 20,000 ug/g, extractable Cu ranged between trace (<1 ug/g)- 55 ug/g. The soils from Parys are unusual in that they are derived from weathered chalcopyrite, and would be expected to have high concentrations of both total and extractable Cu. In general, Cu concentrations in mineral soils may range from 0.1-1000 ug/g though less than 1

ug/g is likely to be in solution (Etherington 1982). Cu concentrations as low as 0.25 ug/ml are toxic to Festuca rubra L. in solution culture (Karataglis 1982). It is likely, therefore, that sediment Cu concentrations from the Parys sites are potentially toxic to plants.

Total Zn from the Parys sites exceeded 10-300 ug/g quoted by Etherington (1982) for 'normal' soils. Extractable Zn was high and exceeded concentrations observed by Folsom et al. (1981) for flooded and heavy metal-contaminated freshwater sediments (Table 2.2). Zinc concentrations at the Parys Mountain sites may also be potentially toxic to plants.

Total and extractable Pb concentrations from the Parys sites (approximately, 400-60,000 and 4-700 ug/g respectively) were higher than the values observed by Folsom et al. (1981) (Table 2.1-2.2) and Karataglis (1982). A concentration of 1970 ug/g total Pb was reported by the latter from a Zn/Pb mine. Pb concentrations also appear to be potentially toxic for plant growth.

Ca content of soils is widely variable. Soils derived

from Ca-rich parent material may have Ca contents of 16-20% (Etherington 1982), while acid sandy soils often contain less than 0.5% Ca. Total Ca from the Parys sites contained approximately 0.02-0.08% Ca, and was below the total reported by Taylor & Crowder (1983a) (table 2.0). Total Ca from the Skipwith site was approximately 2% Ca. The total soil content of Mg, like Ca, is widely variable, ranging from 0.003%-0.6% in normal soils. Total Mg from the Parys site was approximately 0.004% compared to 0.3% for the soil from Skipwith. Thus, Mg concentrations do not appear to be deficient.

## 2.4.2 Uptake from and accumulation of heavy metals by plants

### 2.4.2.1 Iron and Manganese

Fe was localized in the root, the shoot base and dead leaves of Phragmites and in the roots and dead leaves of Eriophorum. In Phragmites plants sampled from several Scottish lochs, Ho (1981), also found high concentrations in the root but the above-ground plant parts

did not accumulate high concentrations (table 2.5), Fe appeared to be immobilized in or on the root. Kovacs et al. (1978), found low concentrations in all tissues (table 2.5). Allen & Pearsall (1963) showed that Fe was retained in senescent leaves. However, these workers investigated only plants from uncontaminated wetlands. Plant metal concentrations appear to reflect metal availability in the soil (as seen in the results of this study). Thus, high soil metal concentrations result in high plant tissue concentrations. Mayer & Gorham (1951) suggested that the high availability of Fe in reduced sediments was probably responsible for its high concentration in roots of Phragmites. Taylor & Crowder (1983a), in Typha latifolia, also found high root concentrations of Fe, but rhizome tissue also concentrated Fe. Minimum concentrations were found in stem bases and mid-shoots. Thus, at high concentrations, Fe is apparently localised in the root and senescent tissue of wetland plants. At low soil concentrations Fe is accumulated in the root only.



Despite higher extractable Fe concentrations in the soil from the Skipwith site, Phragmites plants from Parys tended to concentrate more Fe in their tissues. This suggests that perhaps some interaction between metals or an unidentified soil factor was influencing the accumulation of Fe. Auclair (1979) found that Fe and Mn contents in tissues of Phragmites were correlated with soil organic matter content. Folsom et al. (1981), attempted to relate the heavy metal content of Cyperus esculentus, Spartina alterniflora and Distichlis spicata to sediment characteristics. They suggested that a combination of the physical and chemical characteristics associated primarily with sediment organic fraction was related to plant content of heavy metals.

Although extractable soil Mn was as high as 1000 ug/g at the study sites, tissue Mn concentrations in Eriophorum and Phragmites rarely exceeded 150 ug/g. Mn in plant tissues was more evenly distributed than Fe, Cu, Zn and Pb throughout

the plant, although slightly more Mn was concentrated in roots of both species. In Eriophorum, Mn was also concentrated in living leaves. The response of Eriophorum to two closely related heavy metals (Fe and Mn) appears to be different. Generally, Fe is concentrated in dead tissues rather than living leaves. Higher Mn concentrations in living leaves suggests a degree of tolerance to this element. Rice plants also have high tolerance to excess Mn, because their leaves accumulate 5 to 10 times as much Mn as those of other grasses, such as oats, barley, wheat and ryegrass (Vlamis & Williams 1976). The data presented by Taylor & Crowder (1983a) for T. latifolia, also indicated that Mn is localized in the shoot, the remaining organs having lower concentrations. In contrast, Nazrul-Islam (1976) indicated that Eriophorum, the most tolerant of the species he studied, contained least Mn in the shoots and roots, whereas Rumex acetosa (susceptible species) contained more Mn in the shoot (and root). (This experiment was performed under controlled conditions in culture solutions in the absence of other

metals, so it was not directly relevant to the observations in this study).

In Phragmites and Eriophorum, concentrations of Fe reflected availability in sediments. As no evidence was available to suggest Mn was excluded, another factor may be responsible for reducing Mn uptake. Fe and Mn interactions have been observed by many workers (Amberger, Gustav & Wunsch 1982, Tanaka & Navasero 1966a, Reddy, Tucker & Dunn 1987). It was observed that an increase of Fe or Mn in the growth media of rice plants caused a decrease in Mn or Fe content of the plant, possibly indicating that these metals were competing for absorption sites. Ohki (1975) noted reciprocal Fe/Mn relationships in cotton; Mn concentrations of 4 and 247 ug/g in plant tops were associated with Fe concentrations of 270 and 51 ug/g respectively. High Fe concentrations in species in this study may explain the correspondingly lower Mn content of these plants.

#### 2.4.2.2 Copper concentrations in tissues of Phragmites australis and Eriophorum angustifolium

Roots of Phragmites accumulated more Cu than was found in the soil/sediment but other tissues reflected soil/sediment values, suggesting tolerance to high concentrations of this element. In contrast, Taylor & Crowder (1983a) observed, that despite high concentrations of Cu (and Ni), this metal was largely excluded from above-ground tissues of T.latifolia. Total Cu concentration in the sediment was 3738 ug/g, but tissue concentrations were less than 30 ug/g; root concentrations did not reflect soil concentrations. In Phragmites a build-up of Cu on roots was apparent, followed by a gradual build-up in plant tissues. Cu may be immobilized on/in the root of Phragmites. Cu immobilization may be linked to the presence of oxidized Fe coatings (Fe-plaques) observed on the surface of plant roots in the field. Taylor & Crowder (1983b) postulated that the presence of Fe-plaques on the roots of T.latifolia may in turn bind

metals such as Cu and Ni rendering them unavailable for uptake despite relatively high extractable soil concentrations. However, in the presence of a constant external supply of Cu this phenomenon is probably not sufficient to restrict the gradual build-up of Cu in Phragmites under the soil conditions observed in this study. Kovacs et al. (1978) and Larsen & Schierup (1981) also reported high root Cu concentrations with lower values in the stems and leaves.

Contrasting patterns were observed for the distribution of Cu in plants from Parys and Skipwith with the lowest concentrations for plants from the former site greater than those of the latter. It seems likely that these differences are due to soil Cu at Parys being enriched whereas those at Skipwith are close to those found in non-contaminated soils.

The build up of Cu in senescing tissues of E.angustifolium suggests either seasonal concentration (a passive accumulation) or remobilization of this metal. No evidence is apparent for the latter. Lepp (1981) suggested that Cu can be lost from plants via abscission of the various

organs borne on the stem. Rates of loss via this route will depend on the type of plant, the nature of the abscised organs and the rate of abscission. In Fagus sylvatica, Denaeyer De Smet (1973) demonstrated that 50% of the total absorbed Cu was lost in the growing season, the majority of this loss occurring via leaf abscission. It is suggested that early leaf senescence and high turnover of biomass might reduce excess build-up of Cu from physiologically-young to older tissues able to resist Cu in excess- perhaps bound to cell walls and structural material.

#### 2.4.2.3 Zinc in Phragmites australis and Eriophorum angustifolium

The localization of Zn in each species was not similar. In Phragmites it was site-dependent. This is different to the results of Taylor & Crowder (1983a) for T. latifolia where the response to Zn by this species was typified by a lack of correlation between plant and soil-sediment metal concentrations. Species variation and differential response

to high metal concentration could account for the differences in the results. The pattern of Zn localization in Phragmites reflected extractable soil concentrations. Localization of Zn in tissues was the same for each population implying that the strategy of Zn distribution in plant tissues was the same irrespective of external concentration.

In contrast, localization of Zn in Eriophorum was site-independent and high internal Zn concentrations were observed in both populations despite differences in soil extractable Zn concentrations. This implies a high Zn requirement, or passive accumulation of this element perhaps initiated by the influx of another metal. Total metal content in plant tissues may not be as significant as the ratio of metals to one another, as inferred with Fe and Mn.

#### 2.4.2.4 Lead concentrations in plant tissues.

In Eriophorum, Pb content of plant tissues was site-dependent. The nature of Pb localization was different according to external soil concentrations. Excess Pb was

diverted to dead or senescing leaves and the root in Pb-rich sites and concentrated in both live and dead leaves in the Pb-poor site. This was also true of Phragmites. Kufel & Kufel (1980) also observed a direct relationship between this species and the substratum in which it grew, observing that the concentration of Pb in shoot tissue was proportional to the amount of that element in the soil. They concluded that the uptake of elements by Phragmites was specific in the case of Pb. Koeppel (1981) also concluded that the Pb content in and/or on plants reflects the extractability of Pb in soils of what can be termed 'biologically available' Pb. Arknrog & Lippert (1971) reported that in barley plants which were exposed to Pb-contaminated rooting media, the roots contained Pb at considerably greater concentrations than other above-ground tissues. The present work suggests that roots do not appear to be the major site for internal regulation of Pb concentrations (in both species) in contrast to the work of Malone, Koeppel & Miller (1974), who suggested that large quantities of Pb are bound to the outside of the roots in Zea



mays L., the 'free space', as well as cell walls throughout the root. However, the bulk of Pb present in the species studied here may be concentrated and consequently immobilised in maturing leaf tissue. Both species appeared to be tolerant to Pb, despite high tissue concentrations.

#### 2.4.2.5 Calcium concentrations in plant tissues.

Because of the abundance of Ca in soils (Rorison and Robinson 1984), and the fact that relatively small changes in Ca<sup>2+</sup> activity can have significant effects upon the concentration of other plant nutrients and of potentially toxic metals through pH-mediated effects, it was considered necessary to include this element in the analyses. Several authors have investigated Ca accumulation by plant species in metal-contaminated sites (Johnston & Proctor, 1977; Kufel & Kufel, 1980; Taylor & Crowder, 1983a) The results of this investigation suggest that Ca concentration by Phragmites was site-dependent. Ca content in Phragmites from both

populations was governed by Ca extractability in the sediments. Many investigators (Ho 1981, Ksenofontova 1988) of Ca nutrition in Phragmites have recorded the same localization of Ca in plant tissues as in the present work.

In Phragmites Ca was found to concentrate in senescent leaves. Ca localization in dead tissues may merely reflect immobility of Ca within the plant. (Ca may enter passively towards a transpirational sink. Phloem-immobile elements will be subject to large-scale redistribution, through the transpirational demands of different organs and tissues. Because the oldest tissue represents the largest transpiration sink, accumulation of immobile elements may be greatest at this point. Other authors who have recognized this were Van Goor and Wiersma, 1974; Dykyjova, 1978; and Taylor and Crowder 1983a).

In Eriophorum Ca contents of plant tissues were site-independent and did not differ between populations. Eriophorum appears therefore to be able to maintain internal Ca concentrations over a range of external concentrations.

#### 2.4.2.6 Magnesium concentrations in plant tissues

Concentration of Mg in Eriogonum plants was site-dependent, unlike the uptake of Ca. In contrast, Phragmites showed no relationship between site and plant tissue. Mg concentrations were maintained at a constant level at a range of soil-sediment concentrations and were localized in different organs. In the same species, Ho (1981) observed that Mg was found in higher concentrations in above-ground rather than below-ground tissues. Mg concentrations in Phragmites were similar in plants from different sites and tended to be more-or-less evenly distributed throughout the tissues. Higher extractable Mg from the Skipwith site did not correlate with higher Mg uptake in Phragmites. As soil Mg concentrations were similar to concentrations observed in normal soils (see section 2.4.1), plants were probably not

deficient in this element. The uptake of Mg can be strongly depressed by other cations such as  $\text{Ca}^{2+}$  and  $\text{Mn}^{2+}$  (Heenan & Campbell 1980), as well as by  $\text{H}^+$ , that is, by low pH. Mg uptake in Phragmites does not appear to be affected by soil chemical conditions.

#### 2.4.2.7 The relationship between soil metal concentrations and uptake by plants

Hitherto all the soil-sediment-plant tissue-metal relationships have been described independently. However, in normal soils metal ions interact, at the interface between roots and soils and are seen to influence the growth of a plant collectively. Synergistic and antagonistic responses of groups of metals (Fe and Mn, Fe and Cu, Zn and Cu) have been revealed and are described by Foy et al. (1978). Little evidence was available on the possible interactions between metals in this study but the presence of interactions cannot be ruled out and is an area for future investigation.

#### 2.4.2.8 Sampling of plant material

A further point highlighted by this study was the differences amongst authors in sampling times of plant material for metal and nutrient analysis reported by various authors. Numerous workers have observed seasonal variations in nutrient and metal content (Ca, Mg, Fe, Cu, Zn and Pb) particularly the seasonal fluctuations in element status of Phragmites (Bayley & O'Neal, 1972; Dykyjova, 1978; Ho, 1981; Kaul, 1984). Conclusions based on dormant plants at the end of the season (such as in the present work) are not likely to give any indication of the effect of changes in element concentrations over critical periods in the growing season. Under field conditions, the chemical conditions of the environment fluctuate and the effects of these fluctuations on critical stages in plant development may be more important for the success of a plant/species in that habitat than total concentrations of potentially toxic metals. To elucidate more clearly the factors which govern the tolerance to excess metal concentration it would be necessary to monitor metal-status of

the soil and the plant on emergence , at the point of maximum growth rate and at peak biomass. The exchange of nutrients between soil-sediments and plants is a function of several physical, chemical and biological processes. Sediment pH influences the concentration of dissolved components in sediments but sediment redox-potential also has a marked effect on the nature of that soil and associated vegetation. For example, large quantities of Fe in solution were found under low pH, redox- potential conditions by Delaune, Reddy & Patrick (1981). Mn reactions in sediments are apparently regulated not only by chemical constraints, especially at low pH values but also by microbial oxidation-reduction processes. It is therefore essential that any further investigation should involve measurements of redox-potentials.

## 2.5 Conclusions

One of the most notable findings of this study was the high concentrations of Fe and Mn in soils (both total and

extractable) and plant tissues collected from both sites. High concentrations of Fe and Mn in solution are a common characteristic of waterlogged environments (Armstrong 1982, Jones 1972) and it is suggested that the response of both populations of Phragmites and Eriophorum to Fe and Mn is an intrinsic feature of plants from wetland habitats. A general resistance to Fe and Mn is implied.

Plants of Phragmites from the Parys site accumulated more Cu with respect to plants from the Skipwith site. Roots from these plants appeared to accumulate Cu concentrations higher than the external medium. Cu immobilization may be linked to Fe-coatings on the root surfaces of this plant. The Parys population appears to be resistant to high internal Cu concentrations. A similar pattern of Cu localization was found in plants of Eriophorum.

Zn localization was similar in both populations of Phragmites despite differences in extractable concentrations. Localization of Zn in Eriophorum was

site-independent and high internal concentrations were observed despite differences in soil extractable concentrations.

The pattern of localization of Pb was also dependent on the availability of Pb for uptake by the plant. At higher external concentrations this element was not localized in metabolically active tissue (living leaves).

It is apparent that in some soils excessive concentrations of metals may be present without adversely affecting the growth of plants. The lack of toxicity in such cases may be attributed to the interactions of these metals with other soil factors and with each other. Direct correlations between competing and interacting metals are more difficult to observe and would require standardized conditions and is a further area for study. The balance of metals in relation to the ratios of one metal to another may be a more important consideration than absolute concentrations of metals and a plant may have high internal concentrations without experiencing physiological damage (for example Fe and Mn).



In soil the availability of metals varies through a growing season and measurements of plant- and soil-metal concentrations at any one time will not be sufficient to determine seasonal cycles. Therefore, it is necessary to take a number of such measurements if the nature of the relationship between plant and soil metal concentrations is to be understood. This should help elucidate mechanisms of plant resistance to metals in these systems.

## CHAPTER 3

### SEASONAL DYNAMICS OF HEAVY METAL CONCENTRATIONS IN CONTAMINATED AND UNCONTAMINATED WETLANDS

#### 3.1 Introduction

Literature exists on the concentrations of heavy metals in plants, water and bottom sediments in aquatic systems (Kufel & Kufel 1980, Schierup & Larsen 1981, Simmers et al. 1981, Van der Werff 1987) but much less is known about the pathways and dynamics of these elements in aquatic environments. Many of the data on mineral composition and metal concentration in Phragmites and other aquatic plants are based on material collected at the time of peak biomass (Allen & Pearsall 1963, Kufel & Kufel 1980, Taylor & Crowder 1983a). However, the chemical composition of plants

such as Phragmites, Typha spp, and Eriophorum varies during the growing season. (Bayley & O'Neill 1972, Chapin, Van Cleve & Tieszen 1975, Mason & Bryant 1975, Dykova 1978, Kufel 1978, Ho 1981).

Kufel (1978) demonstrated marked seasonal changes in Cu and Pb concentrations from May to September, in Phragmites collected from two unpolluted lakes. The concentration of Cu and Pb increased at the beginning of the growing season, reaching a maximum at the end of May and then decreasing. The Cu content of leaves of Phragmites is apparently well regulated by the plant at levels below the threshold of toxicity, as Cu concentrations were similar in the leaves of plants growing in a variety of soils of different Cu contents (Chiaudani 1969).

Ho (1981) found no consistent seasonal variation in Phragmites of levels of Ca or Fe in the root and rhizome but found that Ca in both the stem and leaf rose to a peak before declining for the rest of the growing season. Mn differed from all the other elements examined by Chapin

et al. (1975) in that the concentration showed virtually no seasonal change in Eriophorum. Alberts, Newell & Price (1987) found that Fe increased with time while Cu and Mn decreased or remained unchanged in Spartina alterniflora. Jones (1967), measured seasonal fluctuations in extractable Fe and Mn in wet sand dune slacks, finding very high values during the late spring and early summer months. These high concentrations coincided with peak growth rates in Agrostis stolonifera.

High concentrations of metals in plant tissues reflected both the relative availability to plants of the metal in sediments (Schierup & Larsen 1981) and the degree of contamination of a sediment (Chiaudani 1969). Plants growing in more contaminated areas generally have elevated levels of heavy metals in the tissues in comparison to plants growing in less contaminated areas (Chiaudani 1969, Schierup & Larsen 1981). Substrate characteristics may influence significantly the uptake of trace metals in Phragmites. Higher pH, lower redox values, higher organic content and high cation exchange

capacity (CEC) were found to reduce the availability of heavy metals despite relatively high total concentrations in the sediments (Schierup & Larsen 1981). Re-distribution of metals between tissues also varies with species.

Various authors have also shown that, generally, concentrations of trace elements in aquatic macrophytes may vary significantly from year to year and between sites. Kufel (1978) concluded that concentrations of heavy metals in Phragmites and T. latifolia may change considerably during the growing season. The observed seasonal changes in concentration were due to the different rates of accumulation of heavy metals by plants. The highest rate of Pb and Cu accumulation by Phragmites occurred at the end of May and the beginning of June. This period corresponded with the main stage of shoot elongation. During the rest of the growing season Pb and Cu are accumulated at slower rates; apparent negative values of the rate of accumulation were likely to be due to a corresponding rise in plant mass which 'diluted' the element (Kufel 1979).

This part of the study had four main aims; (a) to collect comparative data on the contents of mineral elements in plants from different wetland habitats; (b) to illustrate the variation in the uptake capacity of different species and their different organs; (c) to monitor the seasonal dynamics in concentrations of Fe, Cu, Mn, and Ca in the root, stem (culm) and leaves of Phragmites and Eriophorum and to compare these with seasonal variations in element status; and (d) to raise further points for consideration on the possible nature of resistance of these two species to potentially toxic concentrations of metals.

Fe, Mn, Cu and Ca were selected for the following reasons. High Fe and Mn concentrations are characteristic of wetland sites and both Phragmites and Eriophorum were apparently tolerant to high tissue concentrations of these elements.

Cu was selected as plants of both species from the Parys site were suspected of being tolerant to Cu.

Ca concentrations in soils are widely variable

(Etherington 1982) and a distinction was made between low Ca sites and high Ca sites in Chapter 2. Unlike Mg, Ca concentrations reported in Chapter 2 were below those quoted for normal soils (section 2.2.1). In addition to P and K, Ca is regarded as being essential in the maintenance of resistance of rice plants to high Fe concentrations (Ottow et al. 1983). Therefore, it was thought necessary to investigate the possible relationship between Ca and metal uptake.

## 3.2 METHODS

### 3.2.1 Sites

In addition to the sites studied in Chapter 2, a further subsite and an extra site was included in the present investigation to add further detail to the study. These were:

(i) Parys subsite 4. (ii) Crymlyn Bog, near Swansea (S. Wales).

### 3.2.2 Field determinations of soil oxidation-reduction potentials (Eh)

Soil redox potential <sup>measurements</sup> were made with a millivolt meter in the field using a platinum/saturated calomel electrode pair corrected to pH 7 using the following formula (Bohn 1971):

$$\text{Redox E7} = (\text{redox} + 260) - (59 \times (7 - \text{soil pH}))$$

Standardization to pH 7, made comparisons between sites easier. Eh readings (along with pH) at two depths were



determined following a 10 minute equilibration period (<250 mm and >250 mm depth) and replicated five times.

### 3.2.3 Collection, preparation and analysis of plant and soil samples

#### 3.2.3.1 Plant material

The same procedures as outlined in Section 2.2.2 were used in the preparation of plant material for analysis. However, the plants were further divided into the following sections, taking into account growth and differentiation:

(a) Phragmites australis (i) upper, middle, lower and dead leaves (ii) culm (iii) culm base

(b) Eriophorum angustifolium (i) upper, middle, lower and dead leaves (ii) culm Harvests were made in April (the initial period of growth), July (the period of maximum growth rate) and September (peak biomass).

The thorough cleaning of samples to reduce external metal contamination proved so time consuming that only five replicates were taken. Four elements were analysed; Fe, Mn,

Cu and Ca.

### 3.2.3.2 Soil samples

Total (nitric acid) and extractable (acetic acid) elemental fractions were determined for the soil/sediments on the sampling dates given above and prepared using the methods outlined in Section 2.2.4.1 and 2.2.4.2. pH was determined at each harvest (see Section 2.2.4) to complement soil redox potentials.

### 3.2.4 Statistical analysis

Analyses of variance were performed on the results using the GENSTAT statistical package. Data were transformed using natural logarithms, since the variances were related to the means. For ease of interpretation the values on the y-axis have been back-transformed.

Unless otherwise stated significance was tested at the 5% level ( $p < 0.05$ )

### 3.3 Results

#### 3.3.1 Seasonal oxidation-reduction potentials (upper and lower sediments)

Oxidizing and reducing conditions are defined according to the definitions outlined by Patrick and Mahapatra (1968). Oxidized soils are at redox potentials of  $>400$  mV, moderately reduced soils about 100 to 400 mV, reduced -100 to 100 mV and highly reduced -100 to -300 mV (all measurements at pH 7).

There was a clear difference between the upper and lower soil sediments and between sites with respect to redox potentials (Table 3.1). For April and July, upper sediment redox potentials were moderately reduced at Parys with the exception of subsites 1 and 4. In contrast, the redox potentials of the lower sediments were moderately reduced to reduced, reaching minimum values in July. However, in September the redox potentials of the lower sediments became more oxidizing with respect to the upper

Table 3.1 Redox (Eh7) values for surface (100 mm) and subsurface (250 mm) sediment samples collected in April, July and September 1986 (means of 5 replicates).

Site	Redox Potential (mv)					
	April		July		September	
	100 mm	250mm	100 mm	250 mm	100 mm	250 mm
Parys 1	224	44	220	55	468	444
Parys 2	332	244	332	159	205	361
Parys 3	353	198	201	29	361	470
Parys 4	174	140	111	379	415	402
Skipwith	108	32	8	29	-116	-97
Crymlyn 1	33	-	121	111	-34	-45
Crymlyn 2	21	127	87	84	-40	-94

Table 3.2 pH values for surface (100 mm) and subsurface (250 mm) sediment samples collected in April, July and September 1986. (Least significant difference =0.1).

Site	pH					
	April		July		September	
	100 mm	250mm	100 mm	250 mm	100 mm	250 mm
Parys 1	2.7	4.9	2.6	2.6	4.2	5.3
Parys 2	2.8	3.6	3.5	4.6	3.2	4.4
Parys 3	2.8	5.1	3.5	5.4	2.8	4.9
Parys 4	4.9	5.3	6.0	5.5	5.3	5.7
Skipwith	5.5	5.5	5.2	5.0	5.1	5.2
Crymlyn 1	5.6	-	6.2	6.2	5.9	5.9
Crymlyn 2	5.6	5.6	6.2	6.2	6.2	6.2

sediments. Parys subsite 4 had redox potentials different to those of the other subsites. Conditions were more reducing at the beginning of the season and there was little difference between upper and lower sediments. In mid-season the sediments were oxidizing with respect to the values recorded for April, and were similar to the other subsites. September sampling indicated a much greater increase in redox potential than previously measured (greater oxidation of the sediments). Values for upper and lower sediments were similar.

Sediment redox potentials for Skipwith Common were reduced in April and July becoming highly reduced in the surface peat in September. The Crymlyn soil/sediments were moderately reduced to reduced. Both subsites showed a trend towards more reducing conditions at the end of the season.

### 3.3.2 Seasonal pH determinations of soil-sediments

More acidic conditions were found in the upper sediments

at Parys with the exception of subsite 4. pH values remained similar throughout the sampling period but remained different for the upper and lower sediments. In contrast Skipwith and Crymlyn showed no change in pH with depth and were less acidic (Table 3.2).

### 3.3.3 Soil/sediment total and extractable values for Fe, Cu, Mn and Ca in soil-sediments sampled in April, July, and September

As observed in Chapter 2, the upper sediments of Parys were generally more Fe enriched than the lower sediments (Table 3.3) with the exception of Parys subsite 4 where the opposite was true. Total Fe concentrations for Skipwith were consistently lower than the other sites and more consistent with depth. Maximum values were measured for Crymlyn where the surface sediments were also more Fe enriched. The general increase in total Fe concentrations observed over the sampling period is likely to be due to variation in sampling. Only a small fraction of the total

Table 3.3 Total (1) and Extractable (2) iron concentrations (ug/g) in upper (a) and lower (b) soil/sediments collected in April, June and September 1986 from Parys Mountain, Crymllyn Bog and Skipwith Common. Values followed by the same letter are not significantly different (comparisons made within sediment layers only).

Site	Fe (concentration ug/g)		
	April	June	September
<hr/>			
(1) Total Fe			
(a) Upper sediments			
Parys 1	43088 f	84796 e	359331 b
Parys 2	48485 f	170758 d	567502 a
Parys 3	46630 f	184425 cd	85819 e
Parys 4	1250 g	93620 e	53051 f
Skipwith	1067 gh	775 h	716 h
Crymllyn 1	476170 a	576079 a	244752 c
Crymllyn 2	463228 a	482627 a	77575 e
(b) Lower sediments			
Parys 1	7762 h	140365 a	62755 cd
Parys 2	39895 ef	109316 ab	23063 f
Parys 3	8333 h	95511 b	34441 ef
Parys 4	32112 e	34892 ef	73792 bc
Skipwith	1194 i	6124 h	318 i
Crymllyn 1	16482 g	84582 bc	16882 g
Crymllyn 2	24637 ef	126374 a	57068 d
<hr/>			
(2) Available Fe			
(a) Upper sediments			
Parys 1	207 cd	225 c	667 ab
Parys 2	89 e	882 a	137 d
Parys 3	62 e	245 c	156 d
Parys 4	41 f	460 b	39 f
Skipwith	5 g	27 f	86 e
Crymllyn 1	979 a	483 b	1022 a
Crymllyn 2	975 a	296 c	968 a
<hr/>			
(b) Lower sediments			
Parys 1	774 d	233 f	1055 c
Parys 2	789 d	13739 a	2981 b
Parys 3	1326 c	3008 b	1343 c
Parys 4	144 g	425 e	121 h
Skipwith	5 i	102 h	97 h
Crymllyn 1	183 fg	957 c	590 de
Crymllyn 2	198 fg	1038 c	539 de

pool of Fe present in soil/sediments at the sites was potentially available for uptake by plants. For the lower sediments from Parys subsites 2, 3 and 4 and Skipwith, extractable Fe values peaked mid-season and declined thereafter (Table 3.3). For the latter sites and subsites the extractable Fe concentrations for the upper sediments were lower than for the lower sediments although they increased by mid-season and then levelled off. In general, extractable Fe concentrations from the Crymlyn sediments were more consistent and did not vary through the growing season.

Total Mn concentrations (Table 3.4) for upper and lower sediments from Parys subsite 4 and Crymlyn were greater than for the remaining sites. Total Mn values for surface sediments were more variable compared to lower sediments. Extractable Mn showed the same trend outlined for total Mn concentrations.

As observed previously (Chapter 2), the Parys Mountain site was more Cu enriched, total Cu concentrations of soil/sediments confirmed this observation. The lower



Table 3.4 Total (1) and Extractable (2) manganese concentrations (ug/g) in upper (a) and lower (b) soil/sediments collected in April, June and September 1986 from Parys Mountain, Crymlyn Bog and Skipwith Common. Values followed by the same letter are not significantly different (comparisons made within soil layers only).

Site	Mn (concentration ug/g)		
	April	June	September
<b>(1) Total Mn</b>			
<b>(a) Upper sediments</b>			
Parys 1	1 k	37 f	26 g
Parys 2	3 j	22 g	1 k
Parys 3	6 i	68 c	1 k
Parys 4	32403 b	49415 a	3278 c
Skipwith	15 h	12 h	1 k
Crymlyn 1	7564 c	7383 c	1580 d
Crymlyn 2	7999 c	8358 c	163 d
<b>(b) Lower sediments</b>			
Parys 1	161 d	50 e	160 d
Parys 2	3 f	45 e	1 g
Parys 3	3 f	633 a	32 e
Parys 4	355 abc	332 bc	623 ab
Skipwith	37 e	148 d	51 e
Crymlyn 1	41 e	167 d	49 e
Crymlyn 2	58 e	230 cd	140 d
<b>(2) Extractable Mn</b>			
<b>(a) Upper sediments</b>			
Parys 1	1 g	10 d	7 e
Parys 2	1 g	6 e	1 g
Parys 3	1 g	18 d	1 g
Parys 4	2191 b	12358 a	820 c
Skipwith	3 f	4 f	1 g
Crymlyn 1	1648 b	1848 b	396 d
Crymlyn 2	1998 b	2090 b	409 d
<b>(b) Lower sediments</b>			
Parys 1	41 c	13 e	41 c
Parys 2	2 f	12 e	1 g
Parys 3	2 f	159 a	9 f
Parys 4	97 b	84 b	156 a
Skipwith	13 e	38 c	18 de
Crymlyn 1	22 d	45 c	20 d
Crymlyn 2	18 de	53 c	36 c

sediments had higher Cu concentrations than the upper sediments. Extractable Cu concentrations were highly variable but more Cu was available at Parys. The extractable Cu from upper sediments were less variable with time (Table 3.5).

Total Ca concentrations of the upper soil/sediments from the Parys Mountain site with the exception of Parys subsite 4 (which were intermediate in value) were lower than the upper soil/sediments from the remaining sites (Table 3.6). Extractable Ca concentrations were remarkably constant throughout the sites, samplings and depths (Table 3.6).

#### 3.3.4 Analysis of Iron, Manganese, Copper and Calcium in tissues of Phragmites australis.

The Fe concentrations of leaves (upper, middle and lower) were all rather similar but showed some seasonal changes (only upper leaf values are presented here (see Figure 3.1a) At Parys subsite 1 there was a significant

Table 3.5 Total (1) and Extractable (2) copper concentrations (ug/g) in upper (a) and lower (b) soil/sediments collected in April, June and September 1986 from Parys Mountain, Crymllyn Bog and Skipwith Common. Values followed by the same letter are not significantly different (comparisons made within sediment levels only).

Site	Cu (concentration ug/g)		
	April	June	September
<b>(1) Total Cu</b>			
<b>(a) Upper sediments</b>			
Parys 1	35 k	403 e	1508 c
Parys 2	168 g	483 d	187 g
Parys 3	45 j	462 d	347 f
Parys 4	1 l	1698 b	1979 a
Skipwith	1 l	1 l	39 j
Crymllyn 1	1 l	1 l	89 i
Crymllyn 2	1 l	1 l	100 h
<b>(b) Lower sediments</b>			
Parys 1	549 e	436 f	1313 b
Parys 2	115 k	1656 a	1846 a
Parys 3	167 i	1572 a	936 c
Parys 4	280 g	232 h	751 d
Skipwith	68 l	187 hi	42 m
Crymllyn 1	70 l	198 h	142 j
Crymllyn 2	154 j	439 f	165 j
<b>(2) Extractable Cu</b>			
<b>(a) Upper sediments</b>			
Parys 1	12 h	20 g	105 c
Parys 2	26 f	239 b	53 d
Parys 3	8 i	3 j	3 i
Parys 4	39 e	156 a	105 c
Skipwith	1 k	1 k	1 k
Crymllyn 1	1 k	1 k	1 k
Crymllyn 2	1 k	1 k	1 k
<b>(b) Lower sediments</b>			
Parys 1	7 ef	136 b	9 e
Parys 2	17 d	1 h	1084 a
Parys 3	1 h	1 h	1 h
Parys 4	71 c	89 bc	100 bc
Skipwith	1 h	1 h	1 h
Crymllyn 1	6 f	11 de	11 de
Crymllyn 2	2 h	3 q	1 h

Table 3.6 Total (1) and Extractable (2) calcium concentrations (ug/g) in upper (a) and lower (b) soil/sediments collected in April, June and September 1986 from Parys Mountain, Crymllyn Bog and Skipwith Common. Values followed by the same letter are not significantly different (comparisons made within sediment levels only).

Site	Ca (concentration ug/g)		
	April	June	September
<b>(1) Total Ca</b>			
<b>(a) Upper sediments</b>			
Parys 1	9 k	119 i	276 h
Parys 2	1 l	61 j	107 i
Parys 3	1 l	75 j	87 d
Parys 4	373 h	3722 c	2345 de
Skipwith	2538 de	2612 d	2028 de
Crymllyn 1	5486 b	5514 b	1154 a
Crymllyn 2	1929 e	1966 de	38292 a
<b>(b) Lower sediments</b>			
Parys 1	211 i	85 j	652 e
Parys 2	1 l	33 k	210 i
Parys 3	41 k	498 fg	276 h
Parys 4	109 j	517 efa	624 def
Skipwith	93 j	408 g	1093 d
Crymllyn 1	37 k	164 i	1935 c
Crymllyn 2	621 ef	2716 b	28170 a
<b>(2) Extractable Ca</b>			
<b>(a) Upper sediments</b>			
Parys 1	79 k	347 ef	265 g
Parys 2	83 k	90 jk	234 h
Parys 3	97 j	90 j	227 h
Parys 4	175 i	358 ef	807 b
Skipwith	419 de	748 bc	1244 a
Crymllyn 1	249 gh	594 c	1578 a
Crymllyn 2	284 fgh	948 b	1360 a
<b>(b) Lower sediments</b>			
Parys 1	296 i	371 g	519 e
Parys 2	982 l	326 hi	1166 c
Parys 3	259 j	102 l	553 e
Parys 4	257 j	353 gh	1166 c
Skipwith	453 f	556 c	1579 a
Crymllyn 1	210 k	257 j	1383 b
Crymllyn 2	359 gh	440 f	1058 d

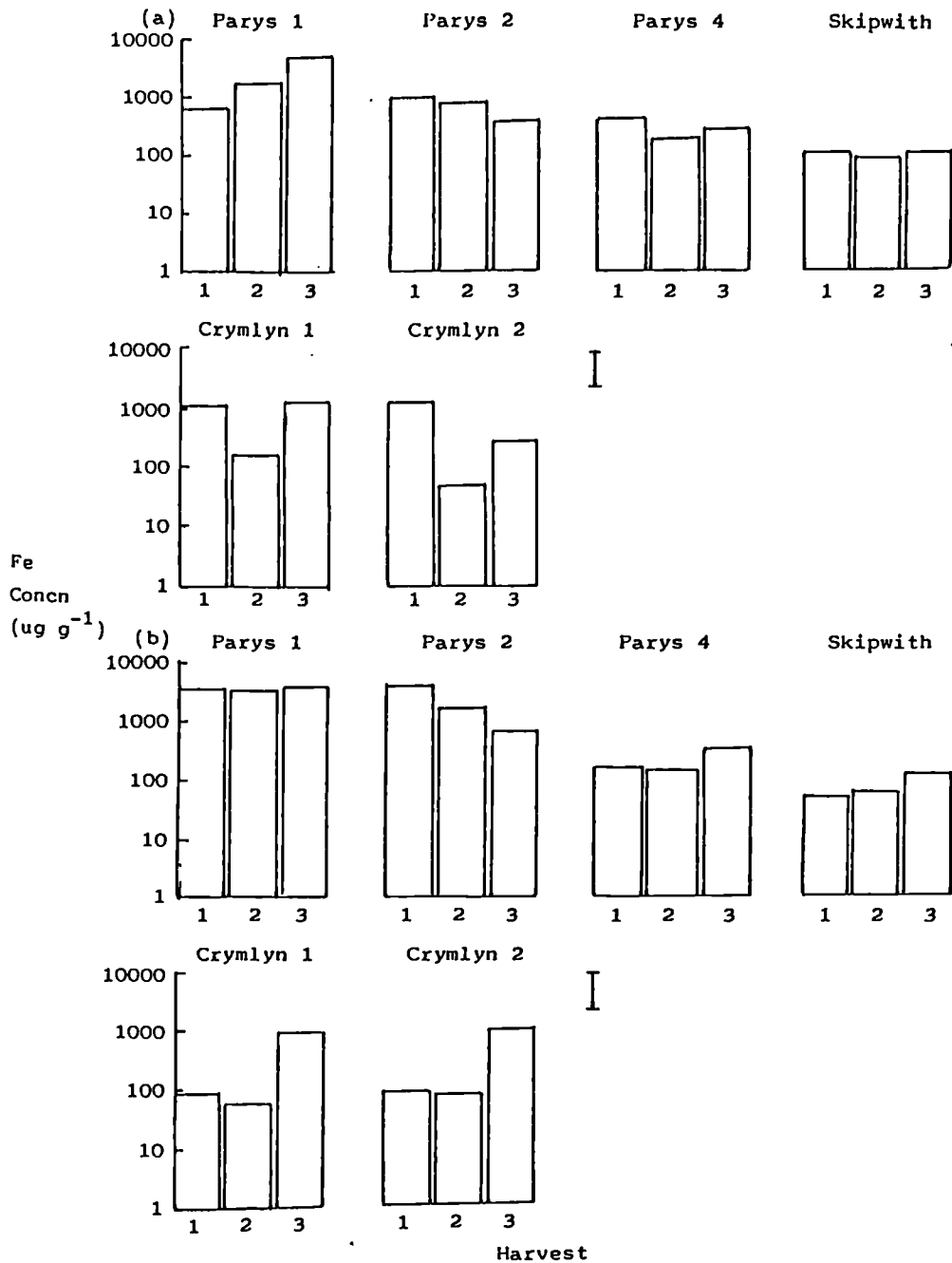


Figure 3.1

Iron concentrations ( $\mu\text{g/g}$ ) of (a) upper leaves and (b) dead leaves of *Phragmites australis* sampled in April (1), June (2) and September (3) 1986 from Parys Mountain, Crymlyn Bog and Skipwith Common. (Vertical bars represent Least Significant Differences). ( $p < 0.05$ ).

increase in Fe concentrations in the upper leaves between April and September but a decrease at Parys subsite 2. The concentration of Fe in the upper leaves of plants from Crymlyn decreased in midseason. The same trends in Fe concentrations of leaves sampled from different sites were observed in all ages of living leaves (Appendix IV). Concentrations of Fe in dead leaves (Figure 3.1b) of plants from Parys subsites 1 and 2 were similar to those recorded for living leaves but did not show any obvious seasonal trend. However, Fe concentrations in dead leaf tissues of plants from the remaining sites remained constant over April and July and increased significantly in September. Overall, culm Fe concentrations (Figure 3.2a) were significantly lower than Fe concentrations observed in leaf samples. The Fe content of the culm showed clear seasonal changes and declined from April to September. The Fe concentrations of the culm bases also showed this trend. No consistent pattern was observed for the rhizome and there was no difference between Parys and Crymlyn. As observed in

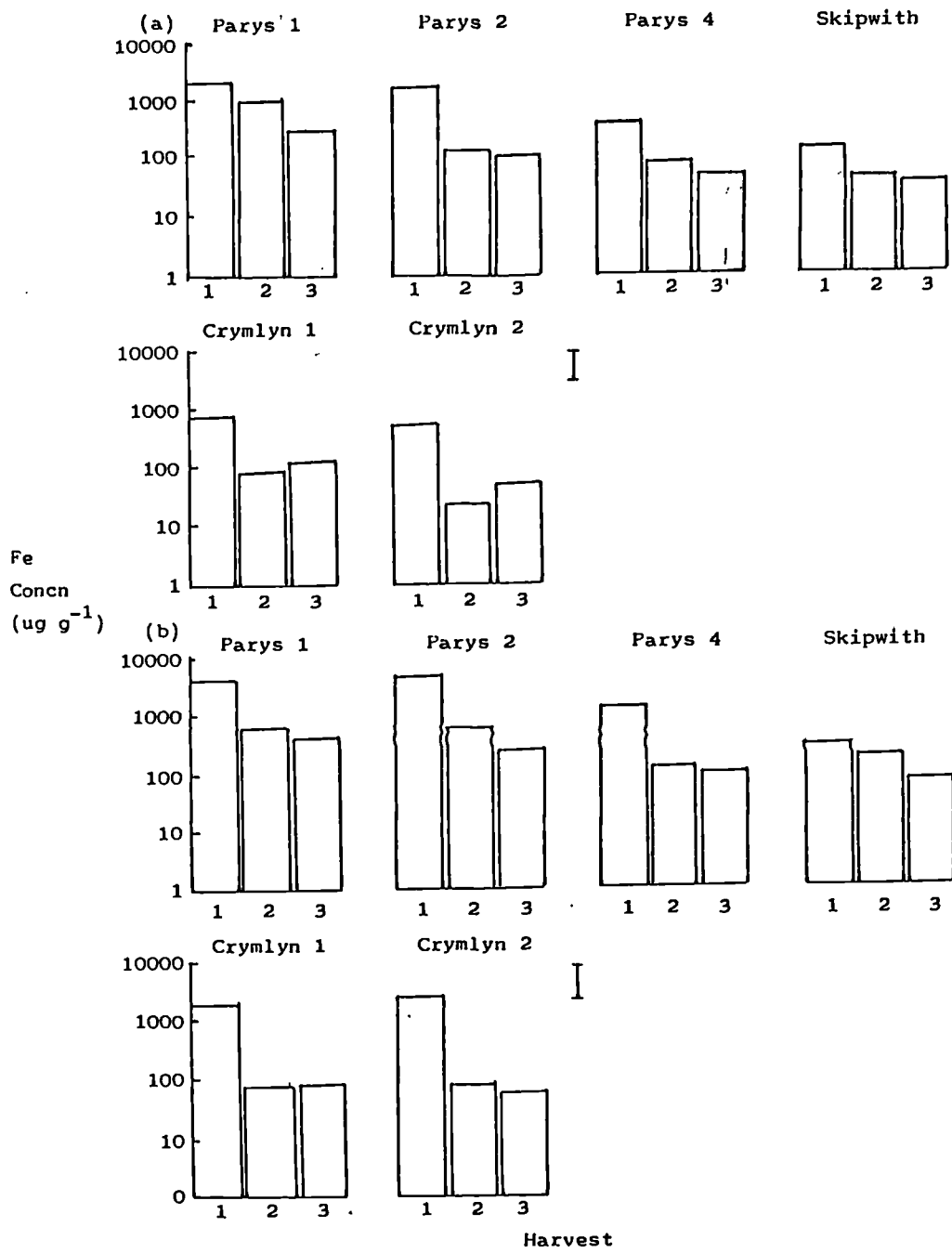


Figure 3.2

Iron concentrations (ug/g) of (a) culms and (b) culm bases of *Phragmites australis* sampled in April (1), June (2) and September (3) 1986 from Parys Mountain, Crymlyn Bog and Skipwith Common. (Vertical bars represent Least Significant Differences). ( $p < 0.05$ ).

Chapter 2, highest Fe concentrations were found in the root which closely reflected Fe in sediments.

The concentrations of Mn in the upper, middle and lower leaves did not differ from one another nor were there any clear seasonal trends or site differences (only upper leaf Mn concentrations are presented, see Table 3.7). The Mn concentrations in dead leaves appeared to increase in September, but this was only significant for Crymlyn subsite 2. The concentrations were approximately equal for all sites (Table 3.7b).

Fe concentrations of plant tissues were compared with Mn concentrations of the same plant part but no relationship was apparent. Fe and Mn concentrations of middle leaf tissue only are presented here (Table 3.8).

Cu concentrations of upper and middle leaves, and culm bases reached minimum values in July. There were no obvious trends for dead leaves (only the Cu concentrations for the upper leaves are presented here, shown in Figure 3.3). With the exception of Parys subsite 4 Cu content of the



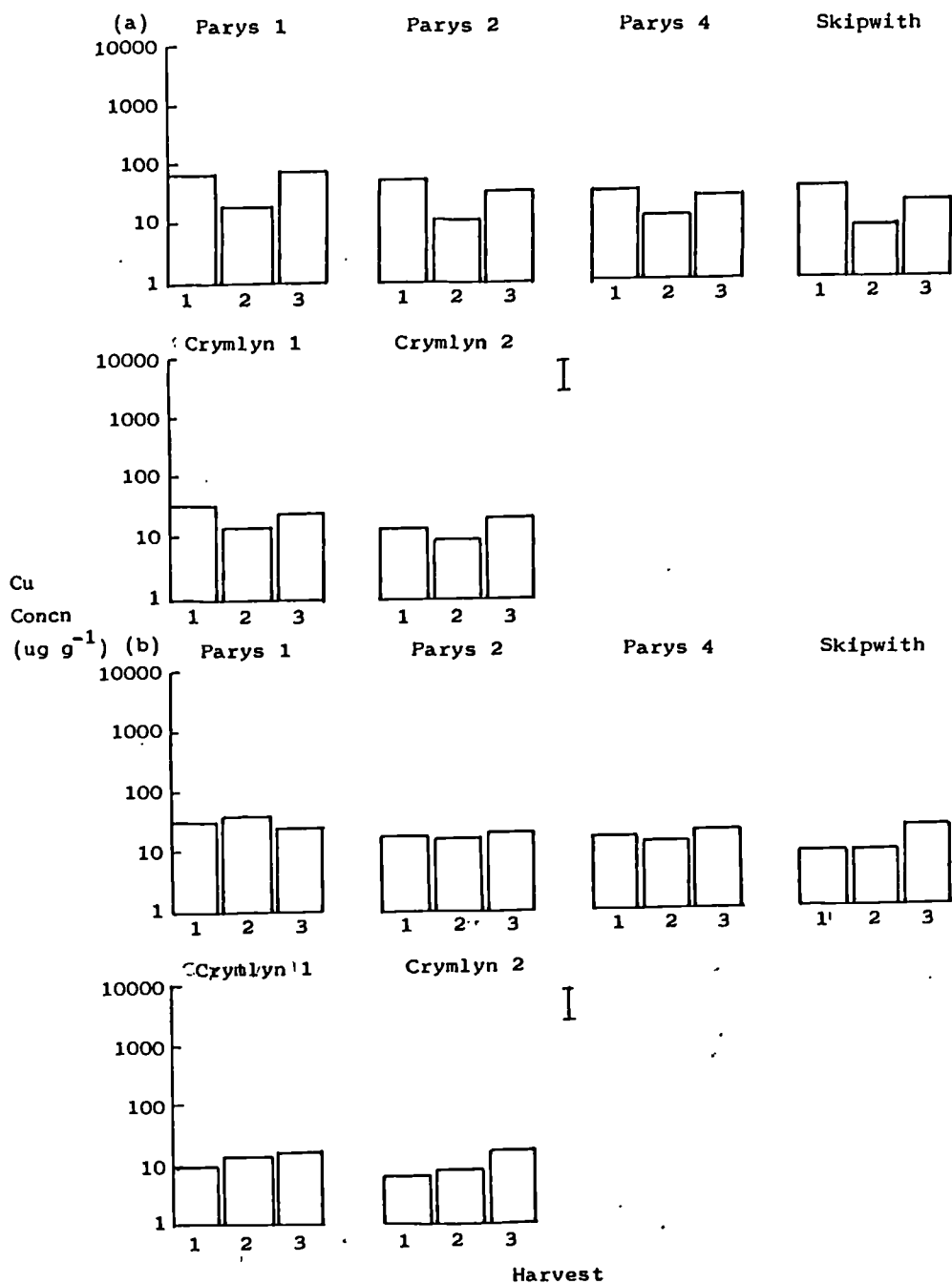


Figure 3.3

Copper concentrations ( $\mu\text{g/g}$ ) of (a) upper leaves and (b) dead leaves of *Phragmites australis* sampled in April (1), June (2) and September (3) 1986 from Parys Mountain, Crymlun Bog and Skipwith Common. (Vertical bars represent Least Significant Differences). ( $p < 0.05$ ).

Table 3.7 Manganese concentrations ( $\mu\text{g/g}$ ) of (a) upper leaves and (b) dead leaves of plants of *Phragmites australis* collected in April, July and September 1986 from Parys Mountain, Crymlun Bog and Skipwith Common. Values followed by the same letter are not significantly different ( $p < 0.05$ )

Site	Harvest		
	1	2	3
(a) upper leaves			
Parys 1	109 abc	93 abcd	99 abcd
Parys 2	75 bc	1 i	29 ghi
Parys 4	89 bcd	65 cdef	103 abcd
Skipwith	53 defg	22 hi	145 ab
Crymlun 1	123 abc	28 ghi	45 efg
Crymlun 2	78 bcd	37 fgh	180 a
(b) dead leaves			
Parys 1	216 a	94 bcd	63 cdef
Parys 2	98 bc	48 defg	21 h
Parys 4	126 ab	98 bc	27 g
Skipwith	67 bcdef	73 bcde	21 h
Crymlun 1	112 abc	34 f	19 h
Crymlun 2	91 bcd	41 efg	35 gf

Table 3.8 Comparison between the iron and manganese concentrations (ug/g) of middle leaves of plants of *Phragmites australis* collected in April, July and September 1986 from Parys Mountain, Crymlyn Bog and Skipwith Common. Values followed by the same letter are not significantly different (comparisons made within each element only).

Site	Harvest		
	April	July	August
<b>Fe</b>			
Parys 1	1763 a	1320 ab	1384 ab
Parys 2	3011 a	1195 ab	424 bc
Parys 3	529 bc	184 cd	263 cd
Skipwith	182 d	155 d	87 de
Crymlyn 1	939 bc	127 de	350 cd
Crymlyn 2	951 bc	57 e	368 cd
<b>Mn</b>			
Parys 1	95 bcd	82 bcd	70 de
Parys 2	38 ef	20 f	22 f
Parys 3	96 bcd	72 cde	72 cde
Skipwith	151 a	116 abcd	117 abcd
Crymlyn 1	142 abc	71 cde	70 de
Crymlyn 2	142 abc	122 abcd	233 a

rhizomes increased through the growing period (Figure 3.4).

Ca concentrations for the upper leaf (Figure 3.5a) increased slightly (with the exception of Crymlyn subsite 1) but was significant only at  $P < 0.1$ . The Ca concentration of dead leaves was lower than the concentration of Ca in live leaves (Figure 3.5b). No trend was apparent elsewhere. No differences in Ca concentration of the rhizome were found between sites. The remaining results showed no clear trends and have been placed in Appendix IV.

### 3.3.5 Analysis of Iron, Manganese, Copper and Calcium in tissues of Eriophorum angustifolium

There was a tendency for Fe concentrations in leaves to decrease with age but this was not consistent for all sites and harvests (only middle leaf Fe concentrations are presented, see Figure 3.6). No clear trends were apparent for Mn concentrations in tissues of these plants and these data are not presented. Fe concentrations in plant tissues were compared to Mn concentrations in the same organs, and a

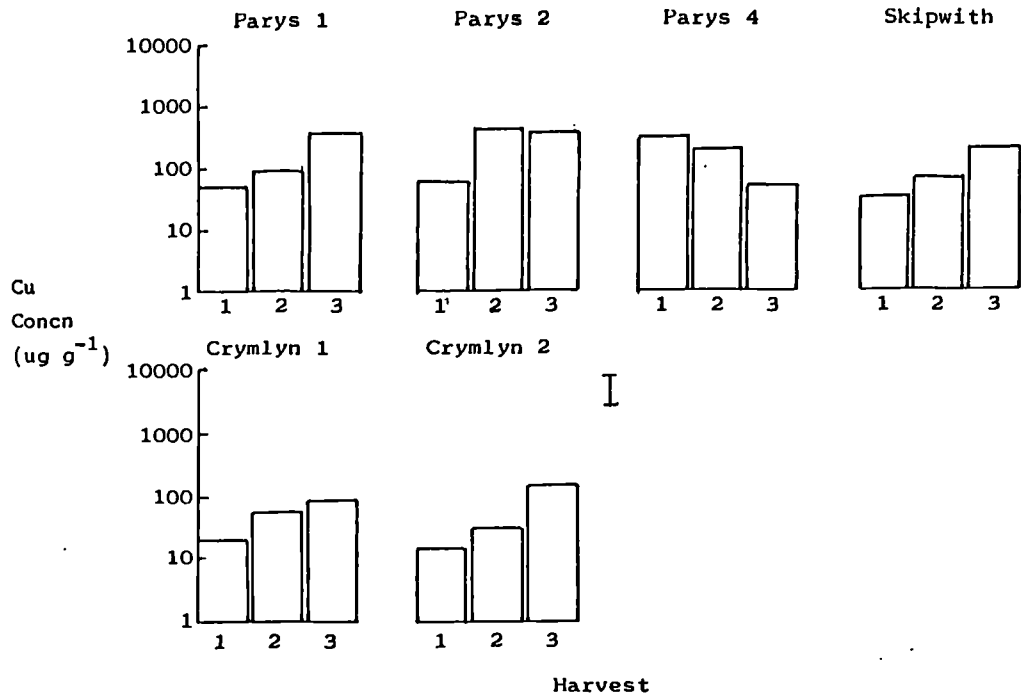


Figure 3.4

Copper concentrations (ug/g) of rhizomes of *Phragmites australis* sampled in April (1), June (2) and September (3) 1986 from Parys Mountain, Crymllyn Bog and Skipwith Common. (Vertical bars represent Least Significant Differences). ( $p < 0.05$ ).

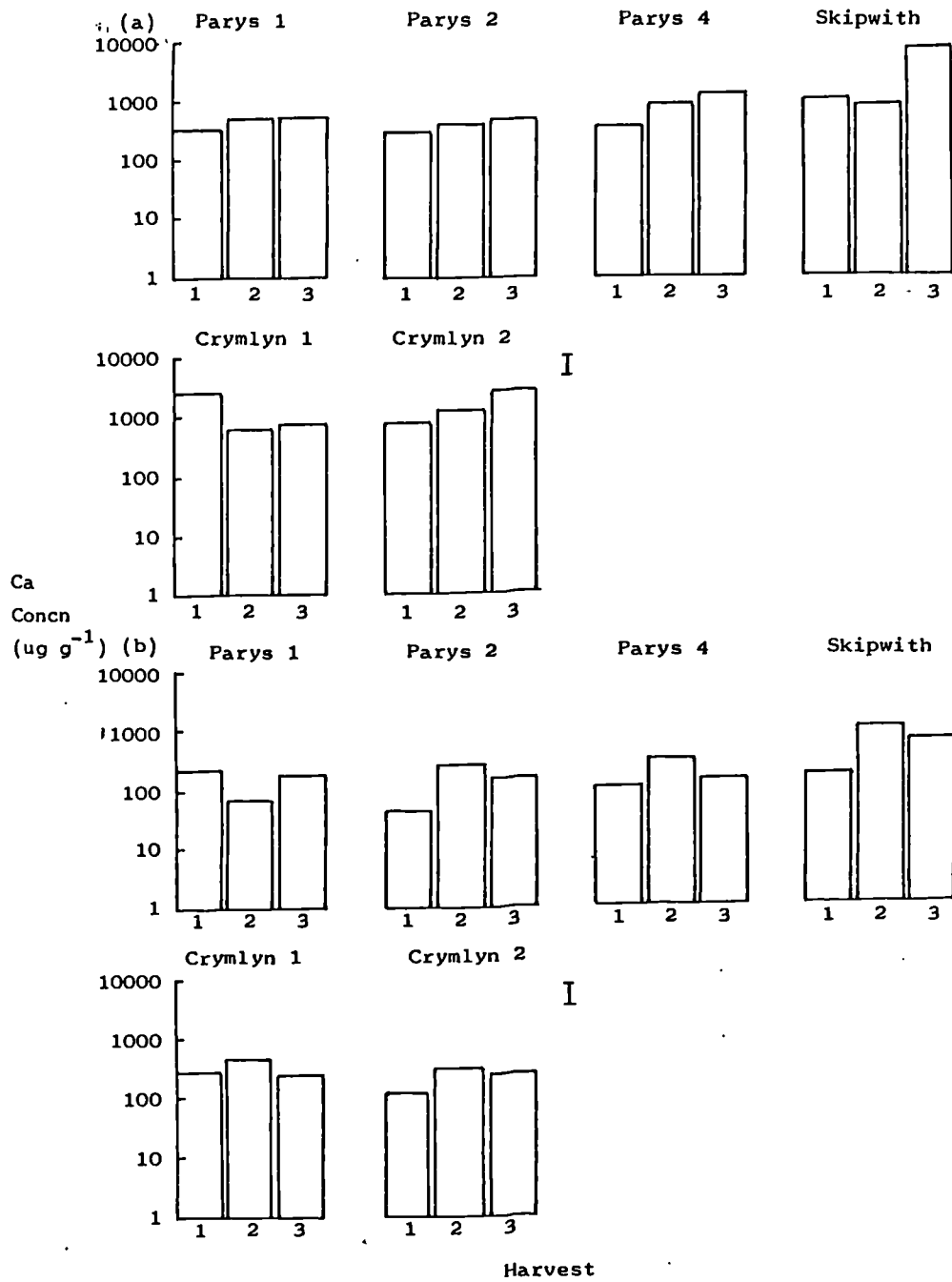


Figure 3.5

Calcium concentrations ( $\mu\text{g/g}$ ) of (a) upper leaves and (b) dead leaves of *Phragmites australis* sampled in April (1), June (2) and September (3) 1986 from Parys Mountain, Crymlyn Bog and Skipwith Common. (Vertical bars represent Least Significant differences). ( $p < 0.05$ ).

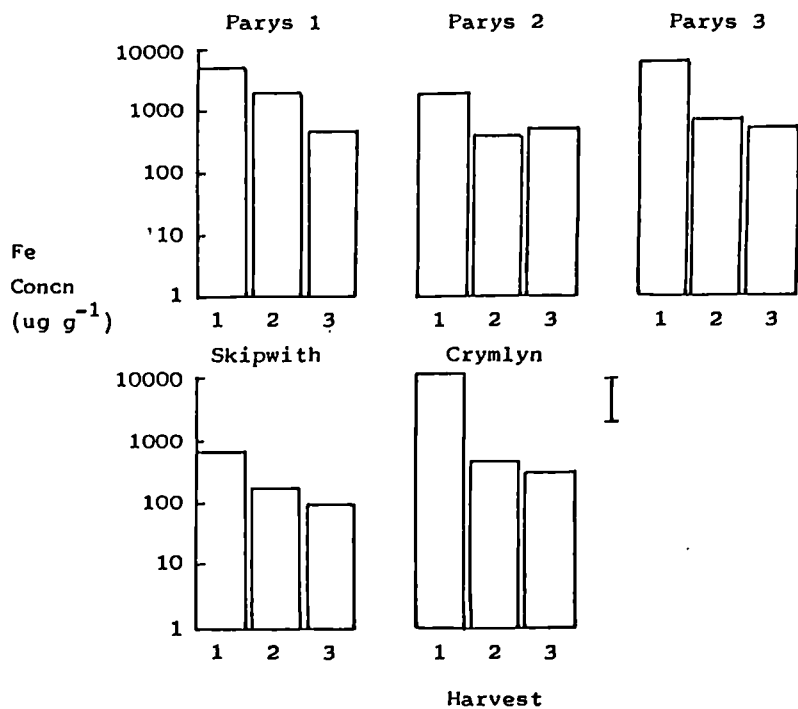


Figure 3.6

Iron concentrations ( $\mu\text{g/g}$ ) of (a) middle leaves of Eriophorum angustifolium sampled in April (1), June (2) and September (3) 1986 from Parys Mountain, Crymlyn Bog and Skipwith Common. (Vertical bars represent Least Significant Differences). ( $p < 0.05$ ).

negative relationship was found: high Fe concentrations in plant tissues corresponded with low Mn concentrations in the same plant part (Table 3.9).

Cu concentrations in living leaves (middle leaves only, Figure 3.7) were higher at Parys than at other sites. Cu concentrations of living leaves increased in the low Cu sites (Skipwith and Crymlyn). There was some evidence of a decline in Cu concentrations over the sampling period in living leaves of plants from Parys in the upper and middle leaf only (only the concentrations of Cu in the upper leaves are presented here). At the low Cu sites the low Cu concentration coincided with maximum growth (Harvest 2).

Ca concentrations in all plant parts were higher at Skipwith and Crymlyn than at Parys. There was a decline in the Ca concentration of leaves sampled in September (Fig. 3.8a) although this was significant only at  $p < 0.1$  (only lower leaf Ca concentrations are presented here). The Ca concentrations in dead leaves (Fig. 3.8b) were highest at the final harvest. As no clear trends were found in the



Table 3.9 Comparison between the iron and manganese concentrations (ug/g) of middle leaves of plants of collected in April, July and September 1986 from Parys Mountain, Crymlyn Bog and Skipwith Common. Values followed by the same letter are not significantly different (comparisons made within one element only)

	Harvest		
	April	July	August
Fe			
Parys 1	1438 bc	1781 bc	626 cdef
Parys 2	1139 bcd	383 bc	1270 bc
Parys 3	40417 a	1687 bc	2128 b
Skipwith	303 ef	274 f	76 g
Crymlyn 1	2705 b	905 bcde	237 f
Mn			
Parys 1	263 cd	112 efg	85 fg
Parys 2	137 def	36 h	38 h
Parys 3	1119 a	145 de	66 gh
Skipwith	403 bc	162 def	205 cde
Crymlyn	830 ab	173 def	255 cd

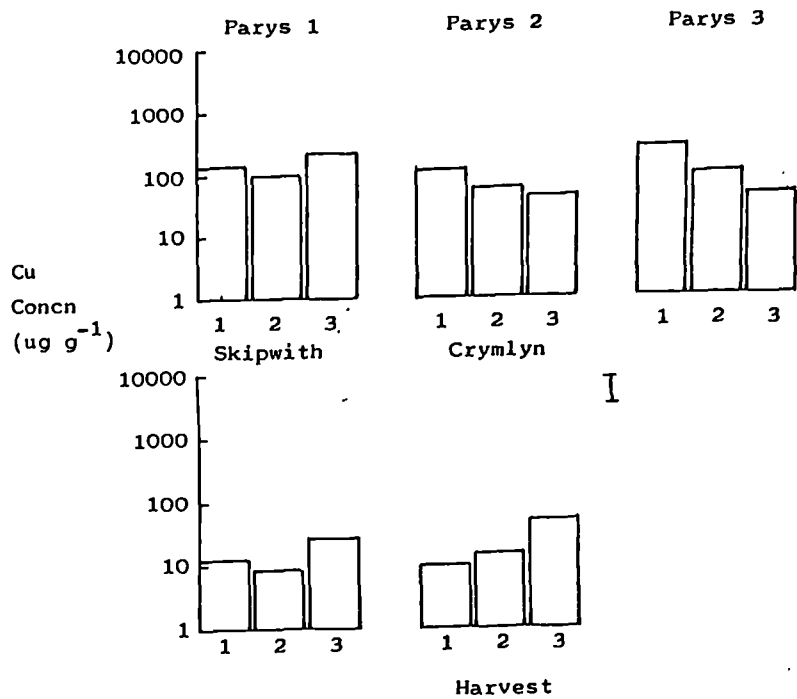


Figure 3.7

Copper concentrations ( $\mu\text{g/g}$ ) of (a) upper leaves of Eriophorum angustifolium sampled in April (1), June (2) and September (3) 1986 from Parys Mountain, Crymllyn Bog and Skipwith Common. (Vertical bars represent Least Significant differences). ( $p < 0.05$ ).

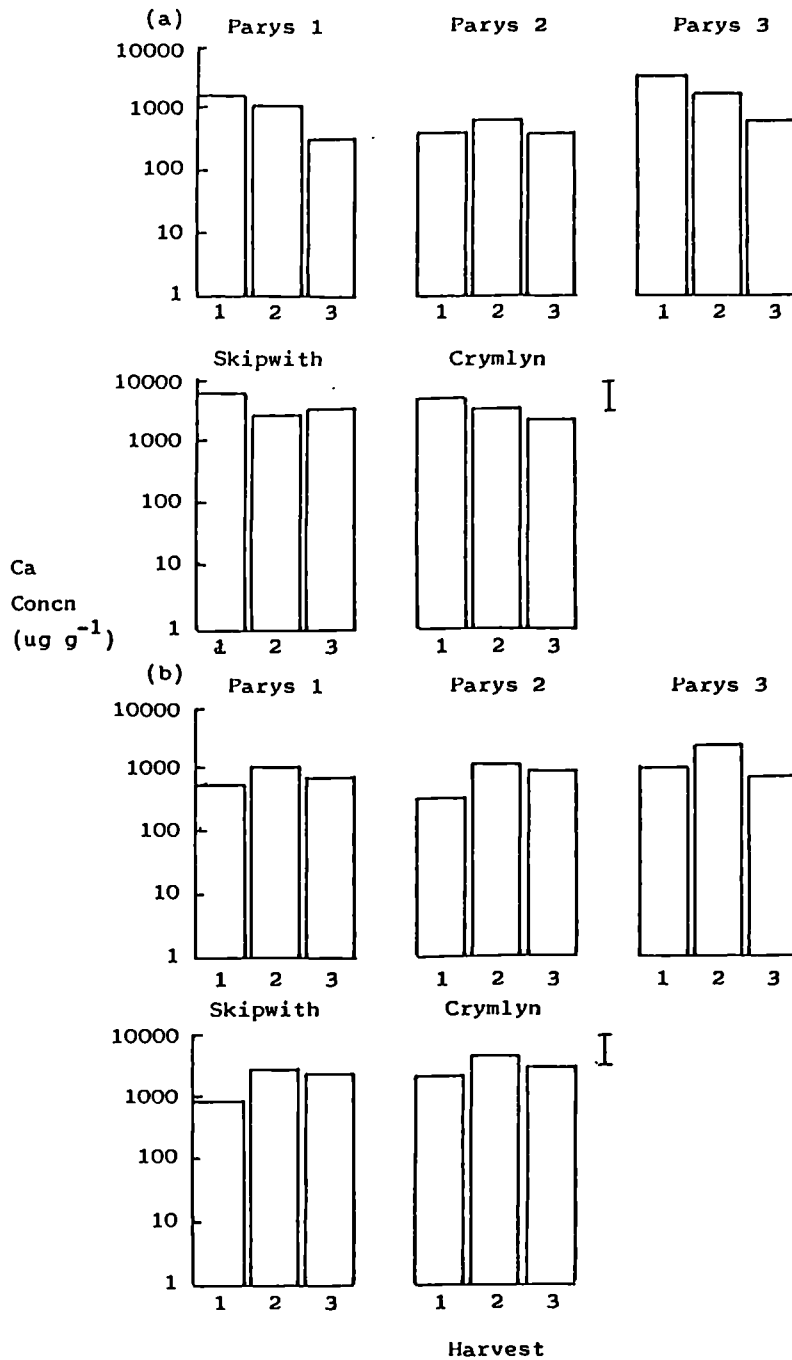


Figure 3.8

Calcium concentrations ( $\mu\text{g/g}$ ) of (a) lower leaves and (b) dead leaves of *Phragmites australis* sampled in April (1), June (2) and September (3) 1986 from Parys Mountain, Crymlyn Bog and Skipwith Common. (Vertical bars represent Least significant differences). ( $p < 0.05$ ).

remaining results, these have been placed in the appendix.

### 3.4 Discussion

#### 3.4.1 Soil/Sediments

##### 3.4.1.1 Oxidation-reduction potentials and pH

At low pH and low redox conditions there was an increase in the concentrations of soluble Fe in Estuarine sediments studied by Delaune et al. (1981). Soluble Fe concentrations were over 100 ug/ml at -200 mV and pH 5.0. There was a drastic reduction in the amount of Fe in solution as the redox potential was changed from 0 to 250 mV. Contrary to that observed by Delaune et al. (1981), availability of Fe was not low at redox values between 0 and 250 mV in the present work. This may be explained by the differences in pH of the sediments studied by Delaune et al. (1981) and the present work, the former maintained pH at 5.0, 6.5 and 8.0; whereas pH's lower than 5.0 were studied in the present work. pH has a marked effect on the solubility of Fe (Ponnamperuma et al. 1969). Soluble Fe increases

with decreasing redox potential and pH (Delaune et al. 1981). At low pH the effect of acidity may be greater than the effect of redox potential on Fe in solution.

Increases in the level of oxidation through the season at Parys subsite 4 may perhaps be attributed to the oxidizing capacity of well-established roots of Phragmites. Root oxidizing activity has been acknowledged and investigated by several workers and has been observed in many species (eg Menyanthes trifoliata L., Molinia caerulea (L.) Moench, Eriophorum (Armstrong 1967), Oryza sativa L. (Tadano 1975, Trolldenier 1988), T. latifolia and Phragmites (Armstrong & Armstrong 1988). Such root oxidizing power may be sufficient to increase the level of oxidation of the sediment if roots were numerous. The ability to oxidize the surrounding sediment by roots has been shown for several aquatic macrophytes. Wium-Anderson & Anderson (1972) considered that redox potential was elevated in sediments in the presence of roots of Lobelia dortmanna L.. Teal & Kanwisher (1966) and Hayes, Havarth, Teal & Valiela (1981)

have shown that Spartina alternifolia Loisel. oxidized the sediment around the root zone. Hansen & Anderson (1981) found that the redox potential was highest in the Phragmites swamp sediment where large roots and rhizomes were present. At Parys subsite 4, Phragmites was well established and the root system was extensive. (In fact, difficulties arose in obtaining sediment samples without obtaining large quantities of root material). In July, a vertical cross-section through the substrate indicated a reduced darker surface with a lighter, more oxidized, subsurface. This was supported by the redox potentials. This appeared to confirm the relatively high levels of oxidation of the rooting medium by a well established stand of Phragmites and the evidence of oxygen transport from aerial parts. However, oxidation by roots and rhizomes is often very local and the majority of the sediment remains anaerobic unless there is a very high density of plant roots; as seen at Parys subsite 4.

In addition to the oxidizing capacity of the roots it is

suggested that the difference between the redox potentials at these sites was caused by the extent to which the sites were waterlogged. Direct observations at Parys showed a loss of surface water in mid-season (July), this correlated with an increase in measured redox potential of the surface sediments for the period, reflecting oxidizing conditions. More reduced conditions appeared to correlate with more waterlogged conditions observed in April and September.

The stand of Phragmites at Skipwith Common was also well-established but here redox potentials indicated more reducing conditions from April to September. This site remained waterlogged throughout the season and the soil-sediment depth was greater. The majority of roots of Phragmites grew to depths exceeding those at which redox was measured.

Crymlyn Bog also remained waterlogged throughout the year and conditions were found to be more reduced relative to the Parys site.



#### 3.4.1.2 Soil total and extractable metal concentrations

Davy & Taylor (1975) found little evidence of distinct seasonal trends in total element availability. The apparent variation observed in the present work was probably largely spatial rather than seasonal in origin (Frankland, Ovington & Macrae 1963, Ball & Williams 1968). The concentration of any metal reflected the origin (locality) and geochemical nature of the site.

In most sites there was evidence of seasonal change in extractable Fe concentration. Davy & Taylor (1975) also found that exchangeable Fe concentrations in acid mull showed striking seasonal patterns. Low winter values gave way to a pronounced peak in April which declined throughout the summer and autumn. The later peak of exchangeable Fe observed here may be due to differences in the nature of acid mull soils and the soil/sediments in this study. The peak in exchangeable Fe appeared to coincide with maximum growth of plants. Jones (1967), also noted seasonal fluctuations in exchangeable Fe and Mn in wet sand dune slacks. High values

were found during late spring/early summer. These high concentrations coincided with the period of maximum growth in Agrostis stolonifera. The rate at which waterlogged soils warm after winter chilling is also likely to affect exchangeable Fe concentrations. Increases in temperature will enhance the activity of chemo-autotrophic bacteria, such as Thiobacillus ferrooxidans, responsible for mediating the conversion of Fe (III) to Fe (II) (Van Breeman 1972, Vear and Curtis 1981), and thus affecting its availability to plants.

#### 3.4.2 Seasonality of plant metal concentrations

There were few general seasonal trends in metal concentrations in plant tissues. There was a decline in the concentration of Fe in culms of Phragmites coinciding with peak growth rates and an increase in exchangeable Fe in the soil/sediments. The 'negative' value in the rate of accumulation of Fe is probably caused by the intensive rise in plant biomass which 'diluted' the element, rather

than reaction of plants to increases in exchangeable Fe. However, the 'dilution' of Fe in culms caused by increasing biomass may serve as a 'cross-resistance mechanism' effectively maintaining lower Fe concentrations in plant tissues. The absence of seasonal variation in roots and rhizomes, and the decline in Fe concentration in culms accords with the results of Ho (1981) for the same species in uncontaminated Scottish lochs. In general, the concentration of Fe in the roots and dead leaves of the same plants was in agreement with the results of the preliminary study.

In Eriophorum Fe concentration of leaves increased with leaf age. Chapin et al. (1975) also found an increase in Fe concentration in this species as the season progressed. Mn differed from all elements examined by Chapin et al. (1975) in that its concentration showed virtually no change, in contrast to the observations made from the present study (where there were changes in Mn concentration despite the absence of measureable differences in the Mn concentration of sediments although no clear trend was

apparent.

Antagonism between Fe and Mn uptake was apparent in Eriophorum. Plants from 'Fe-rich' sites (Parys and Crymlyn) had lower leaf Mn contents (relative to Fe) than plants from sites with lower Fe. The importance of the ratio of Fe to Mn in plant tissue has been highlighted by several workers including Tanaka & Navasero (1966c). These workers reported a number of interactions between Fe and Mn particularly in the root of rice plants. It was found that an increase of Fe or Mn in the growth media caused a decrease in Mn or Fe content of the plant respectively. An increase in Mn concentration in solution caused a decrease in Fe content of young leaves in agreement with the results of this study. Beauchamp & Rossi (1972) found that this interaction was only significant when the Mn supply was above the optimum concentration for growth in barley plants. Mn concentrations at both mine sites (Parys and Crymlyn) were likely to have been in this category (in excess to plant

requirements).

Fe-Mn antagonism was not detected in Phragmites. This may be due to the lack of seasonal trend in Fe content.

In Eriophorum, but not Phragmites, Cu concentrations in plant tissues reflected the availability of Cu in the soil/sediments. In contrast, Larsen & Schierup (1981) found that uptake of Cu (Zn, Pb and Cd) by Phragmites was greater in an oligotrophic, non-polluted lake than in an eutrophic, sewage and heavy metal polluted lake. The greater part of the heavy metals taken up were retained in the roots and rhizomes and only small amounts were translocated to the above-ground parts. Cu concentrations of upper and middle leaf sections and culm bases reached minimum values in July, coinciding with maximum growth rates, while Pb concentrations increased indicating a gradual buildup over the season. Larsen and Schierup (1981), similarly reported a dramatic decline in Cu in stems, and Zn in stems and leaves, of the same species from July (1977) to April (1978) in a polluted lake and an unpolluted lake. Kufel (1978) found that

concentrations of Cu, Pb, Co and Mo in Phragmites changed considerably during the growing season. These seasonal changes in concentration could be related to differential uptake of heavy metals by the plants. Higher Pb and Cu concentrations in Phragmites occurred at the end of May and the beginning of June. This period corresponded with the initial phase of Phragmites growth. For the remainder of the growth period Pb and Cu were accumulated at slower rates; apparent negative values of the rate of accumulation were probably due to the corresponding rise in plant biomass which diluted the element, the results for this study support this hypothesis. Although Cu (and Zn) are generally considered to be immobile in plants, Larsen and Schierup (1981) considered Cu (and Zn) to be mobile, to a certain extent. Accepting this, the decrease in the concentration of Cu (and Zn) after the growing season could be explained as a result of either translocation of these elements to the rhizome for storage or of leaching from the standing litter. The results of the present study support the first suggestion

as there was a rise in Cu content of the rhizome throughout the growing season. In Eriophorum there was a decline in Cu concentrations in leaves corresponding to maximum growth rates.

Ca concentrations in living leaves of both species reflected total concentrations in the sediments. Allen & Pearsall (1963) found more Ca (N and K) in Phragmites growing in more eutrophic conditions. Higher Ca concentrations in dead leaves of Eriophorum may be attributed to the greater immobility of the element (Chapin et al. 1975). In Phragmites the lack of difference between the Ca content of living and dead leaves suggested either the absence of remobilization of elements from senescing leaves or the replacement by elements as Mn or Pb (see Chapter 2). The general lack of seasonal changes in Ca content in Phragmites is not in agreement with the findings of Bollard & Butler (1966), Bayley & O' Neill (1972) and Dykyjova (1979). These authors found increasing levels of Ca in the shoot material reflecting continued uptake and

deposition of this element during the growing season.

The principal difficulty in analysing leaf material to investigate site effects is that it is such a metabolically active tissue. Which varies in chemical composition and with aging (Allen & Pearsall 1963). Some of the differences between the present study and previous work may relate to sampling strategies used. In the present study the sampling aimed to take material of a uniform physiological age rather than following the progress of an individual leaf or cohort of leaves. Consequently, changes in element concentration related to physiological age would be less likely to be detected.

The general lack of large changes in metal concentration in the plant material probably reflects the lack of seasonal variation in soil metal status. Why the results of the present study should differ from some other studies is not clear, but may be due to the nature of the environment in which the study took place, being generally much more metal-rich than the sites studied by other workers. Cu



concentrations in tissues of Phragmites in this study, often exceeded 20 ug/g, whereas Larsen & Schierup (1981), found a maximum of 3 ug/g Cu in stems and leaves of Phragmites in a sewage polluted lake. Such high tissue concentrations may 'mask' internal fluctuations in metal content.

### 3.5 Conclusion

There was an increase in exchangeable Fe which correlated with a peak in plant growth rates, presumably related to increasing temperature and microbial activity. No marked seasonal patterns for Mn concentrations were observed in soils/sediments across the sites sampled. The differences between sites were probably largely spatial rather than seasonal in origin.

Tissue concentrations of any metal in Phragmites reflected the origin (locality) and nature of the site. This was true for Fe at all sites. Decrease in Fe content of culms coincided with peak growth rates and an increase in

exchangeable soil Fe. Apparent negative values in the rate of accumulation were probably due to the corresponding rise in plant biomass which 'diluted' the concentration of the element in plant tissues.

Cu concentrations in upper, middle leaves and stems of Phragmites reached minimum values in July, also coinciding with peak growth rates. Rhizome concentrations reflected extractable Cu in the soil/sediment. Ca concentrations in live and dead leaves suggested that remobilization of elements from senescing tissue was inhibited or there was exchange for other elements such as Mn which was found to increase over the sampling period. The increase in Ca in rhizomes corresponded to peak growth rates in mid-season (July).

In Eriophorum, antagonism between Fe and Mn uptake was apparent. Plants from 'Fe-rich' sites showed the lowest Mn concentrations in the leaves. Cu concentrations in tissues of this species reflected extractable Cu in the soil/sediment. There was evidence of a decline in Cu

concentrations in the leaves of plants sampled from 'Cu-rich' sites and an increase in Cu concentrations in leaves from 'Cu-poor' sites through the season, suggesting a degree of internal regulation. Ca concentrations in plant tissues showed no consistent trends over the growing season.

This investigation has highlighted several points which warrant further study:

(i) there is a need to evaluate the physiological and biochemical effects of toxic metal concentrations

(ii) the dynamics and rates of Fe uptake over a range of concentrations (using field concentrations as a guide), the mobilization of Fe within the plant, as well as the effects of external concentrations of the other elements, also require clarification.

(iii) possible population effects should be identified under controlled conditions. It was not possible to identify population differences, in terms of the effects of Fe, Mn, Cu and Ca under field conditions.

## CHAPTER 4

### I. THE EFFECT OF INCREASING SUPPLY OF SELECTED HEAVY METALS ON UPTAKE, CONCENTRATION IN TISSUES AND GROWTH OF ERIOPHORUM ANGUSTIFOLIUM, AND PHRAGMITES AUSTRALIS.

#### 4.1 Introduction

Toxicity effects caused by high concentrations of Fe to some plant species is well known, if not well understood (Foy et al. 1978, Woolhouse 1983, Verkleij & Schat 1988). The erratic performance of wetland rice varieties in the tropics has been identified in part as the effects of Fe toxicity and this is well documented in the literature (Ponnamperuma 1955, Tanaka, Loe & Navasero 1966, Tadano 1975 and Van Breemen & Moormann 1978). It has been suggested that one mechanism of tolerance of plants to waterlogged, Fe-rich conditions is associated with oxidation and immobilization of soluble Fe by roots (Green & Etherington 1977, Armstrong 1979, Talbot & Etherington 1987, Heathcote, Davies & Etherington 1987), a

capacity restricted or lacking in species intolerant to waterlogging. Little is known of the possibility of variation in tolerance to high Fe concentrations in waterlogged soils of typical wetland plants, though it is understood that some rice cultivars display a degree of differential toxicity as reported by Nagai and Matono (1959). More recently Wheeler, Al-Farraji & Cook (1985) gave evidence for considerable difference in the tolerance of Epilobium hirsutum L. and Juncus subnodulosus Schrank. to high Fe concentrations.

On the whole, the literature on Fe toxicity is sparse and without general direction. Many examples are basic correlations of high leaf Fe content with visible toxicity symptoms (mostly in rice) often confounded with the effects of other potential toxins such as Mn and sulphide (Foy et al. 1978, Woolhouse 1983). In contrast, Mn toxicity is better understood. Increased Mn availability may occur in various metalliferous sites, particularly in acidic and waterlogged conditions. It is not surprising, therefore,

that increased Mn resistance is observed among wetland plants (Verkleij & Schat 1988). Mn resistance is often attributed to decreased Mn uptake (Robson & Loneragan 1970, Horiguchi 1987). This may be effected by the increased oxidizing capacity of the root system (which is often characteristic of wetland plants; Engler & Patick 1975). Plants also vary in their capacity to tolerate increased Mn concentration in their shoots (Foy et al. 1978), and tolerance mechanisms may therefore have a role in Mn resistance. However, the actual mechanism/s remain unclear.

Cu tolerance in higher plants is well documented (McNeilly 1968, Gartside 1973, Karataglis 1980a,b). For crop species the critical level of Cu in the leaves is considered to be well above 20 to 30 ug/g dry weight (Robson & Reuter 1981). There are, however, marked differences in Cu resistance among plant species and these differences are attributed to the Cu content of the shoots (Bachthaler & Stritesky 1973). Excess Cu supply inhibits root growth before shoot growth (Karataglis 1980d, Lexmond & Vorm 1981).

Nonetheless, roots are not necessarily more sensitive to high Cu concentrations, but rather they are the sites for preferential Cu accumulation when the external supply is large (Marschner 1986). In plants receiving an enhanced Cu supply, the Cu content of the roots is reported to rise proportionally to reflect the external supply, whereas transport to the shoot remains greatly restricted. Therefore, without an analysis of the roots, critical levels in the shoots are not necessarily a direct indication of Cu resistance. Non-tolerant plants show inhibition of root elongation and damage to root cell membranes; an immediate response to an increased Cu supply (Wainwright & Woolhouse 1977). Cu toxicity has also been well studied and is discussed by Foy et al. (1978). Cu toxicity in plants is generally manifested as chlorosis and stunting of growth. Crop stunting due to Cu-excess can arise from a combination of factors. These include specific effects of Cu on the plant, antagonism with other nutrients, or reduced root growth and penetration into the soil (Foy et al. 1978). In

the case of Cu, toxicity is initially experienced in the root tips (manifested as blackening) followed by a subsequent inhibition of the lateral roots. Such a restriction of root elongation would potentially lead to macronutrient depletion in the limited rooting zone and lead to a reduction in growth (Lepp 1981).

The importance of phosphorus (P) interactions in the ability of plant species to tolerate high concentrations of toxic metals has been discussed by several workers including Baker (1978a), Alva et al. (1986). DeKock, Hall & Inkson (1979) reported that the total amount of Fe in leaf tissue may not be an adequate indication of the Fe status of the plant. They found that the ratio of total P to total Fe was a more accurate indicator of Fe status. Howeler (1973), studied an orange disease of rice associated with flooded soils. The symptoms were attributed to the indirect effect of Fe toxicity causing an Fe-induced deficiency of mainly P, K, Ca and Mg. Hence any interference with nutrient uptake would probably reduce the plants capacity to grow successfully



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under conditions of high metal-status .

The work reported in this chapter was designed to further investigate observations on soils and tissue concentrations of metals in plants from wetland sites (which have been described in the last two chapters). The experiments were designed to relate the response of the plants in culture solution to those observed in field material and also to identify causal factors which collectively equip Phragmites and Eriophorum to resist high concentrations of heavy metals.

The aims can be summarized as follows:

(i) to investigate the nature of the response of Phragmites and Eriophorum to increased external concentrations of Fe and to identify possible mechanisms of resistance

(ii) to investigate the nature of the response of Eriophorum to increased external supply of Mn and Cu and to identify where possible mechanisms of resistance

(iii) to collectively consider these responses and relate them to measurements on field material.

## 4.2 Materials and methods

### 4.2.1 Response to increased iron

#### 4.2.1.1 Choice of sites and species

The sites were Parys Mountain, Anglesey and Skipwith Common, Yorks. The details of the sites and species, and the reasons for their selection are given in Chapter 2.

#### 4.2.1.2 Treatments

Both species and populations were subjected to four concentrations of Fe (1, 10, 100, 1000 mg/l) supplied as ferrous iron sulphate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ).

#### 4.2.1.3 Culture Systems

The plants were grown in 0.1-strength Horison nutrient solution with additional Fe (Appendix I and II) at pH 4.0 (adjusted by addition of 1M HCl or NaOH) in 8 l coloured

plastic bowls (Eriophorum angustifolium) or 16 l plastic bins (Phragmites australis) (Stewart Plastics Ltd). The plants were supported on 'styrofoam' floats with plugs of non-wetting cotton wool. Algal growth was reduced by ensuring that the growth vessels were opaque. This was achieved by using dark coloured plastic bowls only. The solutions were not aerated.

#### 4.2.1.4 Growth conditions

All plants were grown in a controlled environment room, with a 16 hour day and a 8 hour night and a constant air temperature of 25° C. Relative humidity was approximately 60%. Photon flux density was 88.6  $\mu\text{mol. m}^{-2} \text{ s}^{-1}$

#### 4.2.1.5 Experimental design

The populations were grown together in tubs arranged randomly on the controlled environment room bench and placed centrally under a light bank.

##### (i) Eriophorum

Populations (2) x treatments (4) x replicates (8) x  
harvests (1) x tubs (8). Total number of plants (120)

(ii) Phragmites

Populations (2) x treatments (4) x replicates (8) x  
harvests (1) x tubs (8). Total number of plants (120)

4.2.1.6 Procedure

Seeds of Eriophorum were germinated as described in Appendix II and grown for a further 12 weeks in 0.1-strength Rorison solution at pH 4.0 (Appendix I) before being used in the experiment. The Phragmites plants were derived from stock clones (see Appendix II). Each consisted of root, rhizome and shoot. These were grown for two weeks in 0.1 strength Rorison solution before the start of the experiment.

Culture solutions were changed every three days. After three weeks both species were harvested. The plants were divided into root and shoot (Eriophorum) and root, rhizome and shoot (Phragmites). In addition, final root length

measurements were made for Eriophorum. The plant sections were dried in at 50° C for 48 hours, weighed and digested as described in Appendix III. The samples were then analyzed for Fe, Ca by Flame- AAS and P by colorimetry (see Appendix III).

P and Ca concentrations in plant tissues were analyzed to determine the effect of Fe toxicity on P and Ca nutrition.

#### 4.2.2 Response of Eriophorum angustifolium to increased Manganese and Copper supply

##### 4.2.2.1 Treatments

Both populations of Eriophorum (Parys and Skipwith) were subjected to the following concentrations of Mn and Cu:

##### (i) Cu

0.1 (normal concentration 0.1-strength Rorison solution), 0.25, 0.5, 1.0 mg/l supplied as copper nitrate ( $\text{Cu}(\text{NO}_3)_2$ )

##### (ii) Mn

0.5 (normal concentration of 0.1-strength Rorison solution), 5, 25, 50 mg/l supplied as manganese sulphate ( $\text{MnSO}_4$ .)

#### 4.2.2.2 Culture system

The culture system was identical to that in the previous system for Eriophorum but 0.1-strength Rorison solution was replaced with full strength Rorison solution as a precautionary measure, as nutrient deficiency was suspected in plants used for the first experiment (leaves were pale green instead of dark green and there were signs of yellowing in the lower leaves).

#### 4.2.2.3 Growth conditions

See Section 4.2.1.4

#### 4.2.2.4 Experimental design

Populations of Eriophorum were grown together in tubs arranged randomly:

(i) Eriophorum

Populations (2) x treatments (4) x replicates (5) x

harvests (1) x tubs (8). Total number of plants (80).

#### 4.2.2.5 Procedure

Seedling material was again used (see Appendix II). At the start of the experiment the plants were 15 weeks old. Culture solutions were not aerated but were changed every 3 days. The plants were harvested after three weeks. To remove excess Mn and Cu from the root surface the plants were washed thoroughly in distilled water and blotted dry. Plants were then divided into root and shoot and dried in an oven at 50° C for 48 hours. All the material was digested as described in Appendix III. The samples were then analyzed for Mn or Cu and Ca by Flame-AAS.

#### 4.2.3 Seed weight determination

Seeds of Eriophorum were collected in June and July 1988 and cleaned by the method detailed in Appendix II. 100 seed weights were obtained for each population on pre-weighed



foil boats.

#### 4.2.4 Data presentation

Element concentrations of plant parts (shoot, root) are expressed on a ug per g dry weight basis. Total element concentration was calculated using the following formula:

$$\text{Total element concentration} = \frac{(\text{shoot dry weight (g). concentration (ug/g)} + \text{root dry weight (g). concentration (ug/g)})}{(\text{weight of shoot} + \text{weight of root (g)})}$$

In addition, Shoot/root concentration ratios were also calculated.

#### 4.2.5 Data analysis

The data were transformed using the GENSTAT statistical package (Alvey et al., 1982). Full analyses of variance (completely randomized design) were executed for all data. For ease of interpretation the values on the y-axis

have been back-transformed. Unless otherwise stated  
significance was tested at the 5% level ( $p < 0.05$ )

## 4.3 Results

### 4.3.1 Response of Phragmites australis to increased iron supply

#### 4.3.1.1 Yield

In the absence of high Fe concentrations, Phragmites plants from Skipwith achieved greater biomass than plants from Parys, but showed the greatest depression of growth at higher Fe concentrations (Figure 4.1a). The Parys population had lower initial yields but declined to half the biomass of the controls at 100 mg/l Fe and did not decline significantly thereafter. Thus yields of culm, leaf and total plant biomass were found to decrease significantly beyond the 10 mg/l Fe treatment.

Both leaf and culm biomass were found to decline significantly with increased Fe concentration (Figure 4.1b and c). There was also a significant difference in yield between the populations studied. Plants from Skipwith had

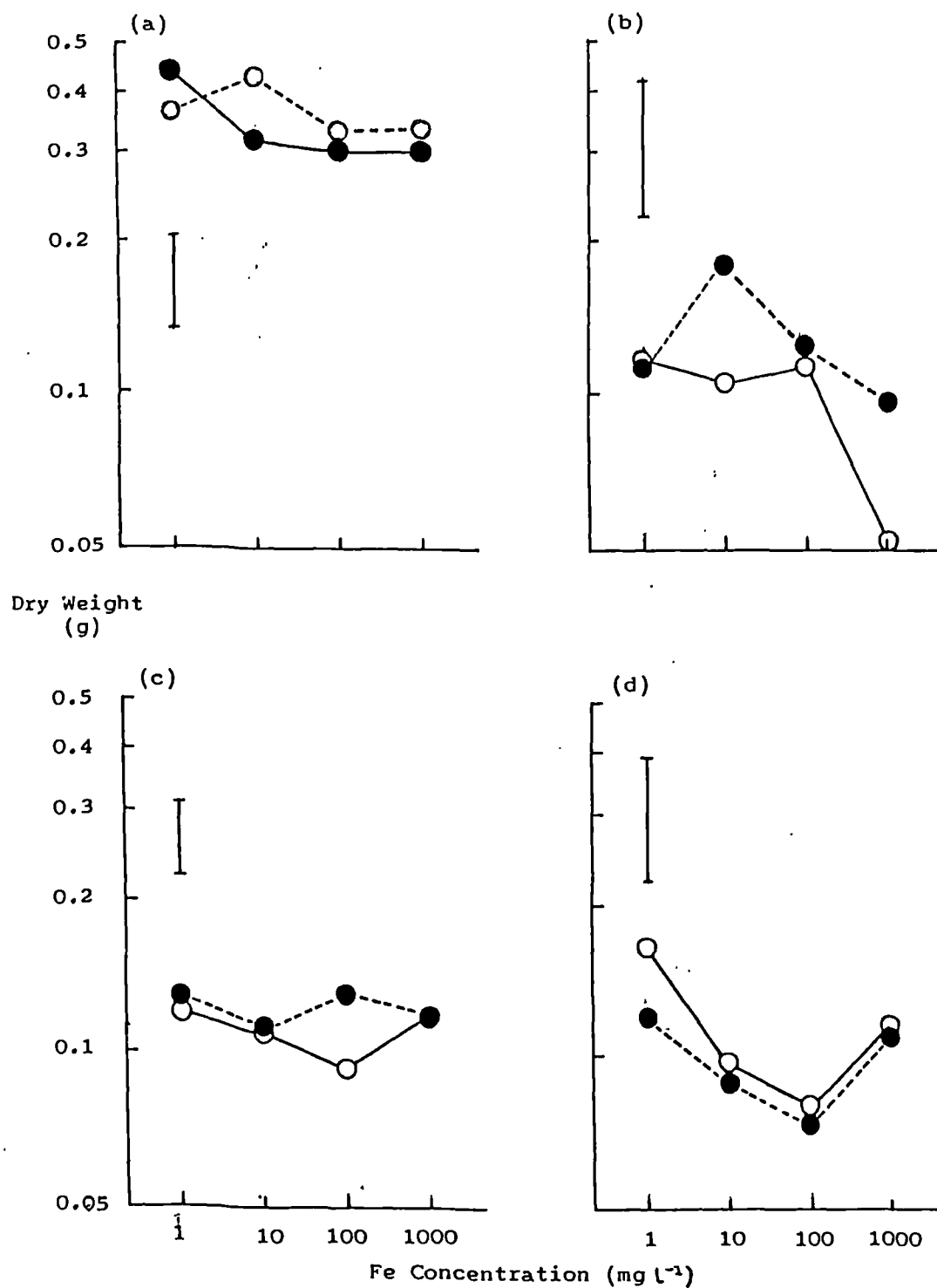


Figure 4.1

Dry weights (g) of (a) whole plant, (b) leaf, (c) culm and (d) root of *Phragmites australis* for plants from Parys Mountain (O) and Skipwith Common (●) exposed to range of concentrations of iron (Fe). (Vertical bars represent Least Significant Differences.). ( $p < 0.05$ ).

greater leaf and culm biomass and were higher yielding than plants from the Parys populations.

Plants from Skipwith had greater root biomass but the response of root biomass of both populations of Phragmites was the same to increased Fe concentration (Figure 4.1d). Fe concentration did not have a significant effect on the root yield of the plants until the highest treatment (1000 mg/l).

#### 4.3.1.2 Iron concentration of tissues of Phragmites australis

Populations of Phragmites were found to respond identically to increased Fe supply (Figure 4.2). Total plant Fe concentrations did not change significantly with increasing Fe concentrations (Figure 4.2a). Leaf Fe concentration (Figure 4.2b) was found to increase with increased concentration of Fe in the treatments. Leaf Fe concentration was found to be approximately half of the Fe concentration in the root (Figure 4.2d). Culm Fe concentrations (Figure 4.2c) were intermediate to the values

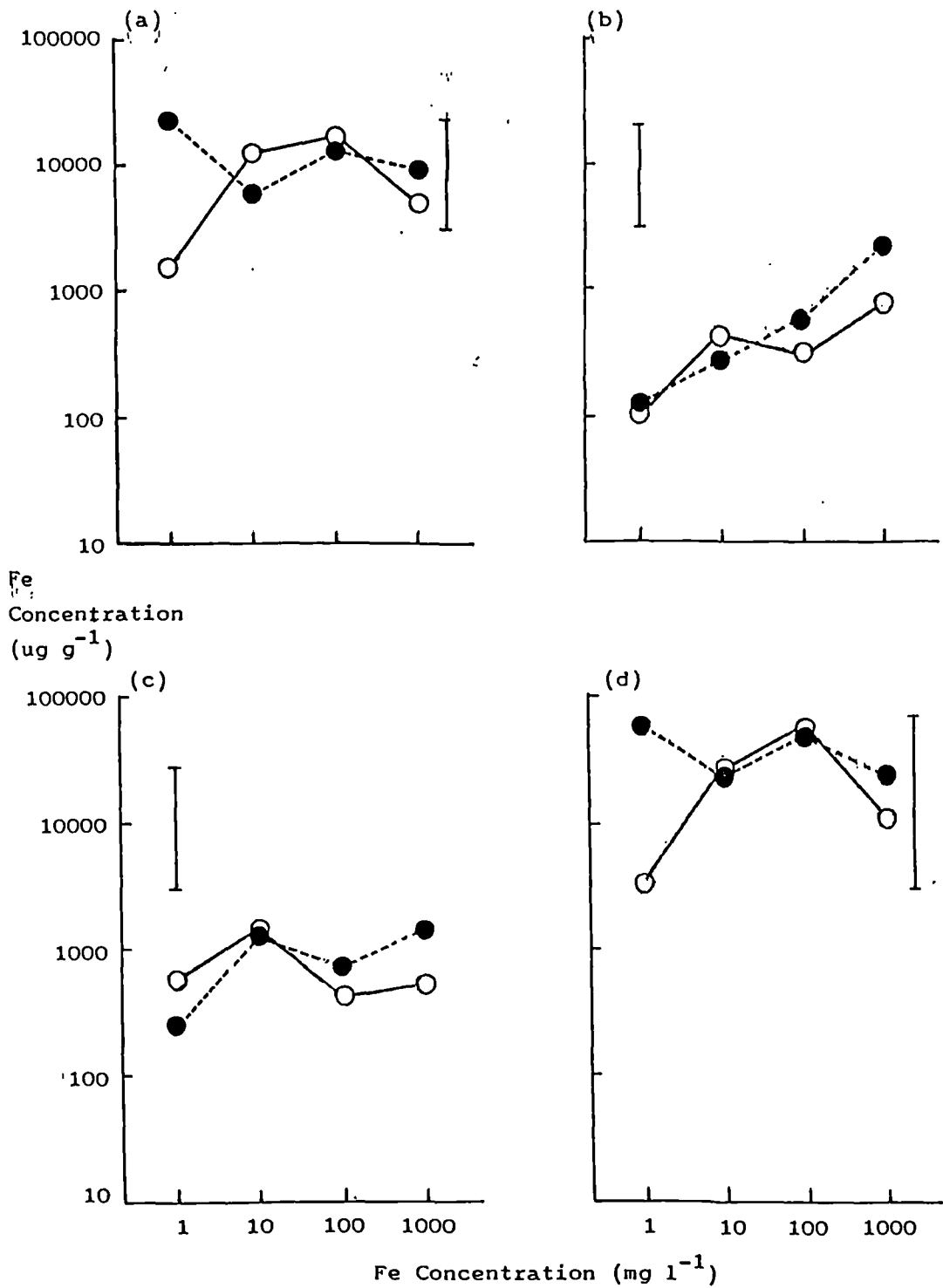


Figure 4.2  
 Iron concentrations (ug/g) of (a) whole plant, (b) leaf, (c) culm and (d) root of *Phragmites australis* for plants from Parys Mountain (O) and Skipwith Common (●) exposed to range of concentrations of iron (Fe). (Vertical bars represent Least Significant Differences.). ( $p < 0.05$ ).

for the root and leaf and did not change significantly between sites or treatments.

Roots of Phragmites was found to accumulate significant amounts of Fe irrespective of treatments and populations.

#### 4.3.1.3 Calcium concentration of tissues of Phragmites australis

Total Ca concentrations were unaffected by increased Fe concentration (Figure 4.3a).

Leaf Ca concentrations (Figure 4.3b) decreased with the highest concentrations of Fe and were the same for both populations. An almost significant difference ( $p < 0.085$ ) was observed for Ca concentrations in culm tissue between populations: the Ca concentrations of the Parys population were stable at 1 and 10 mg/l Fe but declined at above 100 mg/l Fe (Figure 4.3c). Ca concentrations of the Skipwith population were reduced by one half at 10 mg/l Fe. Overall Ca concentrations were higher in the latter population.

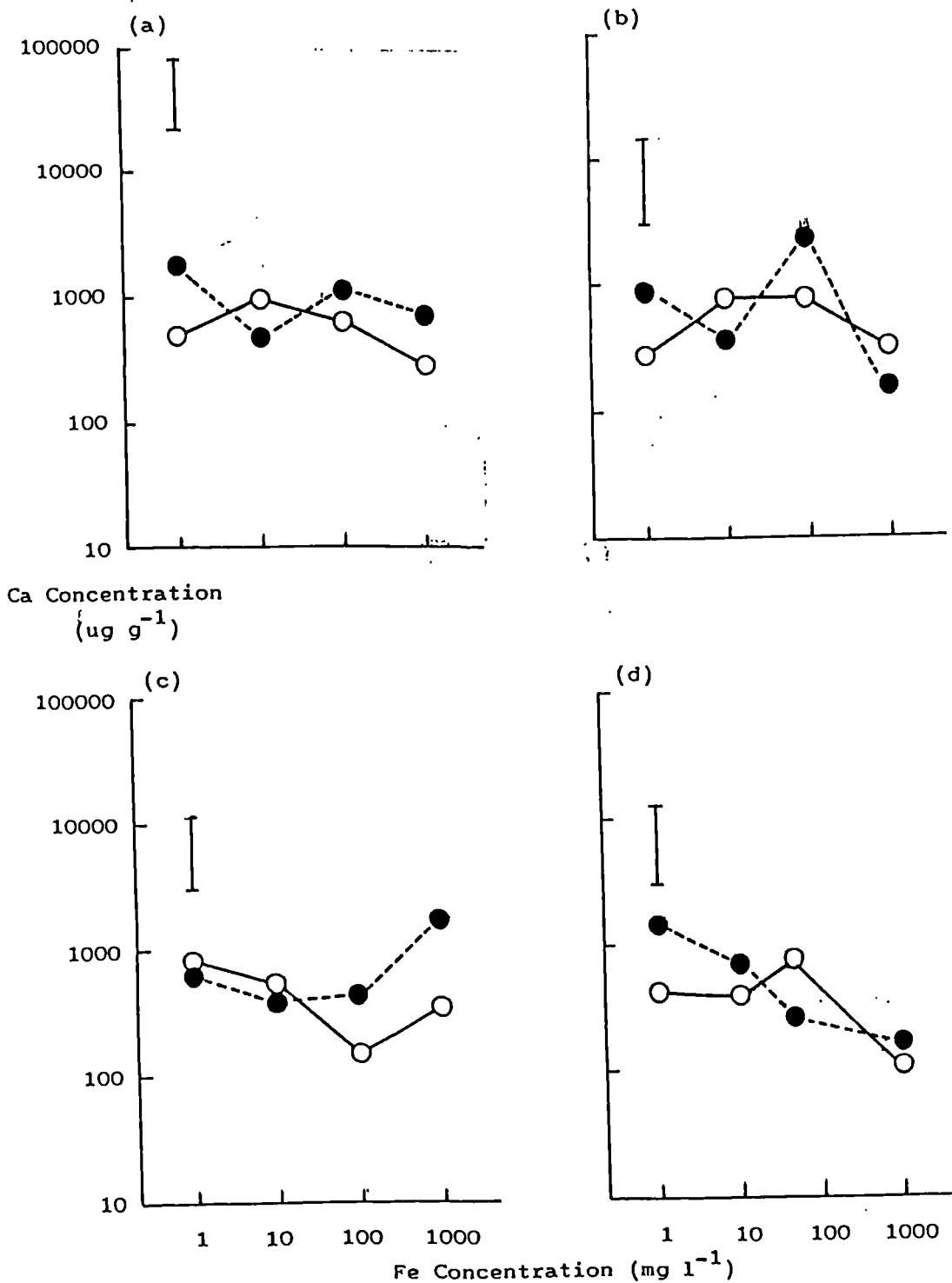


Figure 4.3

Calcium concentrations ( $\mu\text{g/g}$ ) of (a) whole plant, (b) leaf, (c) culm and (d) root of *Phragmites australis* for plants from Parys Mountain (O) and Skipwith Common (●) exposed to range of concentrations of iron (Fe). (Vertical bars represent Least Significant Differences.). ( $p < 0.05$ ).



Root Ca concentrations declined significantly ( $p < 0.001$ ) with increased concentration of Fe (Figure 4.3d). There was a greater reduction in the Ca concentration of the root in the Skipwith population which had a greater initial Ca concentration.

#### 4.3.1.4 Phosphorus concentration of tissues of Phragmites australis

There was a highly significant ( $p < 0.001$ ) decline in Total P concentrations for both populations with increasing Fe supply (Figure 4.4a). There was an almost significant interaction ( $p < 0.080$ ) between populations and increasing Fe concentration. Plants from Skipwith showed a greater decline in P concentration over the range of Fe concentrations. Total leaf P contents declined with increasing Fe concentration and were significantly different between sites (Figure 4.4b). The Skipwith population was found to have the higher P concentrations overall. The two populations of Phragmites differed significantly in P concentration in the culm

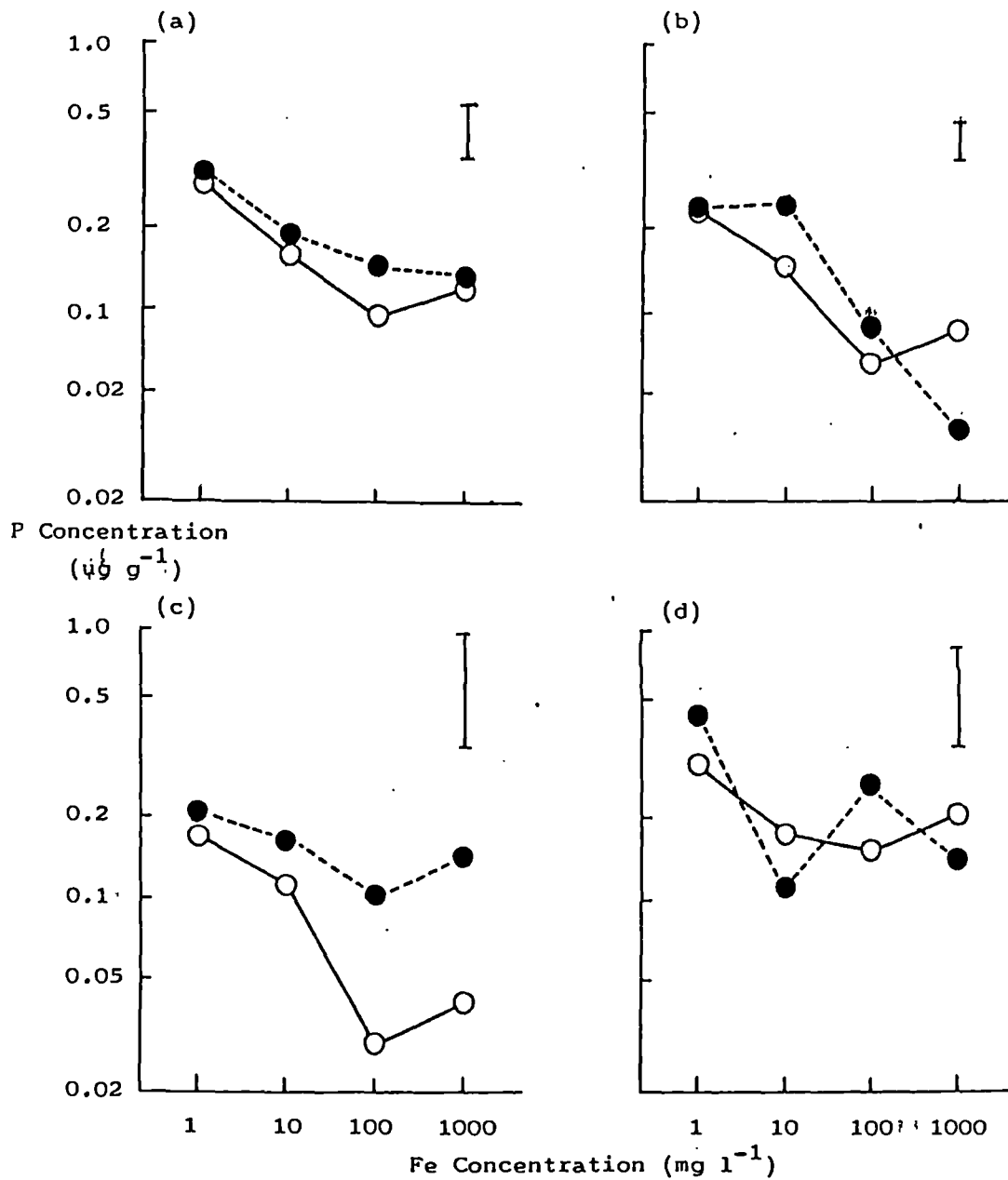


Figure 4.4

Phosphorus concentrations (µg/g) of (a) whole plant, (b) leaf, (c) culm and (d) root of *Phragmites australis* for plants from Parys Mountain (O) and Skipwith Common (●) exposed to range of concentrations of iron (Fe). (Vertical bars represent Least Significant Differences.). ( $p < 0.05$ ).

(Figure 4.4c), with the highest values for plants from Skipwith. In general, there was a decrease in P concentration with increasing Fe supply, levelling off at 100 mg/l Fe. A similar trend was observed for root concentrations of P (Figure 4.4d).

#### 4.3.2 Eriophorum angustifolium

##### 4.3.2.1 Dry weight

The total yield of Eriophorum did not change significantly with increasing concentration of Fe (Figure 4.5a). There was a significant negative effect of Fe concentration on the yield of shoot tissue. The greatest reduction in shoot biomass was at 1000 mg/l Fe (Figure 4.5b). Root biomass, like total yield did not change significantly with increasing concentration of Fe (Figure 4.5c). No population differences were observed. It was also evident that despite a reduction in the size of the plants no

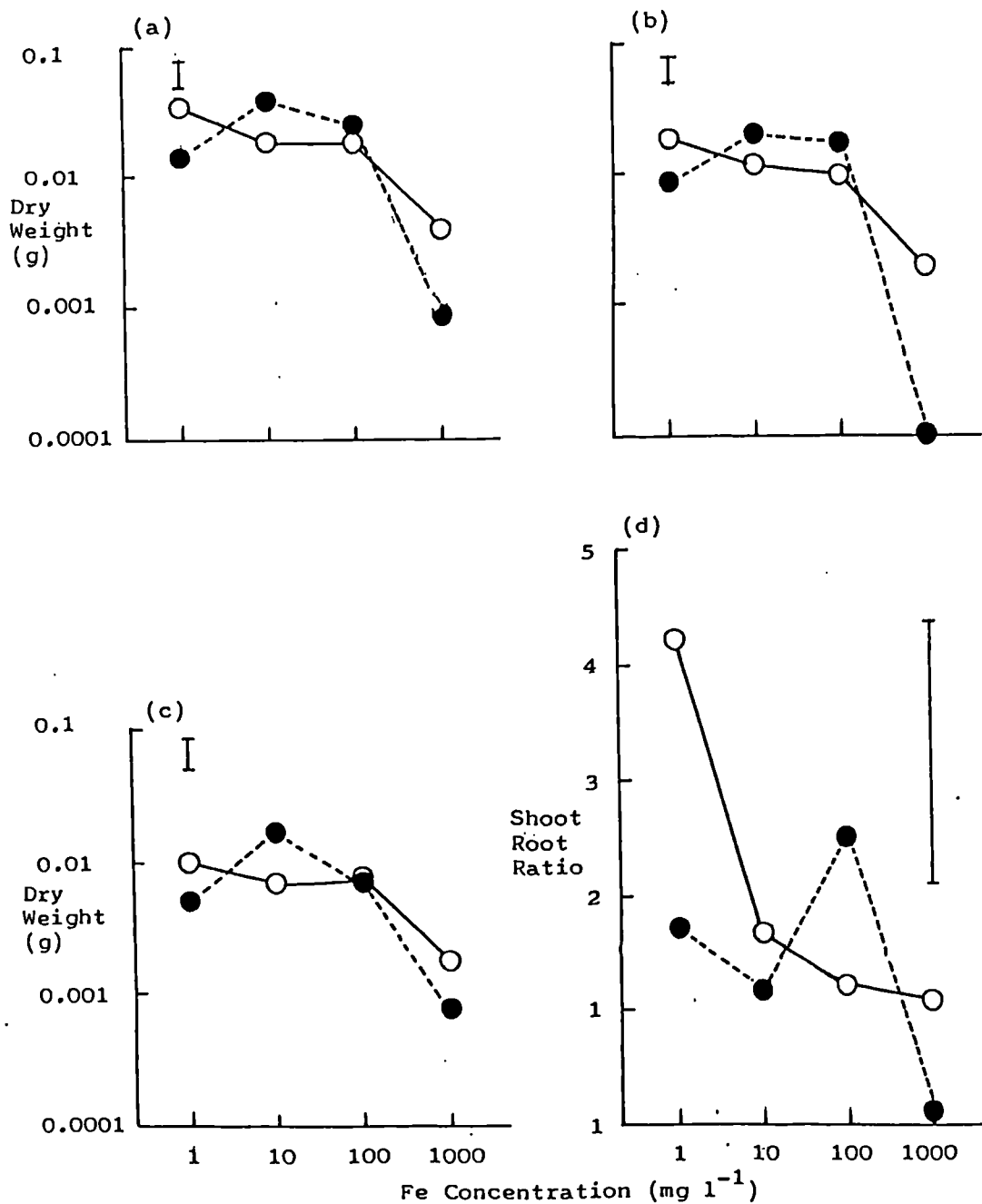


Figure 4.5

Dry weight (g) of (a) whole plant, (b) shoot, (c) root and (d) shoot/root ratio of *Eriophorum angustifolium* for plants from Parys Mountain (O) and Skipwith Common (●) exposed to range of concentrations of iron (Fe). (Vertical bars represent Least Significant Differences.) ( $p < 0.05$ ).

increase in mortality was observed with increased Fe supply.

Shoot root ratio declined with increased Fe (Figure 4.5d). Although, there was no significant population effect the shoot root ratio for the Parys population was less variable compared to the Skipwith population.

#### 4.3.2.2 Root length

Although there was no change in root biomass with increased Fe supply there was a decrease in root length. The proportional decreases were similar for the two populations but the root lengths were approximately 50% greater for plants from Skipwith (Fig. 4.6).

#### 4.3.2.3 Seed weights

Seeds of Eriophorum were significantly heavier from Skipwith than those from Parys (Figure 4.7).

#### 4.3.2.4 Iron concentrations of tissues of Eriophorum angustifolium

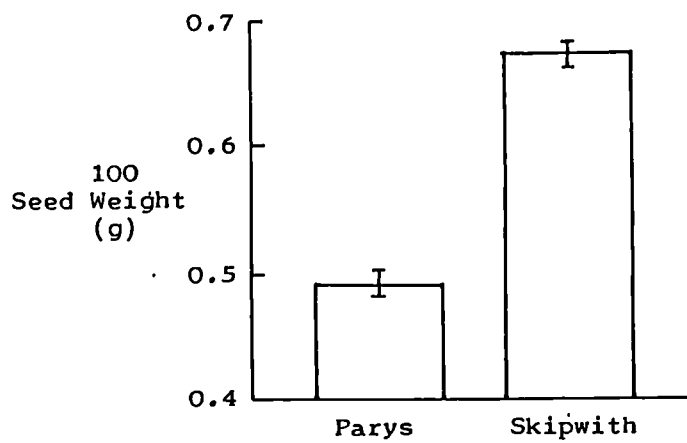


Figure 4.6

100 seed weights (g) of Eriophorum angustifolium sampled from Parys Mountain and Skipwith Common. (Vertical bars represent Least Significant Differences.) ( $p < 0.05$ ).

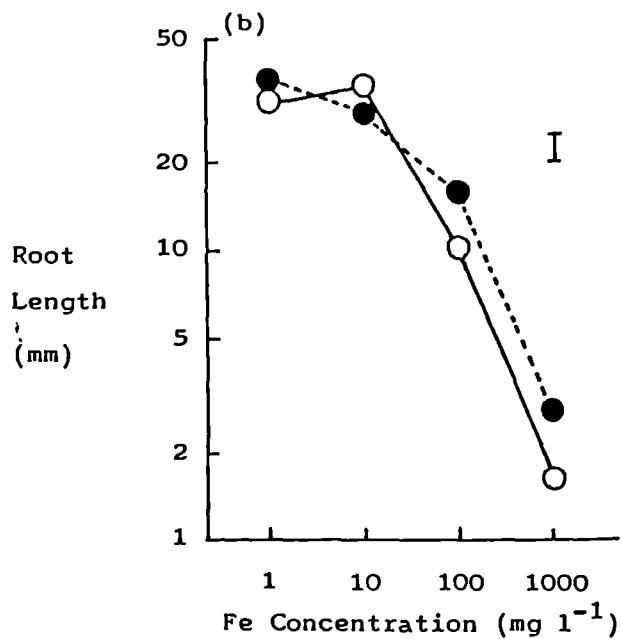


Figure 4.7

Root lengths (mm) of plants of *Eriophorum angustifolium* from Parys Mountain (O) and Skipwith Common (●) exposed to a range of concentrations of iron (Fe). (Vertical bars represent Least Significant Differences.) ( $p < 0.05$ ).

A highly significant ( $p < 0.001$ ) increase in total plant Fe concentration followed an increase in external Fe supply, in both populations (Figure 4.8a). There was no detectable difference in Fe concentrations in shoots of plants from the two populations until Fe concentrations exceeded 100 mg/l Fe in the treatment solutions (Figure 4.8b). At this concentration, plants of Eriophorum from Parys accumulated one third more Fe than plants from Skipwith. (Values for the 1000 mg/l treatment were not available as there was insufficient material.) Root Fe concentrations (Figure 4.8c) increased with increasing Fe supply in both populations.

The shoot root concentration ratios declined with increased concentration of Fe for both sites (Figure 4.8d).

#### 4.3.2.5 Calcium concentration of tissues of Eriophorum angustifolium

Total Ca concentration of plant tissues did not decrease significantly until the 100 mg/l treatment in which the



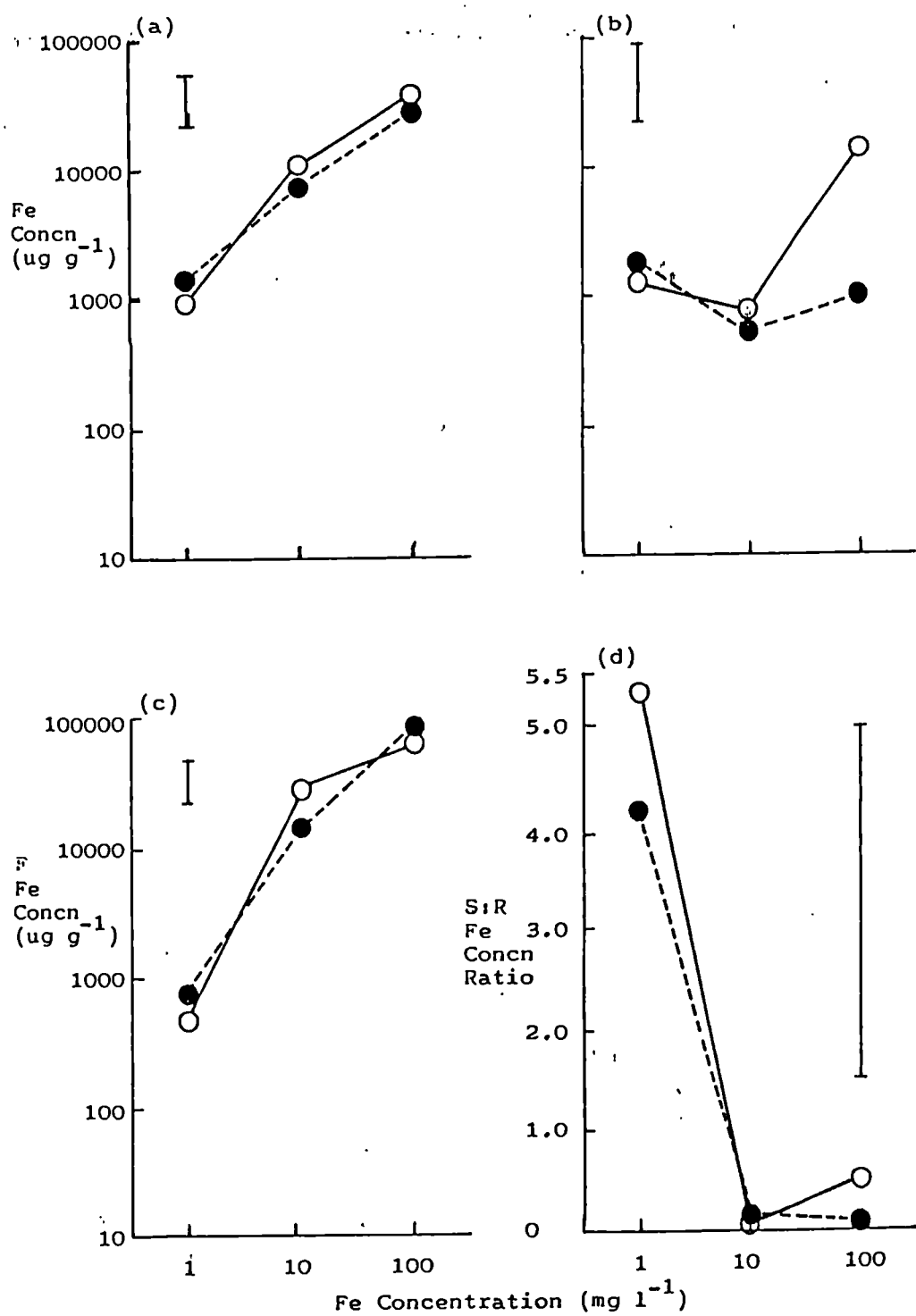


Figure 4.8

Iron (Fe) concentration ( $\text{ug/g}$ ) of (a) whole plant, (b) shoot, (c) root and (d) shoot/root ratio of *Eriophorum angustifolium* for plants from Parys Mountain (O) and Skipwith Common (●) exposed to range of concentrations of iron. (Vertical bars represent Least Significant Differences.) ( $p < 0.05$ ).

values for both populations were a quarter of that of the controls (significant at  $p < 0.001$ ) (Figure 4.9a). The shoot Ca concentration was lower with an external Fe supply of 100 mg/l than in the control (1 mg/l Fe) for both populations (Figure 4.9b). The root Ca concentrations for Eriophorum from both sites (Figure 4.9c) were found to decrease by a third at 100 mg/l Fe relative to the control. Root Ca concentrations at 1 and 10 mg/l Fe did not differ significantly for plants from Parys whereas the decrease in root Ca concentration between these two values was large for plants from Skipwith.

Shoot-root concentration ratios for Ca did not vary significantly between treatments or populations (Figure 4.9d).

#### 4.3.2.6 Phosphorus concentration of tissues of Eriophorum angustifolium

No change in total P concentration was observed in plants from the Parys site treated with 1 and 10 mg/l Fe but

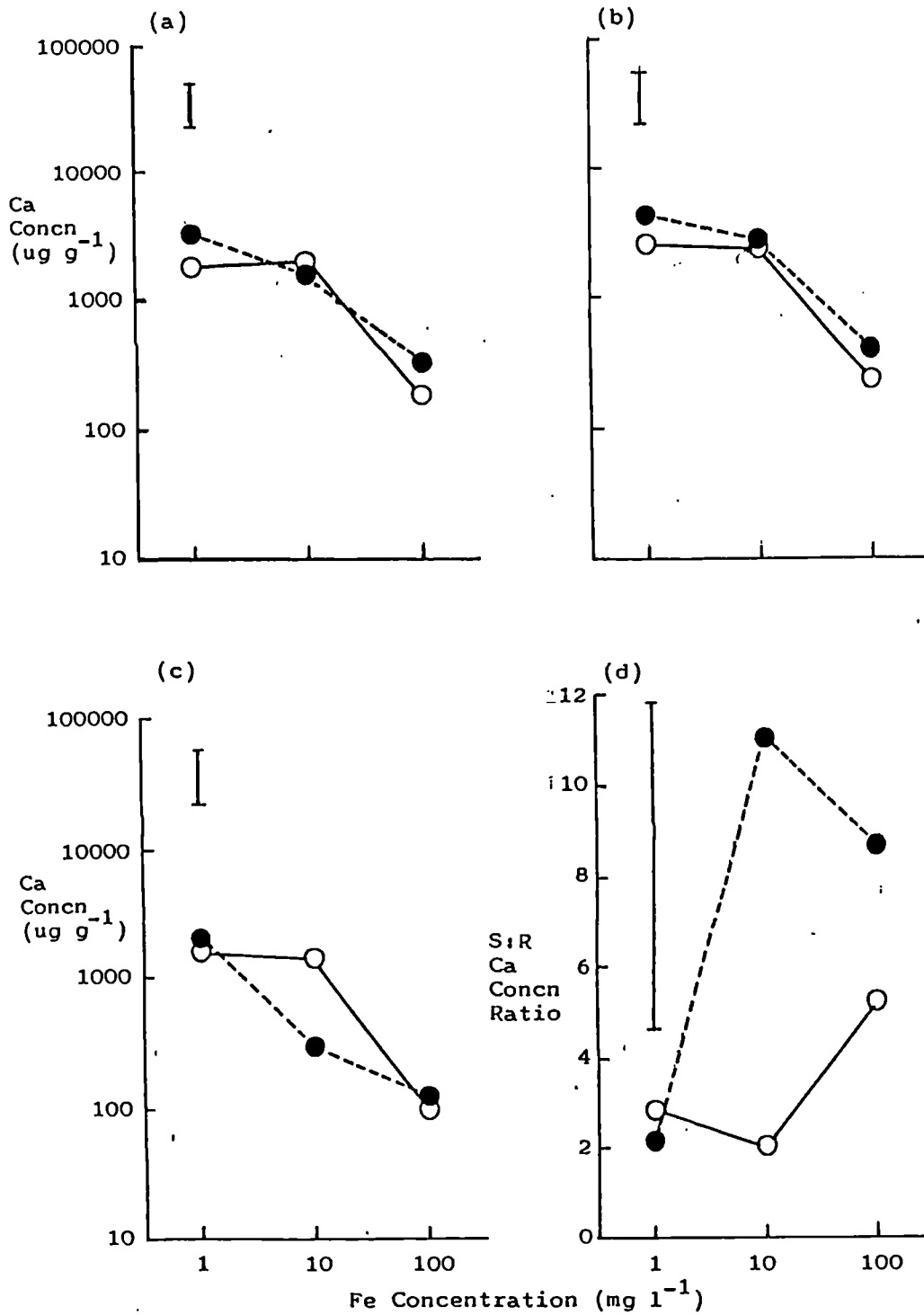


Figure 4.9

Calcium (Ca) concentration ( $\text{ug/g}$ ) of (a) whole plant, (b) shoot, (c) root and (d) shoot/root ratio of *Eriophorum angustifolium* for plants from Parys Mountain (○) and Skipwith Common (●) exposed to range of concentrations of iron (Fe). (Vertical bars represent Least Significant Differences.) ( $p < 0.05$ ).

P concentrations declined thereafter (Figure 4.10a). The response of the Skipwith population was similar but at 100 mg/l Fe, P concentrations in plant tissues declined to the value of the 1 mg/l Fe treatment; significant at  $p < 0.001$ . Both shoot and root concentrations of P showed the same trend as did the totals (Figure 4.10b and c).

There were no clear effects on the shoot-root ratios (Figure 4.10d).

#### 4.3.3 Response of Eriophorum angustifolium to increasing concentrations of Manganese

##### 4.3.3.1 Dry weight

The total dry weight was depressed only at the highest concentration of Mn (Figure 4.11a). This trend was similar for both populations. The same trend was observed for shoot weight (Figure 4.11b). Root dry weights did not change significantly with increased external supply of Mn (Figure 4.11c).

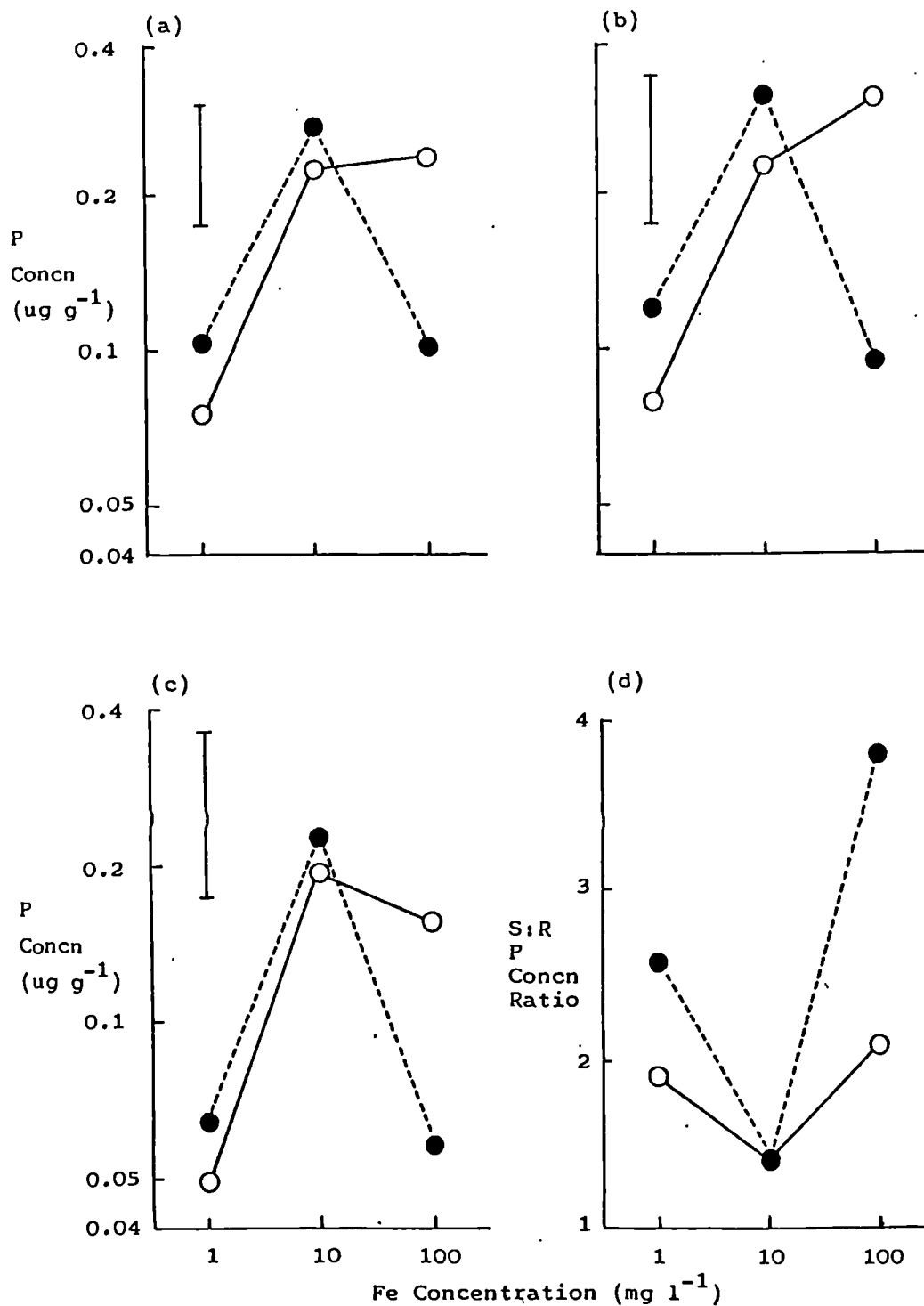


Figure 4.10

Phosphorus (P) concentration (ug/g) of (a) whole plant, (b) shoot, (c) root and (d) shoot/root ratio of *Eriophorum angustifolium* for plants from Parys Mountain (O) and Skipwith Common (●) exposed to range of concentrations of iron (Fe). (Vertical bars represent Least Significant Differences.) ( $p < 0.05$ )

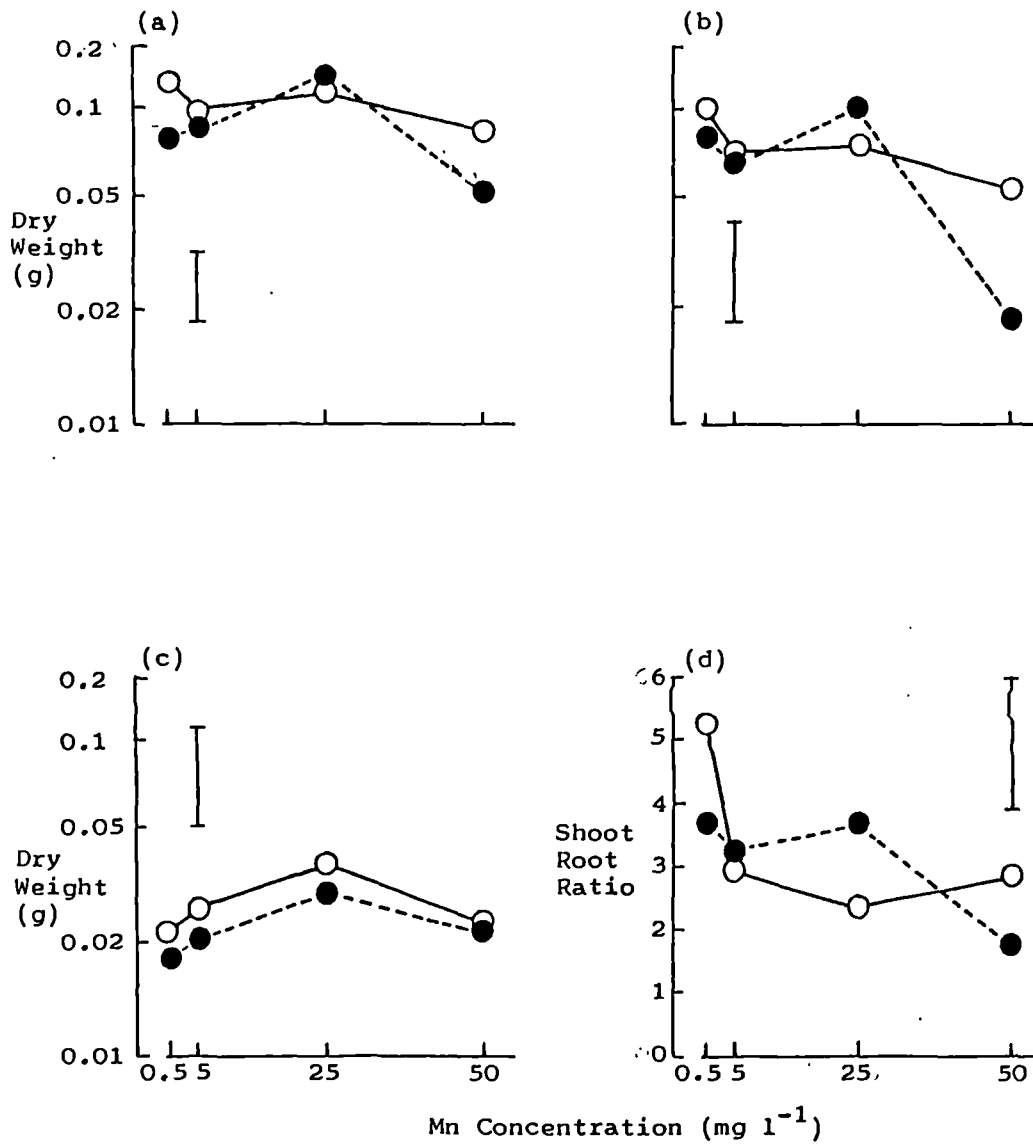


Figure 4.11

Dry weight (g) of (a) whole plant, (b) shoot, (c) root and (d) shoot/root ratio of *Eriophorum angustifolium* for plants from Parys Mountain (O) and Skipwith Common (●) exposed to range of concentrations of manganese (Mn). (Vertical bars represent Least Significant Differences.) ( $p < 0.05$ ).

#### 4.3.3.2 Manganese concentrations of tissues of Eriophorum angustifolium

The total concentrations of Mn (Figure 4.12a) in plants did not differ significantly between populations or external concentrations. There was no significant effect of external Mn concentration on the shoot concentration of plants from Parys (Figure 4.12b). In the Skipwith population significantly elevated concentrations were observed at 15 and 50 mg/l Mn. The concentration of Mn in the root was not significantly affected by the external supply in either population (Figure 4.12c).

Shoot root ratios for Mn increased significantly ( $P < 0.1$ ) at the highest external Mn supply (Figure 4.12d).

#### 4.3.3.3 Calcium concentrations of tissues of Eriophorum angustifolium

Total Ca concentration of plants grown in 15 mg/l Mn was lower than for plants grown at 25 and 50 mg/l Mn with the plants grown in 0.1 mg/l Mn intermediate in value (Figure

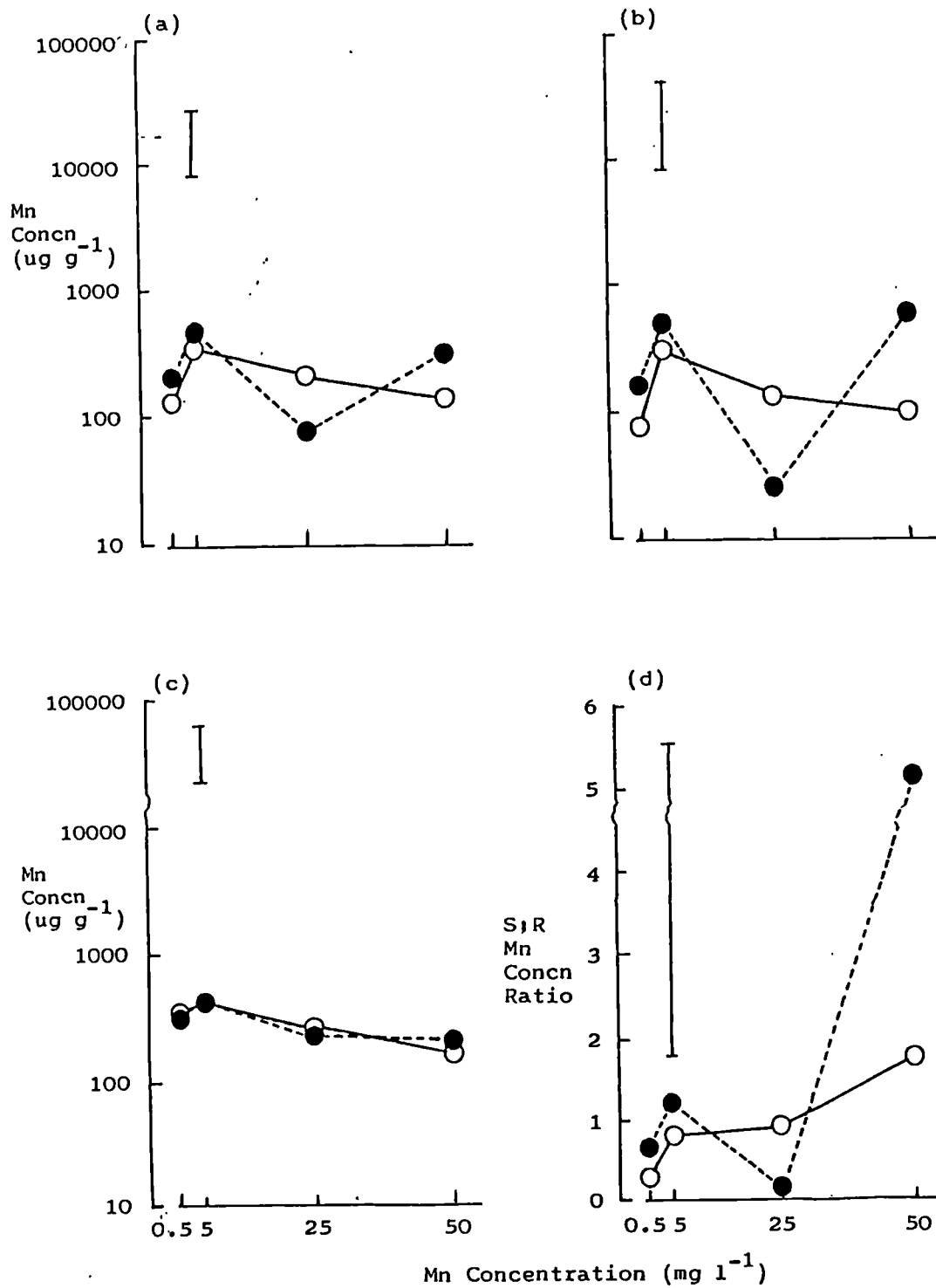


Figure 4.12

Manganese (Mn) concentration ( $\text{ug/g}$ ) of (a) whole plant, (b) shoot, (c) root and (d) shoot/root ratio of *Eriophorum angustifolium* for plants from Parys Mountain (O) and Skipwith Common (●) exposed to range of concentrations of manganese. (Vertical bars represent Least Significant Differences.) ( $p < 0.05$ ).



4.13a). Total Ca concentrations were significantly higher in plants from Skipwith than those from Parys. Shoot Ca concentrations increased with increased external supply of Mn (Figure 4.13b). Plants from Parys showed an increase in Ca concentration in the shoot in the highest treatment (50 mg/l Mn) but in plants from Skipwith the increase was apparent at 25 mg/l Mn. The root Mn concentration of plants (Figure 4.13c) from Parys was significantly lower at the highest external supply of Mn. For plants from Skipwith the Ca concentration was lower in the 25 than in the 0.1 and 15 mg/l Mn treatment, with the plants grown at 50 mg/l intermediate.

Shoot root concentration ratios for Ca concentrations did not show any clear trends with external Mn supply (Figure 4.13d).

#### 4.3.4 Response of Eriophorum angustifolium to increasing concentrations of Copper

##### 4.3.4.1 Dry weight

No differences were observed in total plant or

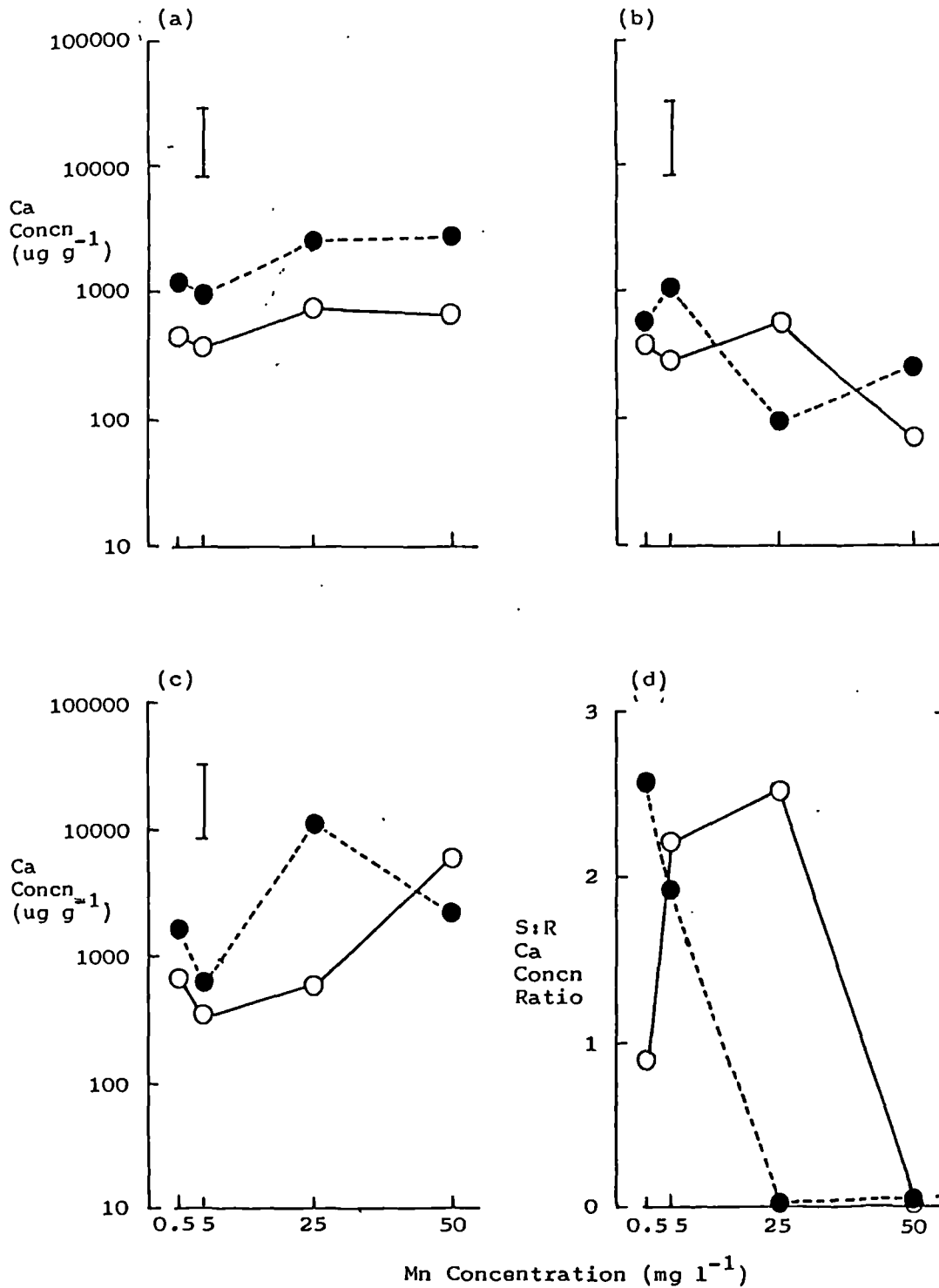


Figure 4.13

Calcium (Ca) concentration ( $\mu\text{g/g}$ ) of (a) whole plant, (b) shoot, (c) root and (d) shoot/root ratio of *Eriophorum angustifolium* for plants from Parys Mountain (O) and Skipwith Common (●) exposed to range of concentrations of manganese (Mn). (Vertical bars represent Least Significant Differences.) ( $p < 0.05$ ).

shoot dry weight of plants from Parys or in total, shoot or root dry weight of plants from Skipwith in response to Cu supply (Figure 4.14a, b and c). There was some evidence ( $P < 0.1$ ) of an increase in root growth of plants from Parys grown in 0.25 mg/l Cu (Figure 4.14c).

#### 4.3.4.2 Copper concentrations of tissues of Eriophorum angustifolium

The total Cu concentrations of plants from the Parys population (Figure 4.15a) were very similar except for those grown with the highest external Cu supply which had significantly higher Cu concentrations. There was a progressive increase in plant Cu concentration with external supply such that those grown in 0.5 mg/l Cu and above differed significantly from those grown with the lowest Cu supply. There were increases in the Cu concentrations in the shoots of both populations in response to increased Cu supply (Figure 4.15b). It was not clear at which concentration this increase became significant for the Parys

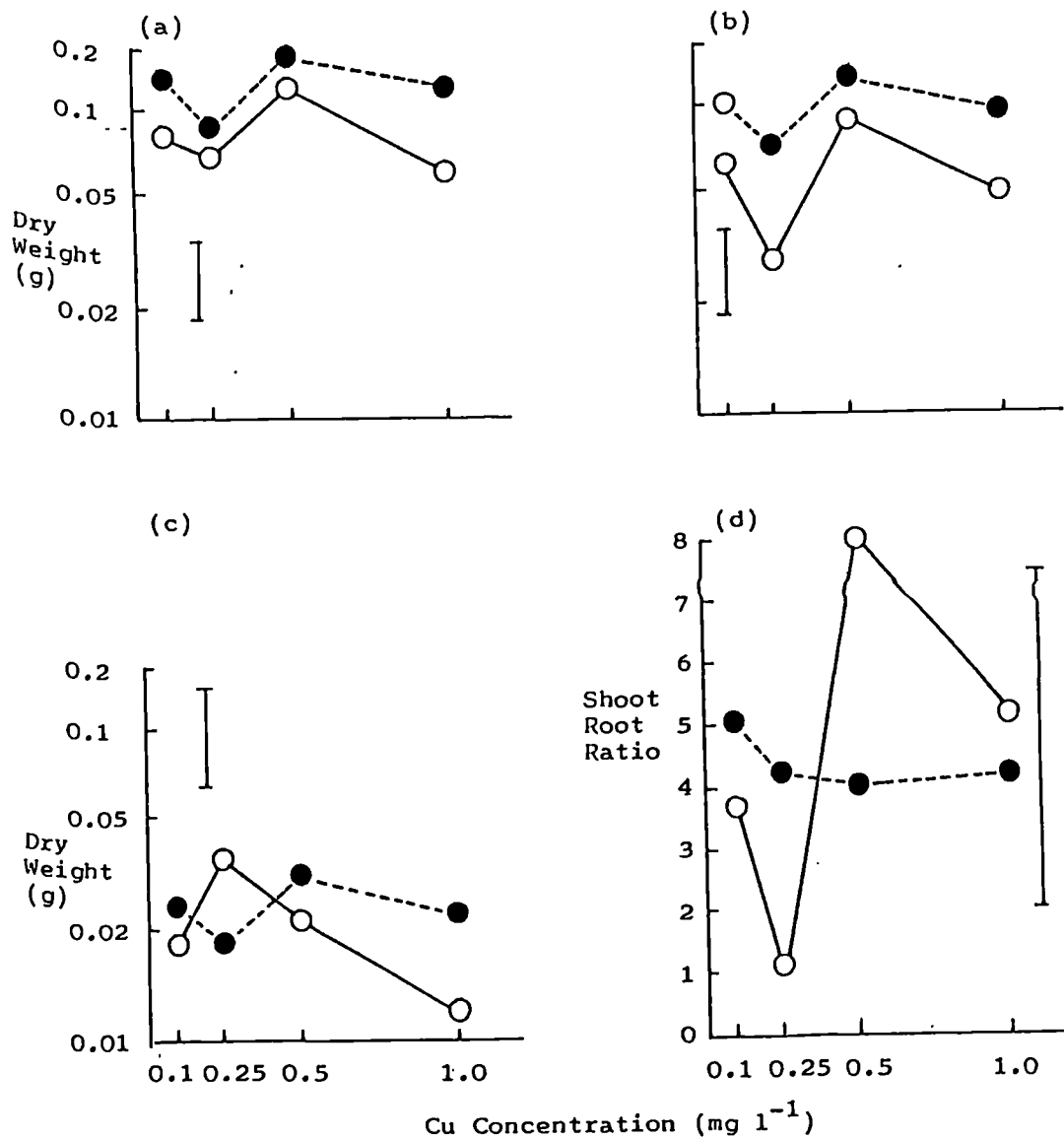


Figure 4.14

Dry weight (g) of (a) whole plant, (b) shoot, (c) root and (d) shoot/root ratio of *Eriophorum angustifolium* for plants from Parys Mountain (O) and Skipwith Common (●) exposed to range of concentrations of copper (Cu). (Vertical bars represent Least Significant Differences.) ( $p < 0.05$ ).

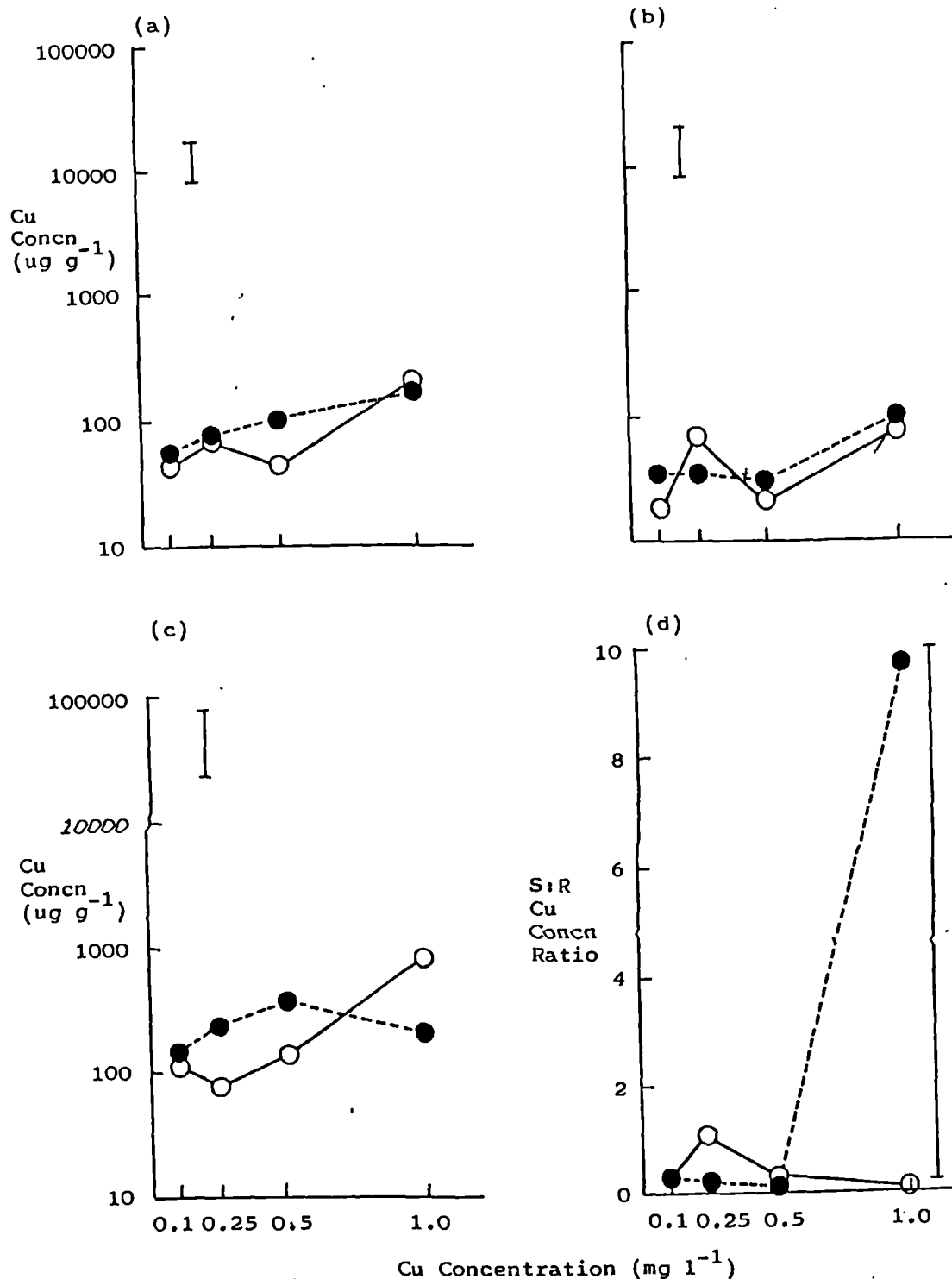


Figure 4.15

Copper (Cu) concentration (ug/g) of (a) whole plant, (b) shoot, (c) root and (d) shoot/root ratio of *Eriophorum angustifolium* for plants from Parys Mountain (O) and Skipwith Common (●) exposed to range of concentrations of copper. (Vertical bars represent Least Significant Differences) ( $p < 0.05$ ).

population but it was not until highest external supply for the Skipwith population. The root concentrations showed the same trends as for the total plant for both populations (Figure 4.15c).

There were no significant changes in shoot-root concentration ratios for either population (Figure 4.15d).

#### 4.3.4.3 Calcium concentrations of tissues of Eriophorum angustifolium

There was no significant increase in the total Ca concentrations of plants from Parys until the highest external concentration of Cu (Figure 4.16a). For plants from Skipwith there was a progressive significant increase up to a Cu concentration of 0.5 mg/l above which there was no further change. The concentrations of Ca in the shoot were not significantly affected by the treatments (Figure 4.16b). Root concentrations followed the same trends as shoot concentration except that the difference between the two lowest values for the Skipwith population was not significant

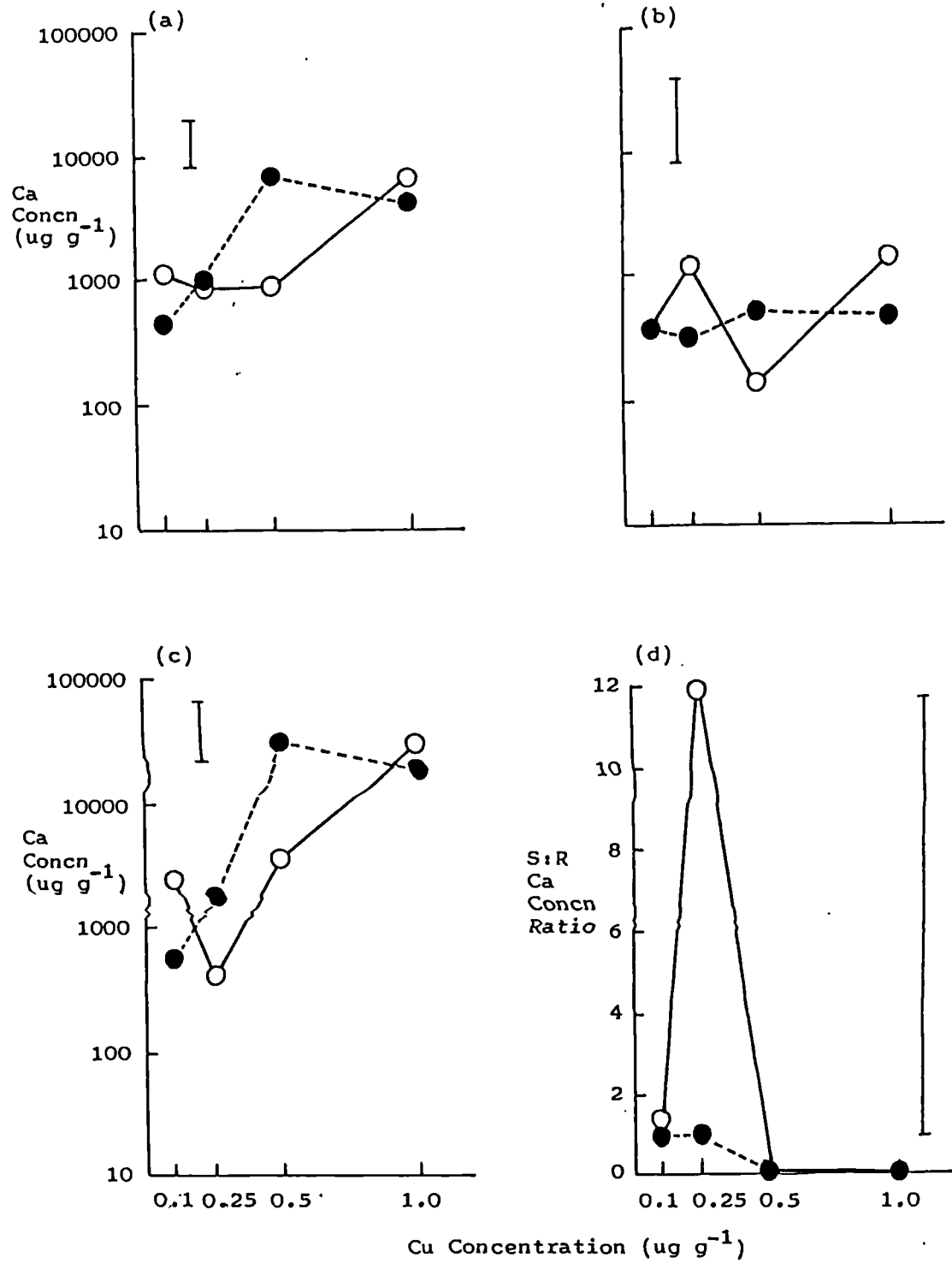


Figure 4.16

Calcium (Ca) concentration ( $\mu\text{g/g}$ ) of (a) whole plant, (b) shoot, (c) root and (d) shoot/root ratio of *Eriophorum angustifolium* for plants from Parys Mountain (O) and Skipwith Common (●) exposed to range of concentrations of copper (Cu). (Vertical bars represent Least Significant Differences.) ( $p < 0.05$ )

(Figure 4.16c). The shoot-root concentration ratios were not affected by Cu supply (Figure 4.16d).



#### 4.4 Discussion

##### 4.4.1 Response of Phragmites australis to increasing iron concentration

###### 4.4.1.1 Population response to increasing iron supply

Both populations were found to respond in the same way to increasing Fe concentration supporting observations made from field data in the previous chapters. Hence there was no evidence for a differential response to increasing Fe concentration between populations. Similarly, in T.latifolia, Taylor and Crowder (1983b), reported that Cu and Ni accumulation in plants from contaminated sites near smelters were similar to plants sampled from uncontaminated locations. Gibson and Risser (1982) found elevated levels of metals in plants of Andropogon virginicus from a mine environment, yet these plants displayed no greater ability to grow in metalliferous soils than plants from uncontaminated

sites. In contrast, Etherington and Thomas (1986), observed a differential capacity to limit Fe (and Mn) uptake in waterlogging tolerant and non-tolerant populations of Dactylis glomerata. This apparent conflict in results may simply reflect the differences in response to high Fe concentrations between species. The degree of variation between plants in the ability to tolerate Fe is not well documented. However, it may be speculated that the discrepancy between the present work and the work presented above may well reflect the difference between wetland plants (for example, Eriophorum, Phragmites and T. latifolia) and plants usually found in relatively well drained soils (such as D. glomerata). Wetland plants may be tolerant to high concentrations of Fe (and Mn) by virtue of growing in waterlogged conditions, while there may be a form of selection and species differentiation involved in D. glomerata, a species generally confined to relatively dry conditions. Further discussion on this subject is beyond the context of this chapter but will be continued in the final

discussion (Chapter 7, part 2.).

In the field, no visual evidence was apparent for the toxic effect of high concentrations of Fe. Fe toxicity is reported as an excessive uptake of Fe causing 'bronzing' and 'yellowing' in wetland rice, retardation of growth of the plant and the poor development of the roots which become blackened rather than red-brown (Tanaka and Yoshida, 1970). Plants grown in hydroponic culture did show indications of yellowing and root death at Fe concentrations of 1000 ug/l.

#### 4.4.1.2 Iron-plaque formation on the roots of Phragmites australis

Despite an increase in shoot (leaf) Fe content, root Fe concentrations were constant irrespective of external Fe supply. Root concentrations of Fe, even in normal soils or in nutrient solutions with trace Fe supplies are often substantially greater than those of equivalent leaf tissues (Jones and Etherington 1970; Jones 1971). However, this does not explain the observation that even at low external

concentrations, root Fe content is comparable to values obtained for higher treatments. Accumulation of Fe onto or into the root is apparent.

Plants differ markedly in their adaptation to poorly aerated environments both in the field and under controlled growing conditions (Bartlett 1961). Several mechanisms have been suggested to explain this difference including the ability of plants to oxygenate the rhizosphere. Root oxidizing activity has been well documented in a variety of species (Bartlett 1961, Armstrong 1967, Green and Etherington 1977, Trolldenier 1988) including Phragmites (Hansen and Andersen (1981). Depending on edaphic conditions, for example total Fe, redox conditions and the presence of reducing or oxidizing bacteria in the rhizosphere (Benckiser et al. 1984) excess O<sub>2</sub> from radial oxygen loss from the roots can oxidize Fe (II) to Fe(III). Ferric hydroxide may then be deposited as a plaque or 'halo' in the soil around the root visible as a reddish brown deposit. It can form up to 8% of the total root dry weight and upto 98%

of root Fe (McLaughlin, Van Loon and Crowder 1985). In hydroponic culture, plaque deposition has been induced on the roots of T. latifolia, P. australis, Oryza sativa and Carex rostrata by Macfie and Crowder (1987), the pre-requisite being a suitable source of  $Fe^{2+}$ , a controlled pH and anoxic conditions. In the present study direct observations confirmed the presence of Fe coatings on the roots of plants after 2-3 days in the culture solutions.

The thickness of the coatings appeared to be related to Fe concentration, with darker, more extensive precipitates on the roots with increasing Fe supply. However, no relationship was evident between the extent of Fe-plaque formation on roots of Phragmites and external Fe concentration. This may be explained in terms of Fe absorption and adsorption. At lower external Fe supply Fe absorption exceeds adsorption as the supply is little greater than the demand by the plant. At high external concentrations adsorption and immobilization onto the surface of roots is more significant and may be given as evidence for

the presence of a mechanism involved in the reduction of Fe uptake by the plant. Jones and Etherington (1972), Howeler (1973) Green and Etherington (1977) and Armstrong (1979) have all implicated rhizosphere oxidation of Fe as important in the possible reduction of Fe (and Mn) toxicity.

#### 4.4.1.3 The possible interference of Phosphorus and Calcium uptake by iron plaque

The reduction in plant growth as a consequence of increasing Fe supply in this study may be identified as a nutrient deficiency rather than the direct effect of Fe toxicity per se. A general reduction in P and Ca content of plant tissues was a feature of plants in culture solutions with high Fe concentrations. Ottow et al, (1983) and Benckiser et al, (1984) both attributed Fe toxicity as a multiple nutritional stress. Among the nutrients involved an insufficient supply of K, P, Ca, and Mg were regarded as the primary causes of Fe toxicity. Moorman and Van Breeman (1978) found that soils low in both P and K with Fe (II)

concentrations as low as 30 ug/g were Fe toxic and P deficiency was suggested as a key factor in Fe toxicity.

Fe plaques may limit uptake of P by the plant by immobilizing P on the surface of the plaque through precipitation of Fe-phosphates or by directly acting as a physical barrier. Ca absorption may also be reduced in the same manner. Howeler (1973) also suggested that the formation of an Fe plaque may diminish the roots capacity to absorb essential nutrients. Plants insufficiently supplied with K, P and Ca would show dramatic changes in their metabolism which could be attributed incorrectly to the direct action of excess Fe.

Fe toxicity caused retardation of growth of rice plants (Tanaka and Yoshida; 1970). In this study at low Fe concentrations ( < 1000 ug/g ) the only visible sign of Fe toxicity was stunting of new roots. However, if some factor was responsible for restricting Ca uptake, it would effectively restrict root growth and result in the stunting of roots, as Ca is essential for root initiation

and elongation. P deficiency also causes a reduction in root growth. Thus, the significance of Fe toxicity in the retardation of root growth of Phragmites in this study may be further complicated by the presence of nutritional stresses and the relationship of Fe with these nutrients must be clarified.

#### 4.4.1.4 Population differences in macronutrient uptake in Phragmites australis

##### i) Phosphorus nutrition

In contrast to observations made in the laboratory, direct reciprocal transplants of Phragmites (Appendix VII) from the contaminated and uncontaminated sites in the field gave indications that plants originating from the respective sites could not grow as successfully as plants indigenous to that site. It is likely that long-term experiments may provide the answer to this phenomenon, short-term experiments of the type conducted here are sufficient to monitor



differences in internal element concentrations but give little indication of growth patterns and biomass accumulation in the long-term. In addition, the conditions experienced by the plants would have been different between culture solution and field. Plants exposed to field conditions would experience temporal variation in for example temperature, redox and mineral element status, whereas conditions experienced by plants in culture solutions would have been relatively constant. It is suggested that although Skipwith plants were able to tolerate Fe in high concentrations, there may be population differentiation in growth rate patterns, biomass accumulation and mineral nutrition. Differences in P-nutrition were evident between populations providing some evidence for this hypothesis. P-uptake in the Parys population was lower than the Skipwith, suggesting a lower P requirement. Differences in P uptake between populations has been detected in a number of species by other workers (Snaydon and Bradshaw 1962, 1969, Goodman 1969) and attributed to edaphic adaptation. Rorison (1968),

examined seedlings of four ecologically-distinct species of grasses in culture solution, observed a relationship between growth rate and P-absorption. Some of these grasses were able to survive conditions of low P because of their inherently low growth rates. Although differences in size between individual of the two populations were obvious in Eriophorum, no evidence was available to suggest this in Phragmites, and relative growth rates were not measured in this study.

Although no analyses of soil and plant P concentration were made in the field material, it seems likely that by virtue of the site's origins (as mine waste) the Parys population was adapted to low P availability relative to the plants from the control site. Extreme macronutrient deficiency is a recognized characteristic of metalliferous mine wastes (Antonovics, et al. 1971) and the evolution of metal resistance in populations able to colonize these extreme edaphic situations has involved independent selection for individuals tolerant to low nutrient availability (Baker

1978c).

The supply of P in the culture solutions was probably sufficient to support the higher P requirements of the Skipwith population over the duration of the study, which may explain the lack of difference in response of the two populations to increasing Fe supply. The inconsistency between the results and direct observations made in the field may thus be explained primarily in terms of a difference in the tolerance of the two populations to low nutrient availability.

ii) Calcium nutrition

A degree of population differentiation was also apparent for Ca as the Skipwith population appeared to absorb more Ca in the control. In addition, Ca content of the plants in this population declined more significantly in the presence of increasing Fe concentration. Thus a greater sensitivity in Ca uptake in the presence of high concentrations of Fe was apparent in the control population. In contrast, Ca

concentration was less sensitive to Fe in the mine population. If increasing Fe concentration reduces the availability of Ca in solution (by precipitation or immobilization) then the difference between the populations could be explained as a differential response to low Ca availability. Such a response was demonstrated by Bradshaw, Lodge, Jowett and Chadwick (1960) and Jowett (1959) in a Pb-tolerant population of Agrostis tenuis which was more tolerant to low Ca than a pasture population. The Parys plants originating from a site with low Ca status relative to the control population are likely to differ in their ability to absorb Ca from low solution concentrations. This may be true for the plants in this study. An investigation of population differentiation within the grass Festuca ovina from widely contrasting sites by Snaydon and Bradshaw (1961) has shown that populations within this species differ markedly in their response to Ca in culture solution. This difference was attributed to the ability to absorb Ca from low concentrations in solution, rather than to differences in

ability to metabolize at low internal Ca concentrations.

4.4.2 The response of Eriophorum angustifolium to increasing iron concentrations

Eriophorum and Phragmites were often closely associated in the field. However, despite growing together in the same areas, differences were evident in the nature of Fe resistance. Eriophorum concentrated more Fe in shoot tissues than Phragmites and Fe plaque formation was more pronounced.

It was suggested that the lack of population differentiation in response to Fe was due to a basic response of plants from waterlogged reduced conditions. The ability of wetland plants to survive under conditions of poor aeration has been attributed to a variety of reasons including active translocation of dissolved oxygen in solution and specific tolerance to toxic (reduced) substances formed under waterlogging (Bartlett 1961). Fe (II) oxidation by Eriophorum has been reported by Armstrong

(1967) and is given as evidence for rhizosphere oxidation by this species. Further evidence from the present study agrees that both populations respond in a similar way to increasing Fe concentration but provides information on the nature of Fe resistance in this species.

In accordance with the definitions of Levitt (1980) and Baker (1987), Eriophorum (from the two populations) therefore shows a general resistance to Fe in excess. Fe is immobilized externally on the surface of plant roots (as an Fe plaque) and when absorption exceeds precipitation, high internal Fe concentrations implies a high internal tolerance.

#### 4.4.2.1 Iron content of the plant

Although there was no corresponding reduction in root biomass, root length decreased with increasing Fe concentration. There are two possible explanations of this: (1) that roots were shorter and thicker and had the same biomass, or (2) more probably that, an external factor was contributing to this apparent contradiction. Fe plaque

formation and accumulation increases with increasing Fe concentration and it is likely that the increasing density of plaque masks the effect of reduction on root length or "biomass".

As observed for Phragmites, at lower external Fe concentrations Fe supply does not exceed demand by the plant. At high external concentrations adsorption and immobilization onto the surface of roots is more significant and suggests the presence of a mechanism for avoiding toxic concentrations of Fe. Talbot and Etherington (1987) suggested that the bulk of the Fe in Salix cinerea was apoplastic in origin and the immobilization of Fe by the root system was part of a mechanism of avoiding potential Fe toxicity as removal of plant roots immediately caused sensitivity to much lower external concentrations of reduced Fe. The same authors found that waterlogging substantially increased the Fe content of S. caprea leaves (waterlogging sensitive) in relation to S. cinerea (waterlogging insensitive). Fe

content of the leaves of the former was more than 2000 ug/g, a level which was considered almost certainly toxic. Leaf (shoot) Fe content of Eriophorum (and Phragmites) over the range of concentrations in this study did not exceed this value. No evidence was available for the direct effect of Fe toxicity other than stunting and a reduction in shoot root ratio but as concluded earlier, reduced growth is not a reliable measure for the toxic action of Fe as this is also associated with nutritional stresses caused by the interference of high external concentrations of Fe. In addition, values quoted as being toxic to woody species such as Salix, may be different to concentrations which would be toxic to other plants.

#### 4.4.2.2 The uptake of other elements in the presence of iron

The stimulation in P-uptake in both populations at 1 and 10 mg/l Fe suggests a correlation between the uptake of these two elements. The existence of toxic metal-P<sub>i</sub> interactions has been reported by Ernst (1968) and Baker



(1978c) for Zn. In contrast to the work reported here for Fe, Baker (1978c) found that where the P-content of the above-ground plant parts increases, the relative and absolute amounts of Zn in the non (Zn) -tolerant population of Silene maritima decreased. Ernst (1968) reported a similar trend in shoots of Thlaspi alpestre for the same metal. However, different species will have different responses to toxic metals in solution. Metal-phosphorus interactions are likely to vary according to the nature of the toxicity and the metal in question.

The difference in the maintenance of total P concentration between populations suggested population differentiation in P-nutrition in Eriophorum (from the two sites studied). The mine population being able to tolerate low P supply and maintain constant values over a range of Fe concentrations. Ca content of tissues from plants from both sites did not show this trend in agreement with the observations made in Chapter 2.

4.4.3 Response of Eriophorum angustifolium to increasing Manganese concentration.

4.4.3.1 Differences in population response to high Manganese concentrations

Although both populations responded by a decline in biomass with increasing Mn supply to the roots (in agreement with the findings of Heenan and Cambell (1980) for soybean cultivars and Horiguchi (1988), who observed a slight decrease in rice shoot dry weights with increasing Mn concentrations up to 3000 ug/g), the Parys population appeared to be less tolerant than the Skipwith population. Mn tolerance in Eriophorum has also been reported by Nazrul-Islam (1976).

The most significant growth reductions occurred only in the maximum Mn treatments but there were no mortalities which could be attributed to Mn toxicity (some root tip blackening was visible but had no dramatic effect on the growth of the plant). The differences between the two populations may be due more to differences in yield (Parys plants were smaller and

weighed less) than to the effects of excess concentrations of Mn. However, differences in the sensitivity of the shoot to increasing concentrations of Mn are implied. Reduction in biomass of the shoot was mirrored by a corresponding increase in Mn content of the shoot. Similarly, Martin (1968) reported that Mn toxicity; manifested as interveinal spotting and necrosis of leaf tissue, in Mercurialis perennis grown in water cultures, affected shoots but not the roots, which continued to grow even at the highest Mn concentrations (> 20 mg/l Mn). Mn unlike Al for example, does not injure the roots directly but affects the shoot (Marschner 1986) regardless of the Mn tolerance of the species or cultivar. The nature of Mn resistance in plant tissues in this study is not clear but Heenan and Carter (1976), for soybean cultivars, related genotypical differences in Mn tolerance not to the differences in uptake or transport in the shoot but to the Mn tolerance of the shoot tissues.

A reduction in Mn concentration in plant tissues at 25

mg/l external Mn concentrations preceded the increase in sensitivity to Mn in the two populations at 50 mg/l Mn. Regulation of internal concentrations by the plant through reduction in Mn uptake may be effective at intermediate Mn concentrations. Further work is required to confirm this observation. Mn resistance in Medicago species and rice was attributed to decreased uptake by Robson and Loneragan (1970) and Horiguchi (1987). Nazrul-Islam (1976) also attributed differences in Mn tolerance to the concentration of Mn in the shoot. The lower sensitivity of Phalaris arundinacea and to a lesser degree, Eriophorum compared to the other plants in his study was attributed to low Mn concentrations in the shoots. The major difference between these studies lies in the maximum concentration of Mn used. In the latter investigations concentrations were limited to 32 and 20 mg/l Mn respectively, which were close to the value for which reduction in uptake was observed.

It is possible to conclude Eriophorum is Mn

resistant and the degree of tolerance is probably related to the shoot. However, in disagreement with Heenan and Carter (1976) differences in uptake and transport to the shoot appear to provide a further level of resistance in some species. The formation of a plaque (possibly of oxidized Mn) was found on the roots of Eriophorum at high Mn concentrations, however, the plaque on the roots were fragile and were lost through washing but may explain the lack of significant change in root Mn concentrations. Engler and Patrick (1975) and Horiguchi (1987) both reported the significance of increased oxidation of Mn by roots in the possible reduction of Mn in rice.

#### 4.4.3.2 Calcium content of plant tissues

Of particular importance for plant growth in acid mineral soils is the inhibition of Ca and Mg uptake by high Mn concentrations. Ca deficiency is a well known symptom induced by Mn toxicity in dicotyledonous plants such as cotton (Foy et al., 1981). Ca content of plant tissues

declined with increasing Mn concentrations in this experiment. Horst-Marschner (1978) similarly found that where the supply of Mn was excessive, the translocation of Ca into the shoot apex was inhibited. This was attributed to the possible effect of Mn on the cation exchange capacity of the leaf tissue.

#### 4.4.4 Response of Eriophorum angustifolium to increasing concentration of Copper

High nutrient concentrations may have masked any immediate effects of high Cu concentration on biomass yield in Eriophorum in both populations. Although no immediate effect on plant growth was observed, there was a significant population difference in terms of Cu uptake. Mine plants had relatively low tissue concentrations up to 1 mg/l Cu before showing an increase in uptake. Contrastingly, the non-mine population showed greater Cu accumulation over the range of concentrations in comparison to the mine population. The

two populations may show differential Cu resistance, but longer-term experiments with a greater range of concentrations are required. The Cu tolerance of populations of Agrostis tenuis from Parys Mountain is well known (Smith and Bradshaw 1972, Karataglis 1980(a and b), Humphreys and Nicholls (1984), and it is not surprising that Eriophorum from the same site appears to show a similar resistance to elevated Cu concentrations. Further differentiation between the two populations may be detectable if the plants are grown in low nutrient solutions as observed earlier in this chapter (differences in P uptake). This may have a greater significance to the resistance of these plants to Cu.

Cu toxicity is manifested in the root as a reduction in cell division and elongation leading to reduced root growth (Karataglis 1980d). Cu toxicity in the study plants was limited to occasional blackening of the root tips suggesting that Cu concentrations were insufficient (in full strength Rorison's solution) to have significant effects on growth. However, inhibition of root elongation and damage to root

cell membranes are immediate responses to a large Cu supply (Karataglis 1980c, 1980d, Karataglis & Babalonas 1985; Symeonidis, McNeilly and Bradshaw 1985). It follows that if root growth is reduced then other factors related to the root might also be affected. Ca concentrations of plants from the non-mine population was found to decline at 1 mg/l Cu treatment, whereas absorption by the roots of the Parys population appears to have been enhanced. It is possible that although no response in biomass in the non-mine population was found, the more immediate effects were at the level of the root membrane, this being reflected in the decline in Ca absorption.

#### 4.5 Conclusion

The results from this study suggest that the nature of metal resistance in Phragmites to Fe and Eriophorum to Fe, Mn, and Cu is general and not limited to a specific mechanism. At low Fe supply, Fe absorption by plant roots



exceeds adsorption as the supply does not exceed demand by the plant. At high external concentrations adsorption and immobilization of Fe onto the surface of roots (plaque formation) are more significant but are not sufficient to prevent the uptake of Fe which concentrates in the shoot. Rhizosphere oxidation and plaque formation may reduce the entry of Fe at intermediate levels but at high Fe concentrations this process is ineffective and tolerance mechanisms may be more important. There was evidence that Eriophorum from the Skipwith population could have a slightly higher requirement for Mn.

There was some evidence for ecotypic differentiation between the non-mine (Skipwith) and the mine (Parys) populations in their response to high Mn and Cu concentrations. The Parys population was more tolerant to Cu but was more sensitive to Mn. The reverse was true in the Skipwith population.

The data presented in this study suggest that the reduction in growth of the plants may be due to the result of

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nutritional stresses rather than the toxic effect of Fe per se. Population differentiation in P and Ca nutrition may be evident. The non-mine population had a higher P and Ca requirement than the mine population but the concentrations used in this study were sufficient to support the higher P and Ca demands of the Skipwith plants.

The role of Fe plaque in the exclusion or reduction of mineral nutrient uptake is obviously an important one. The significance of P and Ca in metal absorption at high metal concentrations is an area of study that requires further attention. It is the aim of the following chapters to examine these subjects in more detail.

## CHAPTER 5

EFFECT OF NUTRIENTS ON IRON UPTAKE AND GROWTH OF  
ERIOPHORUM ANGUSTIFOLIUM AT DIFFERENT CONCENTRATIONS OF  
IRON.

### 5.1 Introduction

Ottow et al. (1983) suggested that most Fe-toxic soils in the tropics were characterized by low and deficient levels of available P, K, Ca and Mg. The lack of, or an unbalanced supply of, available P, K, Ca and Mg was thought to be responsible for an uncontrolled influx and uptake of Fe in rice (Benckiser et al. 1984) caused by an increase in root permeability. If a multi-nutritional stress rather than a low pH and a high Fe supply are responsible for Fe toxicity, then the application of P, K, Ca and Mn is likely to prevent or mitigate the effects of the excessive uptake of Fe.

The investigation of heavy metal tolerance in plants has given rise to many reports of Ca reducing the uptake and toxicity of metals, both in the soil (Cox & Rains 1972, John & van Laerhoven 1972, Simon 1978, Karataglis 1981) and from solution cultures (Wilkins 1957, Jowett 1959, Wainwright & Woolhouse 1977, Baker 1978c, Simon 1978, Garland & Wilkins 1981). However, the results of studies where Ca is often applied as lime to soils may not be directly comparable to culture solutions where Ca is often supplied as calcium nitrate under controlled pH conditions. The addition of lime either as calcium hydroxide or as calcium carbonate (ground limestone) direct to a soil initially raises the pH by neutralizing the free hydrogen ions in the soil solution (Etherington 1982). Therefore the effect of liming is often likely to be an effect of pH rather than directly attributable to Ca concentration.

The effect of Ca appears largely independent of pH-related phenomena but the actual mechanisms of Ca alleviation of metal toxicity remain obscure (Baker 1978c).

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The same author found that the influence of Ca in the accumulation of Zn by Silene maritima in solution could be stimulatory, but more often it is inhibitory in nature, particularly for heavy metals (Rashid, Chaudhry & Sharif 1976, Kannan & Ramani 1978). Baker (1978c) found that increasing external Ca concentration led to an enhancement of Zn uptake by the root of Zn-tolerant Silene maritima and a decline in root Zn levels in the non-tolerant plants. By contrast, there was a decline in shoot Zn concentrations for both populations. The stimulation by Ca of Zn uptake by plants of the tolerant population mentioned earlier was thought to reflect an involvement of Ca in the Zn tolerance mechanism sited in the roots. In contrast, Simon (1978) found that Zn tolerance in Festuca ovina was independent of the ratio of Zn to Ca but in some populations of this grass Pb/Ca interactions were of greater significance in determining the toxicity of the substrate.

The interaction between Fe and P is perhaps better

understood. High concentrations of Fe have been reported to cause the removal of P from nutrient solution by precipitation as insoluble ferric phosphates (Jones 1975), leading to a reduction of P in solution. Howeler (1973) suggested that the formation of oxidized Fe coatings on the surface of rice roots may diminish the capacity of roots to absorb essential nutrients. This was also suggested by Chen, Dixon & Turner (1980) for the same species. Thus Fe in the root tissue may induce P-deficiency and limit growth of the root and shoot.

Shaw (1984) determined the tolerance to Pb of a range of limestone species using three culture solutions, 0.1-strength Rorison, 0.1-strength Rorison solution minus P and 0.5 g/l calcium nitrate. All the populations studied (Pb-mine and pasture) were found to be most sensitive to the nutrient solution without P. The toxicity of Pb, for example has been shown to change inversely with the P concentration of the nutrient solution or soil and the P status of the plant (Miller & Koeppel 1971, Rolfe 1973). Koeppel (1981) cited

evidence to suggest that the effects of Pb were indirect through the deprivation of P.

In experiments reported in the previous chapter, high external supplies of Fe were found to reduce plant uptake of P and Ca. It was the aim of the first investigation reported in this chapter to determine whether it was the effects of Fe toxicity per se, or the disruption of P and Ca nutrition that induced a reduction in the growth of plants studied in Chapter 4. Secondly, if P and Ca (and other nutrients) absorption by plants can be enhanced, can this reduce the uptake of Fe or otherwise ameliorate the effects of Fe toxicity ?

The final section of this chapter investigates in more detail the possible effects of Ca on the regulation of Fe absorption by plants and on the alleviation of Fe toxicity.

## 5.2 Materials and Methods

5.2.1 A split root technique for investigating nutrient uptake in plants of Eriophorum angustifolium in the presence of elevated concentrations of iron

### 5.2.1.1 Method

This culture method could not be applied to Phragmites australis because of its rhizomatous nature and therefore this species was not included in this study.

The basic design used for split root culturing of Eriophorum is illustrated in Figure 5.1. Vegetative offsets (20 weeks old) of Eriophorum from mine (Parys) and non mine (Skipwith) populations were pre-cultured in 0.1 strength Rorisons solution under standard conditions prior to the experiment. Each offset (derived from long-term culture stocks) was placed across two x 1 litre plastic bottles (with the top 5 cm removed). The plant and the bottles were held



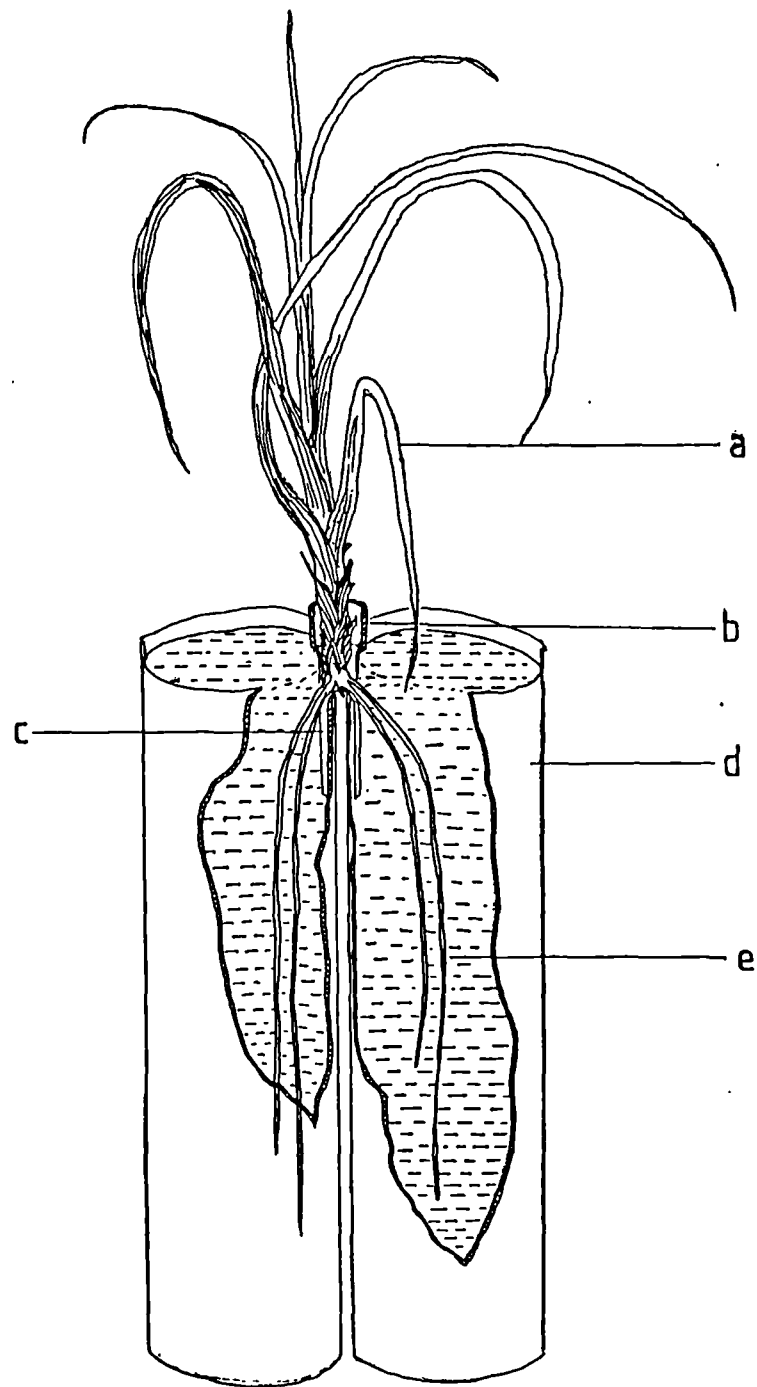


Figure 5.1

Split-root design for supplying plants with nutrients and iron without interference of iron with nutrient uptake showing (a) plant of Eriophorum angustifolium, (b) plastic pipette with distal end removed, (c) strip of filter paper, (d) litre plastic bottle and (e) plant roots.

in position by the base of a plastic pipette tip slit at the sides to fit across the cut edges of the bottles. The offset was held in place within the base of the pipette end by non-wetting cottonwool. The base of the plant rested on top of a strip of filter paper (4 x 1 cm wide) which divided the roots, and by capillary action, prevented the top part of the roots from drying out in the event of a reduction in solution level. The roots were distributed evenly between the two vessels. Duplicates of each treatment pair (2x2 vessels) with an offset from each population were placed in blackened plastic tubs filled with distilled water ( to reduce the problems of warming of a small body of water and the possible effects of temperature on the absorption of nutrients by the roots ). The top of each unit was then sealed with aluminium foil providing a reflective surface in a further effort to reduce warming and evaporation of the culture solutions. Solutions were not aerated.

Plants were left to equilibrate for 14 days under standard growth conditions (refer to conditions used to

culture all the plants, Appendix I and II ). During this period both vessels were supplied with 0.1 strength Rorisons solution and changed every two days. To reduce the risk of disturbance and mechanical damage to plant roots the solutions were removed by siphoning at each replenishment. Following this, plants were treated with the following combinations of solutions:

i) Controls, where both sides of the roots were supplied with either 3.8 mg/l Fe or 100 mg/l Fe (ie 3.8/3.8 and 100/100)

ii) One side of the plant (roots) was supplied with 3.8 mg/l and the other side was supplied with 100 mg/l Fe (ie 3.8/100). This supplied one half of the roots with 0.1-strength Rorisons solution in the absence of a high background Fe concentration while exposing the other half of the root to high Fe concentrations. The aim was to reduce loss of nutrients (ie P) by precipitation and possible interference of nutrient uptake by high background concentrations of Fe. All plants were grown in 0.1-strength

Rorisons solution at pH 4.0 (adjusted by addition of 1M HCl or NaOH) with Fe applied at the concentrations presented above.

#### 5.2.1.2 Experimental design

Populations (2) x treatments (3) x replicates (5) x harvests (1) x tubs (15) (with 2x2 1 bottles). Total number of plants (30).

#### 5.2.2 The response of Eriophorum angustifolium to changes in the ratio of calcium to iron

##### 5.2.2.1 Method

Fifteen week-old seedlings were grown in blackened 2 litre plastic containers (Stewart Plastics ltd) on 'styrofoam' floats. Each plant was secured by heavy duty black plastic strips and inserted via cuts in the sides of the float. A modified Rorison solution (Appendix I) was used omitting P. (Preliminary work indicated that at full strength, nutrient

solutions with Fe concentrations equal to or in excess of 100 mg/l Fe, large-scale precipitation of ferric phosphates effectively reduced available Fe concentrations to less than a fraction of the original concentration. As the experiment was relatively short-term, it was considered not essential to include P additions in the feeding solutions. It was assumed that plants adequately supplied with P prior to the experiment would contain sufficient amounts to meet tissue demands over the period. McCain and Davies (1983) stated that detrimental effects of early P deficiency could not be overcome by an adequate later supply but in contrast, an early supply can largely 'buffer' against later P deficiency (Rorison 1968). (A P 'carry over' effect was observed by the former authors in Agrostis capillaris in support of this assumption).

All nutrients with the exception of Ca and Fe were maintained at a constant concentration equal to full strength Rorison solution. The choice of full nutrient concentrations for this study was made so as to remove the possibilities of

other nutrient deficiencies affecting the responses of the plants.

The experiment was conducted in a controlled environment room under the following conditions: 20°C, 16 hr. day; 15°C, 8 hr. night. Maximum photon flux was 91  $\mu\text{mol m}^{-2}\text{s}^{-1}$ . Solutions were changed every 48 hrs and losses incurred through evapo-transpiration were replaced by additions of distilled water. A value of pH 4.0 was maintained on a daily basis (by addition of either 1M HCL or 1M NaOH). Plants were grown under these conditions for 21 days. Following this period, plants were washed thoroughly in distilled water (3 rinses) and blotted dry. Root and shoot were separated and dry weights determined.

#### 5.2.2.2 Treatments

Plants were grown at 0.08, 0.8, 8 and 80 mg/l Ca with additions of either 3.8, 100 or 1000 mg/l Fe. Ca concentrations were equivalent to values found in 0.001, 0.01, 0.1 and full

strength Borison solution.

### 5.2.2.3 Experimental design

Populations (2) x treatments (12) x replicates (5) x harvests (1).

Total no of tubs	36
Total no of plants	144
Total no of plants used	120

One additional plant was grown per treatment and for each population as replacements for possible losses. All pots were fully randomized.

### 5.2.3 Digestion and analysis of plant material

Root and shoot material was digested using the  $\text{HNO}_3/\text{HClO}_4$  method (Appendix III). Digested samples were centrifuged and analyzed for Fe and Ca by flame-AAS on a Perkin-Elmer 3030 with autosampler. P-determinations for the split-root

experiment were made colorimetrically (appendix III).

#### 5.2.4 Data analysis

Total (calculated on the basis of root and shoot values; see section 4.2.4), shoot, root and shoot/root concentration ratios were calculated for the data and expressed on a ug/g dry weight basis.

The data were transformed using the Genstat statistical package. Full Analyses of Variance (fully randomized design) were executed on all the data sets. For ease of interpretation the values on the y-axis have been back-transformed. Unless otherwise stated significance was tested at the 5% level ( $p < 0.05$ )



## 5.3 Results

### 5.3.1 Experiment 1: Nutrient uptake by Eriophorum angustifolium in the presence of excess iron.

Individual data suggested an inhibition in root growth with respect to the shoot for the full Fe (100/100) treatment (Figure 5.2 d). No trends were observed for dry weight data other than a slight increase in shoot/root ratios at the intermediate split root treatment (3.8/100) (Figure 5.2 a, b, c).

Shoot Fe concentrations (Figure 5.3 ib) were highly significantly different between treatments ( $p=0.001$ ) and increased with increasing external Fe supply ie 3.8/3.8 < 3.8/100 < 100/100. In contrast, root Fe concentrations reached maximum values in the split low/high Fe treatment (3.8/100) (Figure 5.3 ic). Intermediate values were found in roots treated with high Fe concentrations on both 'sides' (100/100) while minimum values were recorded for the 3.8/3.8 split treatment. Shoot/root concentration ratios of

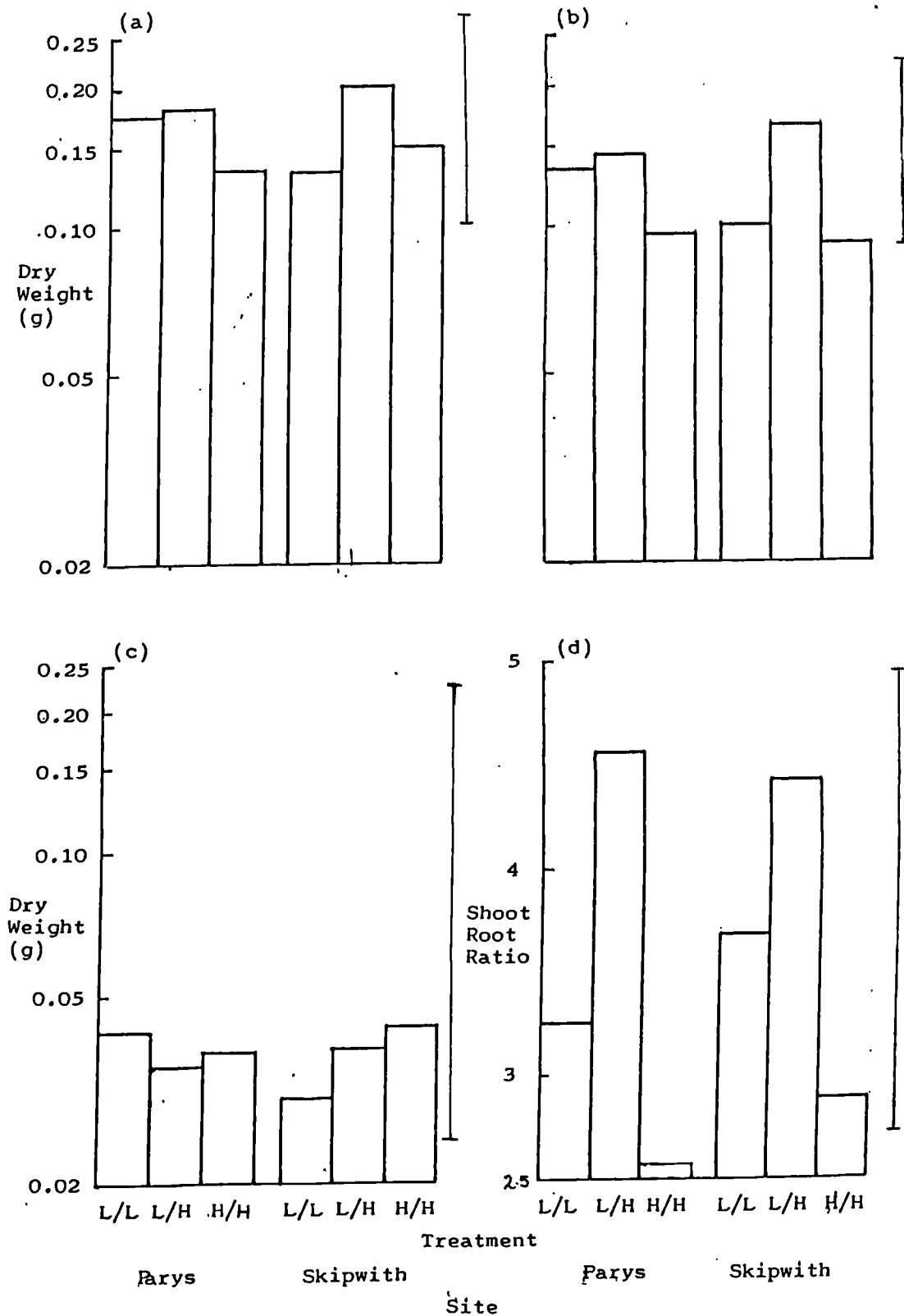


Figure 5.2

Dry weights (g) of (a) whole plants, (b) shoots, (c) roots and (d) shoot/root concentration ratios of *Eriophorum angustifolium* from Parys Mountain and Skipwith Common grown with each half of the root exposed to either (L) 3.8 mg/l or (H) 100 mg/l iron. (Vertical bars represent Least Significant Differences.) ( $p < 0.05$ ).

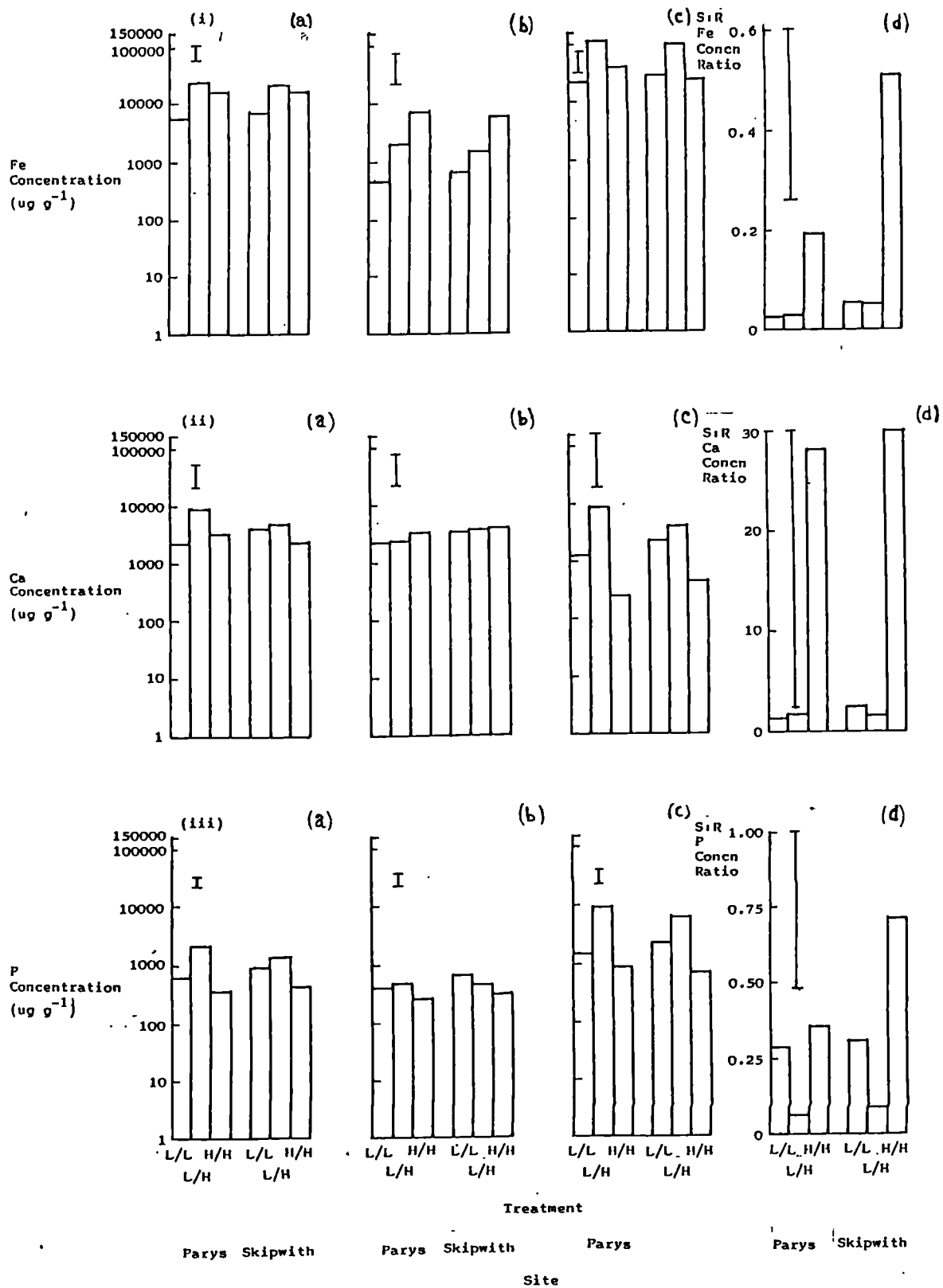


Figure 5.3

Concentrations of (i) iron (Fe), (ii) calcium (Ca) and (iii) phosphorus (P) in plants of *Eriophorum angustifolium* from Parys Mountain and Skipwith Common grown with each half of the root exposed to either (L) 3.8 mg/l or (H) 100 mg/l iron (Fe). (a) whole plant, (b) shoot, (c) root and (d) shoot/root concentration ratio. (Vertical bars represent Least Significant Differences.) ( $p < 0.05$ ).

Fe concentration suggested localization of Fe in shoots rather than roots in the 100/100 Fe treatment (Figure 5.3 id). The split-root low Fe/high Fe treatment was not significantly different from the low Fe control treatments for Eriophorum.

Maximum root Ca concentration was found in plants where the roots were supplied with both 3.8 and 100 mg/l Fe (Figure 5.3 iic). Intermediate values were found in plants grown in 3.8 and 3.8 mg/l Fe and minimum concentrations in plants supplied with high Fe (100/100). Contrastingly, shoot Ca concentration was not significantly different amongst treatments (Figure 5.3 iib). Shoot/root Ca concentration changed only with the high Fe treatment (100/100) with a high shoot/root ratio (Figure 5.3 iic). No population difference was observed for either Fe content or Ca concentration.

Total P-concentration of the plants showed a similar trend to that observed for the root (see below) but with a significant population difference. The non-mine population (Skipwith) treated with 3.8/100 Fe had a higher total-P

content than in the remaining treatments. The same was also true for the mine population (Parys) but the differences were more pronounced (Figure 5.3 iia).

Shoot P concentration showed a significant reduction across the treatments in the order of 3.8/3.8 > 3.8/100 > 100/100 (Figure 5.3 iib). There was an inverse relationship between shoot P concentration and Fe concentration. Root P concentration, however, was significantly greater ( $p < 0.001$ ) in the split 3.8/100 Fe treatment, with a decline in P concentration in the Parys plants treated with the high Fe treatment (100/100) (Figure 5.3 iic).

### 5.3.2 Experiment 2: Response of Eriophorum angustifolium to changes in the ratio of Calcium to iron

Plants of Eriophorum angustifolium subjected to 100 and 1000 mg/l Fe and grown with 80 mg/l Ca showed slight reductions in biomass compared to the remaining treatments ( $p < 0.05$ ). At the highest Fe concentrations slightly greater dry weights were found in plants growing in 8 mg/l Ca. Total

plant dry weights were significantly lower in the 0.08 and 0.8 mg/l Ca and 1000 mg/l Fe treatments (Figure 5.4 i).

Shoot dry weight did not change significantly over the range of Ca concentrations with either 3.8 (control) or 100 mg/l Fe added (Figure 5.4 ii). No population difference was evident. There was only a reduction in dry weight of plants treated with 1000 mg/l Fe. As the response was the same across the range of Ca concentrations, Fe toxicity is implicated rather than a response to Ca availability or a possible interaction between Ca and Fe. There was a significant concentration effect on root dry weight of plants from both populations, with the exception of the controls where there was no response effected by a reduction in Ca concentration (Figure 5.4 iii).

The ratio of shoot to root dry weight data emphasized a highly significant ( $p=0.001$ ) population difference not previously evident. At intermediate Fe concentrations and decreasing external Ca concentrations a reduction in the shoot/root ratio was apparent in the Parys population but no

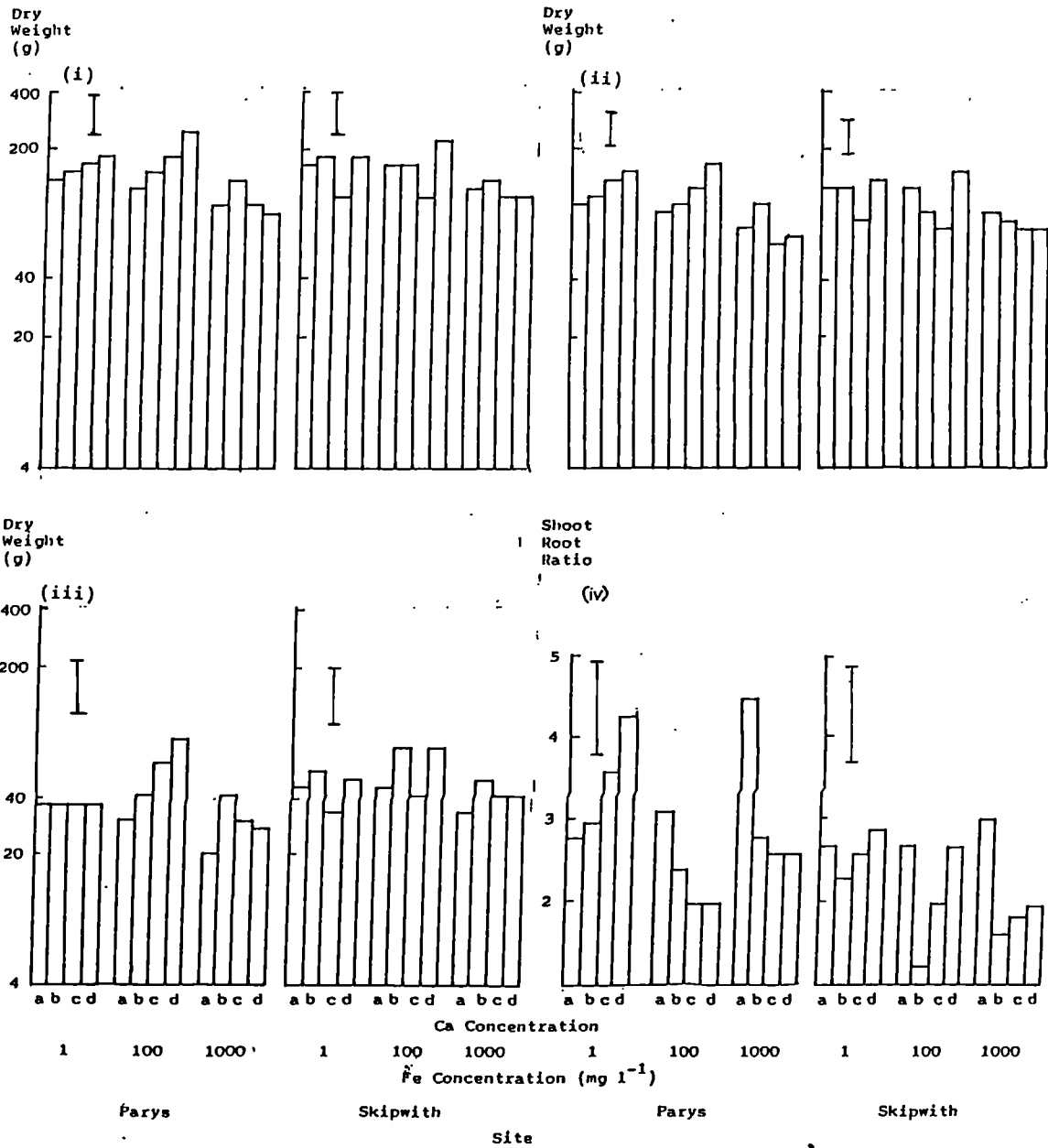


Figure 5.4

Dry weights (g) of (i) whole plants, (ii) shoots, (iii) roots and (iv) shoot/root concentration ratios of *Eriophorum angustifolium* from Parys Mountain and Skipwith Common exposed to 3.8, 100 and 1000 mg/l iron and (a) 80, (b) 8, (c) 0.8, (d) 0.08 mg/l calcium. (Vertical bars represent Least Significant Differences.). ( $p < 0.05$ ).

change was evident in the Skipwith population. At the 1000 mg/l Fe and 80 mg/l Ca treatment high shoot/root ratios were observed in the mine population. At lower Ca concentrations and high Fe concentrations, there was a reduction in shoot/root ratios in the same population. However, individual data suggest that the reduction in shoot/root ratio was due to a reduction in root dry weight and not a decline in shoot biomass. No such changes were evident in the Skipwith population (Figure 5.4 iv).

High external Ca concentration produced a significant decline in Fe content of the shoot for the Parys population only (Figure 5.5 ii). Root Fe data were less variable than those recorded for the shoot (Figure 5.5 iii). Minimum Fe concentrations were found in the controls and these did not differ between populations. High root Fe corresponded with increasing Fe supply rather than to changes in the availability of Ca. Total plant Fe followed the same trend outlined for the root. Shoot/root ratios showed an increase with increasing Fe concentration but did not show easily



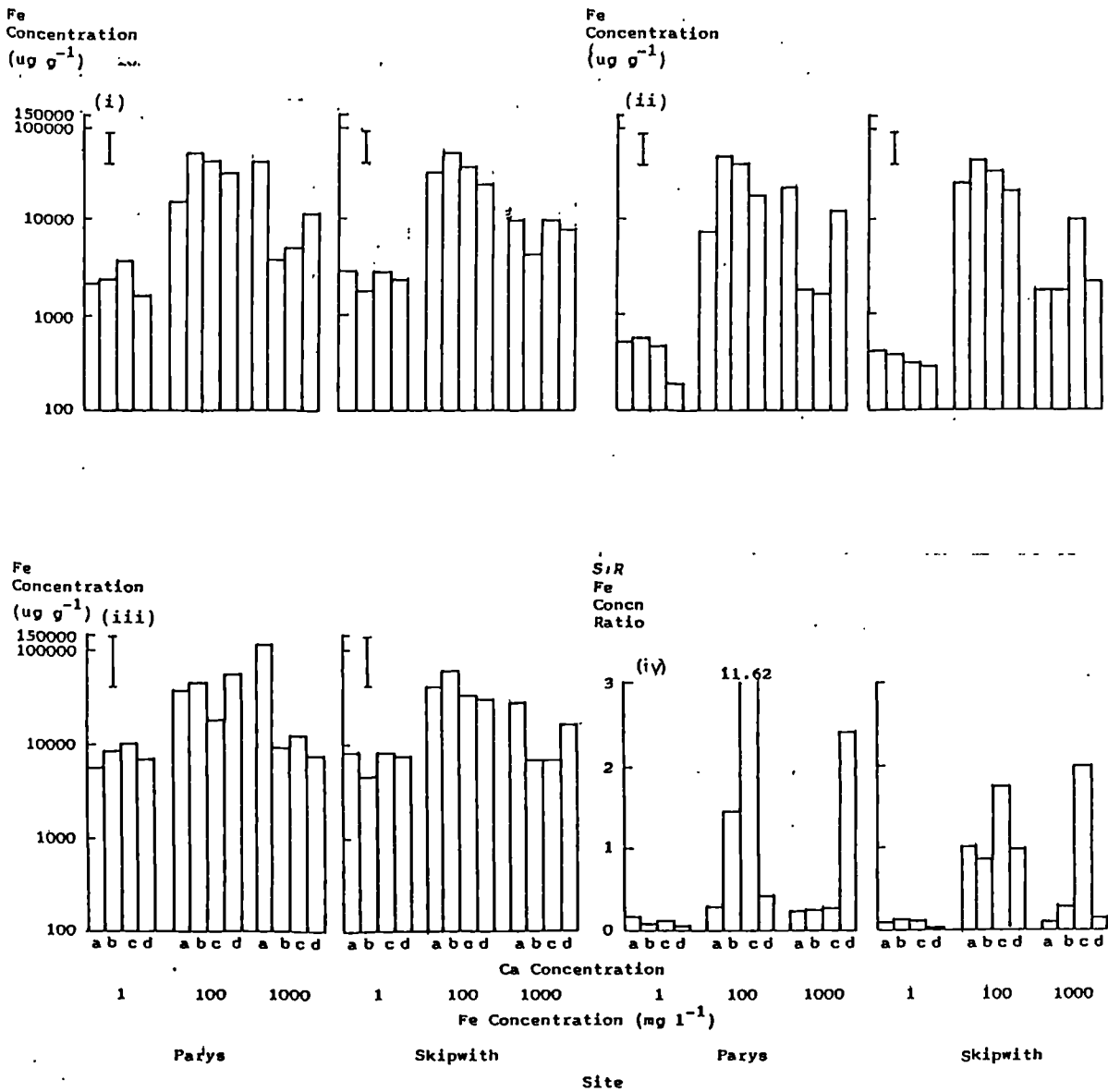


Figure 5.5

Iron concentrations (ug/g) of (i) whole plants, (ii) shoots, (iii) roots and (iv) shoot/root concentration ratios of *Eriophorum angustifolium* from Parys Mountain and Skipwith Common exposed to 3.8, 100, 1000 mg/l iron and (a) 80, (b) 8, (c) 0.8, (d) 0.08 mg/l calcium. (Vertical bars represent Least Significant Differences.). ( $p < 0.05$ ).

interpretable trends with an increase in Ca availability (Figure 5.5 iv).

For the Parys population total Ca concentration of plant tissues were constant at 8 and 80 mg/l Ca but declined thereafter (Figure 5.6 i). This was not observed for the Skipwith population. In the shoot, Ca content of plant tissues remained constant despite decreasing Ca and increasing Fe concentration (Figure 5.6 ii). Root Ca content declined in both populations at 1000 mg/l Fe but did not differ significantly at other concentrations of Fe (Figure 5.6 iii). A decrease in Ca availability had no apparent effect on the Ca content of roots.

Healthy plants were observed in all the control and the control + 100 mg/l Fe treatments. A light precipitate was present on roots treated with 100 mg/l Fe. In contrast, a heavy precipitate was present on the roots of plants treated with 80 mg/l Ca and 1000 mg/l Fe and some root tips were also blackened. Extensive blackening was observed in the lower leaves.

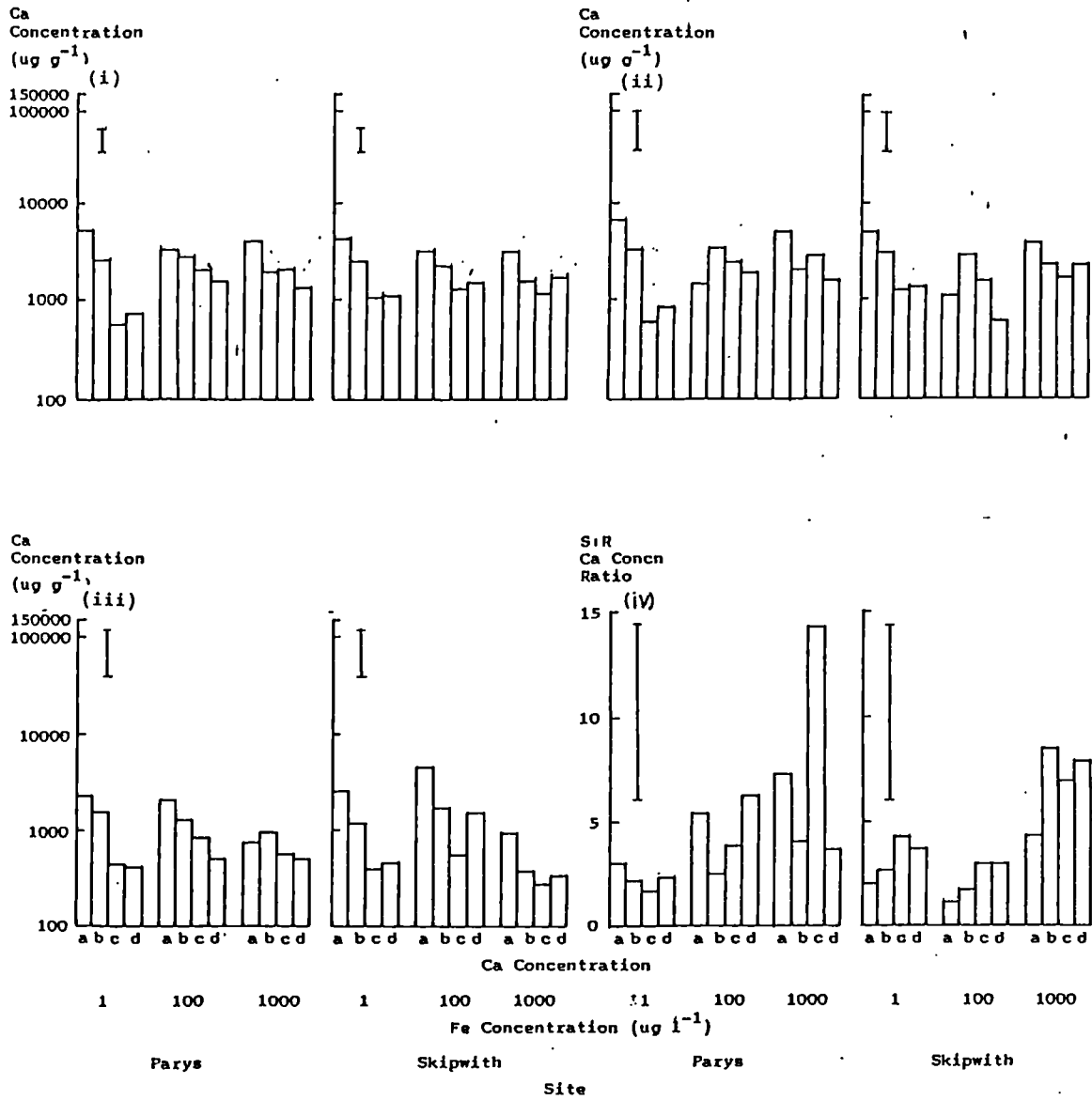


Figure 5.6

Calcium concentrations ( $\mu\text{g/g}$ ) of (i) whole plants, (ii) shoots, (iii) roots and (iv) shoot/root concentration ratios of *Eriophorum angustifolium* from Parys Mountain and Skipwith Common exposed to 3.8, 100, 1000 mg/l iron and (a) 80, (b) 8, (c) 0.8, (d) 0.08 mg/l calcium (Ca). (Vertical bars represent Least Significant Differences.). ( $p < 0.05$ ).

## 5.4 Discussion

### 5.4.1 The effect of increased nutrient supply on iron uptake in Eriophorum angustifolium

High concentrations of Fe have been reported to result in removal of P from nutrient solution by precipitation as insoluble ferric phosphates (Jones 1975). Somers & Shive (1942) suggested that when Fe is absorbed by plants in large quantities, it combines with P and its translocation to shoots is inhibited. Since excess absorption of Fe was found to reduce P concentration in plant tissues in the previous chapter a close relationship between Fe concentration and P uptake in plants was suggested. P-deficiency and Fe toxicity appears to be closely linked (Benckiser et al. 1984).

In view of the earlier observations, it was deemed important to observe the relationship between P and Fe in the absence of precipitation to investigate whether or not the symptoms of Fe toxicity were primarily due to the effects of induced macronutrient deficiency rather than to Fe

toxicity per se. Ca deficiency has also been included as a possible symptom often attributed as Fe toxicity (Ottow et al. 1983). This condition was induced experimentally using the split-root design outlined above (section 5.2.1).

The problem of Fe precipitation (suspected as Fe-phosphates) observed in the full Fe treatment (100/100), was effectively eliminated in the split-root low Fe/high Fe treatment and confirming initial predictions. The only major flaw in the design was thought to be that only one half of the root would be exposed to high Fe, only half the concentration of Fe might be available to plants of Eriophorum compared to solutions where all the roots were exposed the high Fe concentrations (100/100 mg/l Fe treatment). Thus, Fe concentrations in plant tissues might be reduced without amelioration of Fe toxicity by, for example, P and Ca or alleviation in nutrient deficiency. However, plants in the split-root low/high Fe treatment had significantly higher Fe concentrations in the roots than the

control plants so this problem was immaterial in the light of the results.

The two week treatment period in this study was not sufficiently long to produce large differences in biomass, except for a slight increase in shoot dry weight in plants treated with 3.8/100 mg/l Fe and a reduction in shoot biomass in the 'full' Fe treatment. However, it was sufficient to note an enhancement of shoot growth in the presence of higher nutrient availability. Fe content of the shoot in plants treated with 3.8/100 mg/l Fe was not significantly different from the low Fe control treatments. In the light of the comments above, this suggests a depression in Fe uptake. Benckiser et al. (1984) also found that fertilization of rice plants with K, Ca and Mg alone or in combination decreased the uptake of Fe. In nutrient-starved rice plants grown in 300 mg/l Fe there was a nearly two-fold increase in Fe concentration in relation to well-nourished plants grown in complete nutrient solution. In contrast to the effects of K, Ca and Mg, Benckiser et al. (1984)

found that the application of P alone stimulated the accumulation of Fe in culms and leaves of rice plants. No stimulation of Fe uptake was observed in shoots of plants in this study, as Fe concentrations remained intermediate between those of the controls.

Roots of plants in the present study concentrated more Fe with an increase in nutrient availability. P may be implicated in this particular response by the plants. As in all the experiments so far described, Fe-plaque was not removed, and no distinction was made between adsorbed Fe and Fe absorbed by plant roots. However, assuming the Fe content of the shoot is representative of total Fe uptake in the plant, the Fe concentration of roots of plants treated with 3.8/100 mg/l Fe should also be intermediate in value. This was not the case: the Fe content of roots of plants in this treatment was significantly greater than the high Fe treatment (100/100). The results therefore implicate an increase in Fe adsorption and Fe oxidation by plant roots. In addition, Fe concentrations of shoots of plants treated in

the split-nutrient regime was similar to plants grown at low Fe concentrations. Collectively, the results imply that plants supplied with adequate nutrients are able to regulate the uptake of excessive concentrations of Fe. The iron-excluding power (the selective absorption or exclusion) of Fe by rice roots at both low and high Fe concentrations, and the Fe-retaining power (the ability to retain Fe in the roots without excessive translocation of Fe to the shoot) described by Tadano (1975) were found to be lower in plants deficient in P, Ca, Mg, K and Mn than in plants where the nutrient supply was adequate. The combination of nutrient deficiency and a reduction in the capacity of rice plants to regulate the uptake of Fe, resulted in high Fe concentration in shoots.

#### 5.4.1.1 Phosphorus concentration of plant tissues

Root and total P concentrations of plants grown in the high Fe regime were significantly lower than in plants



grown in either the low or the split-nutrient regime. This confirmed that P uptake was affected by Fe concentration. P absorption can be enhanced by increasing the root surface area or the rate at which P reaches the root surface (Gardner, Barber and Parbery 1983). If half the surface area is removed (eg division of plant roots into equal portions in the 3.8/100 mg/l Fe treatment), P absorption should also be reduced. However, this was not found. P concentrations of roots of plants grown in the split-nutrient regime were in excess of values for the 3.8/3.8 mg/l Fe controls ('high' P). Enhanced P uptake was evident in the root only; shoot concentrations of P in the split-nutrient regime were intermediate between the low Fe and the high Fe regimes. Waldren, Etherington and Davies (1987) associated an increase in P content of the roots in flooded Geum urbanum with a corresponding decrease in leaf P, in apparent agreement with the observations made in this study. This is further substantiated by the shoot root concentration ratios presented for Eriophorum where a reduction in shoot P

coincided directly with a marked increase in P concentration of the root.

Increasing the concentration of P from 1-50 mg/l was found to reduce the concentration of radiolabelled Fe ( $^{59}\text{Fe}$ ) in leaves and stems of watercress (Cumbus, Hornsey and Robinson 1977). Similarly, increased application of P was reported to reduce the concentration of Fe, Mn, Zn and N in the foliage of loblolly pine (non-flooding tolerant) by Hock, Debell, McKee and Askew (1983). Loblolly pine under anaerobic conditions was suggested to have incurred an ion imbalance between shoot and root, resulting in toxic levels of some elements in the shoot. P was thought to have beneficial effects, possibly related to lessening the apparent ion imbalance.

In the present study, increased P in the roots of plants grown in 1/100 mg/l Fe coincided with an increase in Fe concentration and plaque formation in the roots of the same plants. The lack of transport from the root to the shoot of accumulated P in the roots, would strongly suggest that it

has been precipitated either internally or adsorbed onto the surface of the roots possibly as a precipitate of ferric phosphates. The net result is a lack of enhanced uptake of Fe into the shoot, in general agreement with the observations of the previous authors.

Rice plants deficient in P are more susceptible to Fe toxicity than plants grown with an adequate nutrient supply (Tadano 1975). Plants of Eriophorum were not exceptional in this case. P appears to have a dual role in the capacity of Eriophorum to resist Fe toxicity in culture solutions: firstly, a direct role in the resistance of these plants to high Fe concentrations, through precipitation of Fe, possibly as ferric phosphates, effectively reducing the concentration of Fe for uptake by the plant, secondly, at a nutritional level, an adequate P-supply appears to contribute to the plant's capacity to regulate Fe uptake. However, almost all sites (including Fe enriched ones) where Eriophorum grows are strongly P-deficient (Wheeler 1989), concentrations used in the laboratory exceeded those

likely to be found under field conditions. It was considered necessary to use such concentrations in order to elicit a statistically significant response in Eriophorum plants grown in culture solutions. Although the responses of Eriophorum to changes in the ratio of Fe to P under field conditions may not be statistically significant, these responses could still be biologically significant.

#### 5.4.1.2 Calcium concentration of plant tissues

The distribution of Ca in plant tissues was not as variable with treatment as that observed for P and it was difficult to draw definitive conclusions from these data. Emmanuelsson (1984) stated that increasing Ca supply to roots of Hordeum vulgare increased nitrate uptake which would then affect growth rate, the shoot to root ratio, the transpiration rate, the uptake rate and hence the concentration ratio between nutrients in the shoot. Such a conclusion could not be drawn from the present data due to the limited nutrient (

no N or K ) analysis undertaken. A slight increase in shoot-root concentration ratio for Ca was observed in the split-nutrient regime but this did not correspond to an increase in Ca content of the shoot. It may be that the plants did not experience a significant deficiency in Ca during the experimental period.

Tadano (1975) observed that rice plants grown in nutrient solutions without Ca showed similar patterns of response to high Fe concentration (100 mg/l Fe) as plants grown in the absence of P. However in the present study, plants of Eriophorum were grown with full nutrient solutions even in the split-nutrient regime. Fe concentration was the only major variable. Thus the effect of P or Ca in plant resistance to high Fe concentration cannot be considered in isolation. A collective effect of enhanced nutrient uptake with P having a more 'active' role in plant resistance to Fe toxicity cannot be ruled out in this case. Benckiser et al. (1984) provide evidence for an increase in yield of rice (variety IR22) grown in Fe-toxic soil with the addition of

K,Ca and Mg in combination. Ca and Mg alone had little effect on the yield of the plants. A similar effect was evident in this study.

#### 5.4.1.3 Population response to enhanced nutrient uptake.

No differences were evident between the two populations of Eriophorum with the possible exception at the level of response to enhanced P availability. The lack of significant response may be due to the relatively short duration of the experiment and the age of the plants as they were older than the plants used previously.

#### 5.4.2 The response of Eriophorum angustifolium to changing calcium and iron concentrations

Karataglis (1981) demonstrated that populations of Festuca rubra from an environment with high concentrations of toxic metals and with high concentrations of Ca in the soil, showed very little or no tolerance against these metals. In contrast, populations from other mine sites with 'normal' Ca

concentrations indicated an increase in tolerance against the toxic metals found there. Wilkins (1957) and Jowett (1964) observed that the toxic action of Pb was ameliorated by the addition of Ca ions to water cultures. Proctor (1971) indicated that the presence of Ca at high concentrations ameliorated the toxic action of Ni and Mg.

Earlier work suggested that Ca concentration and availability may be important in the susceptibility of plants to increasing Fe concentration through Ca depletion. The Skipwith soil-sediments were found to be more Ca-enriched than the Parys sediments in the preliminary study. In the laboratory work, Ca concentrations within the plants were found to decline with increasing availability of Fe in solution. In the light of these observations and the observations of previous workers, it was considered possible that Ca might alleviate the symptoms of Fe toxicity which were reduced growth and root stunting.

The results of the present work, however, indicated that in general Ca had little effect on Fe concentrations in plant

tissues, but had a more significant effect on plant dry matter production. The reduction in dry weight of roots and shoots at 1000 mg/l Fe occurred at all Ca concentrations suggesting that Fe toxicity was the major factor affecting plant biomass yield at very high Fe concentrations rather than Ca availability, although at the lowest Ca concentrations reduced Ca availability appeared to have a greater effect on total dry matter production. Benckiser et al. (1984) found that Ca and Mg did not affect the growth of rice plants when applied together in the presence of toxic concentrations of Fe.

High Ca (80 mg/l) and high Fe concentrations (1000 mg/l) resulted in a depression in total dry weight and shoot root ratios of dry weights in Eriophorum from the Parys site. Reduction in root growth may be responsible for the decline in total dry weight and the shoot/root dry weight ratio, suggesting that high Ca concentration may affect root growth in the Parys population. This could be indicative of a difference in the tolerance of plants pre-adapted to



extreme pH, since the Parys plants were found to grow at lower pH in comparison to plants from the Skipwith site. This is further substantiated by the observation that at high external Ca concentrations (80 mg/l) there was a significant decline in shoot Fe concentrations. Reduction in root growth would decrease the capacity of the roots to absorb Fe (through a reduction in surface area), thereby reducing translocation of Fe to shoots, resulting in a decrease in shoot Fe concentrations.

In the results presented, there was little evidence for an interaction between Ca and Fe, affecting the availability of Fe in Eriophorum. Simon (1978), in contrast, found that the vegetation structure (mainly the Violetum calaminariae Schwick.) in heavy metal contaminated sites was related to the interaction in soils between Pb, Zn and other cations such as Ca, Mg and K, the most striking relationship was between Pb and Ca; the ratio of Pb to Ca determining the toxicity of Pb in the soil. Thus, the relative importance of metal/Ca interactions appears to vary, according to metal and plant

species. Tadano (1975) reported that rice plants deficient in K were more susceptible to Fe-toxicity, compared to plants where K was present. The effect of K on susceptibility of Eriophorum to high concentrations of Fe was not studied and it may prove to have a significant effect. The remaining nutrients including K, were maintained in excess of plant requirements to prevent the possibility of other nutrient deficiencies affecting the possible interaction between Fe and Ca. However, this may have reduced the effects caused by changing Fe/Ca ratios, perhaps by lessening the apparent ion imbalance.

Eriophorum from both populations were able to maintain constant tissue Ca concentrations over a wide range of external Ca concentrations and low Fe (1 mg/l). Plants pre-cultured in 0.1 strength Rorisons solution over a long period may be provided with sufficient Ca to meet internal demands, these concentrations being maintained over the duration of the experiment. This could account for the general lack of difference between Ca treatments.

## 5.5 Conclusion

Deposition of what were suspected to be Fe phosphates in the culture solutions were effectively prevented using the split-nutrient regime, where half the roots of plants of Eriophorum angustifolium were exposed to high Fe supply and the remaining roots were exposed to 0.1-strength Rorison solution with a low Fe supply. Near 'normal' nutrient levels could be maintained, without the problem of direct interference by high concentrations of Fe in solution.

Plants deficient in P were more susceptible to high concentrations of Fe than plants with an adequate supply. Thus plants grown at high Fe concentrations showed reduced tissue concentrations of P which coincided with high internal concentrations of Fe. Increasing P availability in the presence of high Fe concentrations stimulated root oxidation of Fe and the accumulation of Fe plaque. This effectively

reduced the uptake of Fe into the shoot.

A close relationship between P and Fe is apparent in culture solutions. Although P/Fe interactions under field conditions may be less apparent and not statistically significant they may nevertheless be biologically significant.

The influence of Ca on the reduction of Fe toxicity is only apparently a limited one. High Fe concentration is more important in causing the reduction in growth of Eriophorum. High Ca (80 mg/l) affected root growth and dry matter production only in the Parys population of Eriophorum and it is suggested that plants from the Parys site may be adapted to conditions of extreme acidity, characteristic of the site, hence their sensitivity to high Ca concentrations. The ratio of Fe to Ca does not appear to be important in effecting the uptake of Fe in Eriophorum.

## Chapter 6

# FACTORS INFLUENCING THE UPTAKE OF IRON BY ERIOPHORUM ANGUSTIFOLIUM UNDER CONTROLLED ENVIRONMENT CONDITIONS

### 6.1 Introduction

The toxic effect of high concentrations of iron on plant growth is well known (Jones & Etherington 1970, Tadano 1975, Ottow et al. 1983, Benckiser et al. 1984, Waldren et al. 1987). Attempts have been made to explain the mechanisms of Fe toxicity (Tadano 1975, Benckiser et al. 1984, Talbot & Etherington 1987), yet many of the data concerning the processes affecting Fe uptake and translocation in potentially toxic concentrations by plants is conflicting or incomplete. However, several factors have emerged from previous work (Rediske & Biddulph 1953, Tanaka & Navasero 1966c, Tadano 1975, Waldren et al. 1987) and from the present study as being important in the processes of Fe

absorption, including its concentration, the formation of oxidized Fe coatings on plant roots, (Fe plaques) (Taylor & Crowder 1983b, Taylor, Crowder & Rodden 1984, Crowder & Macfie 1986; Chapter 4 & 5), pH and availability of phosphorus.

The concentration of Fe in soils and culture solutions has a profound effect on the amount of Fe absorbed by plants (Rediske & Biddulph 1953). These authors found that as Fe concentrations increased, absorption of Fe (II) by Phaseolus vulgaris increased.

Oxidized Fe coatings on the roots of Eriophorum have already been shown to reduce the capacity of plants to take up nutrients such as phosphorus. Howeler (1973), also found that the roots of rice plants grown in relatively high concentrations of Fe became coated with iron oxides reducing the plants capacity to absorb enough P, K, Ca and Mg. In Chapter 4, it was suggested that oxidation of Fe by plant roots reduced substantial absorption of Fe. Green & Etherington (1977), also suggested that oxidation and

precipitation of Fe by rice roots prevented the uptake of large quantities of iron.

The uptake of Fe from a nutrient solution is considerably influenced by changes in pH, affecting the mobility of Fe (Armstrong 1982) as well as adsorption on root surfaces. Rediske & Biddulph (1953) demonstrated that as hydrogen ion concentration increased, accumulation of iron associated with the roots of Phaseolus vulgaris L. increased and transport of Fe to shoots also increased. This relationship was found when 1.0 mg/l Fe was present as ferric nitrate. It is not precisely understood why accumulation of Fe associated with roots in solution cultures should increase as the hydrogen ion concentration increases, but is probably associated with the type of particle formed in the precipitation of iron, since much Fe becomes insoluble at high pH. The bulk of precipitate formed will be of the ferric oxide type, the formation of which is sensitive to pH changes; affecting the rate of particle aggregation. Variations in rates of aggregation may result in formation of

hematite or gelatinous hydrated ferric oxide particles. It was postulated that the increase in Fe concentration on roots as hydrogen ion concentration increased was due to the difference in ability of these various types of Fe particles to adsorb on roots.

The effect of high concentrations of Fe on the availability of P has already been described in Chapters 4 & 5. Somers & Shive (1942) suggested that phosphorus may be immobilized by high Fe concentrations on the root. Translocation of P in rice plants is inhibited when large amounts of Fe are absorbed (Ota & Yamada 1960). Howeler (1973) observed a coating of ferric oxide on rice roots said to interfere with nutrient absorption, inducing nutrient deficiency. Deposition of iron compounds near roots, probably promoted by oxygen diffusion from roots (Armstrong 1967, 1968) has also been described by Bartlett (1961). Jones (1975) in Carex flacca and C. nigra, found that ferric phosphates may be formed in the oxidized zone around roots, reducing the concentration of available P to



roots.

The present study is concerned with those factors which have been shown to effect the absorption and translocation of Fe, namely Fe concentration, pH, formation of oxidized Fe coatings on roots of Eriophorum and availability of P. An attempt was made to elucidate quantitatively, the effect of these factors. One of the radioisotope  $^{59}\text{Fe}$  offers several advantages for this type of work over  $^{56}\text{Fe}$ . Among these are the speed with which data may be obtained and the absolute measure of uptake which can be made. This provides information leading to a more precise understanding of the resistance of plants to high Fe concentrations.

Since Fe concentrations in solutions and pH are closely linked, the first two experiments have examined the effects of these factors on the uptake of Fe by seedlings of Eriophorum. As 'iron plaques' on roots of Eriophorum appear to affect phosphorus-nutrition and iron-uptake these were investigated as closely related experiments.

## 6.2 Materials and Methods

6.2.1 Experiment 1: The effect of iron concentrations on the uptake of iron by Eriophorum angustifolium from a mine population (Parys Mountain) and a non-mine population (Skipwith Common).

Twelve week old seedlings of Eriophorum angustifolium were transferred to 250 ml Pyrex beakers containing 240 ml of 0.1-strength Horison's culture solution; for culture conditions see Appendix section I. Concentration of Fe in solution was 3.8 mg/l, supplied as  $\text{FeSO}_4$  at pH 4.0 (adjusted by addition of 1M HCl or NaOH). Each beaker contained representatives of 2 populations with 5 replicate seedlings of each (total 10 seedlings). Each seedling occupied a hole punched into a 'styrofoam' float and was held in place with 12 mm long plastic strips. Slits were cut in the floats allowing easy insertion and removal of plants. Floats fitted tightly into the necks of the beakers so that there was

direct contact between float and nutrient solution; reducing risk of exposure of roots to desiccation. Metal hooks, made from plastic coated circuit wire attached to floats, eased the removal of floats after feeding with radiolabelled solutions. A single float could then be transferred with minimum handling and risk of radioactive contamination from solution to solution. This design was used for all further work with radioisotopes.

Plants and beakers were placed in a controlled environment room at 24°C, 16 hr day and 20°C, 8h night, where the experimental studies were performed. Following a 24 h equilibration period, nutrient solutions were immediately replaced with 240 ml of the same nutrient solution containing 3.8 mg/l Fe or 100 mg/l Fe, as FeSO<sub>4</sub> labelled with <sup>59</sup>Fe with specific activity of 27900 Bq/mg and 279 Bq/mg respectively (as carrier-free FeCl<sub>3</sub> (Amersham international)).

Plants were harvested after 6, 24 and 48 hrs. Plants and floats were removed from feeding solutions and transferred to

beakers containing 0.1-strength Rorisons solution for a 10 minute desorption period followed by a 10 minute rinse in deionized water. Having been blotted dry, plants were divided into roots and shoots and sealed in pre-weighed foil envelopes and their fresh weights determined. This was done since preliminary work indicated a significant loss in fresh weight if plants were not sealed.

6.2.2 Experiment 2: The effect of pH on the uptake of iron by Eriophorum angustifolium from a mine (Parys Mountain) and a non-mine population (Skipwith Common)

Twelve week old seedlings of Eriophorum from the Parys and Skipwith sites were transferred to 250 ml Pyrex beakers containing 0.1-strength Rorison solution at pH 4.0. Following a 24 h period the nutrient solution was immediately replaced with 240 ml of nutrient solution adjusted to pH 3.0, 4.0, 5.0 and 6.0 by addition of 1M HCl or NaOH. The Fe concentration of feeding solutions was 20 mg/l Fe; as  $\text{FeSO}_4$ , labelled with  $^{59}\text{Fe}$  (carrier-free  $\text{FeCl}_3$ ,

Amersham International) with specific activity of 2145 KBq/mg. The aim was to observe any differences in Fe-uptake from nutrient solutions over a range of pH. As pH is known to have a significant effect on Fe solubility, an Fe concentration was selected that would be sufficient to have an immediate effect on plant uptake while remaining soluble over the range of pH studied. Each beaker contained 4 replicate plants per population (2 populations per beaker) and two replicates per treatment. After a 24 h feeding period, plants were transferred to 0.1-strength Rorison solution for a 10 minute desorption period, followed by a 10 minute rinse in deionized water. Plants were blotted dry, divided into shoots and roots and sealed in pre-weighed foil envelopes for fresh weight analysis.

6.2.3 Experiment 3. Effect of oxidized iron coatings (iron plaques) on iron uptake by Eriophorum angustifolium from a mine population (Parys Mountain) and in non-mine population (Skipwith Common)

Prior to the experiment, twelve week old seedlings from the Parys Mountain and Skipwith sites were divided into two groups. One group remained in 0.1-strength Rorison solution with concentration of 3.8 mg/l Fe as  $\text{FeSO}_4$ , while the second group was supplied with 100 mg/l Fe at pH 4.0 for one week. These constituted plants with and without plaques (for full description of Fe-plaques see Chapter 4 and 5). Following initial procedures outlined in section 6.2.2, seedlings were transferred to 250 ml Pyrex beakers. After 24 hrs, the nutrient solution was replaced with more of the same solution containing 100 mg/l Fe at pH 4.0, labelled with  $^{59}\text{Fe}$  (carrier-free  $\text{FeCl}_3$ ) with specific activity of 324 KBq/mg. Following a 24 hr feeding period, plants underwent 10 minute desorption and rinsing periods. Plants were divided into roots and shoots and fresh weights were determined.

6.2.4 Experiment 4. Effect of 'iron plaques' on phosphorus uptake by Eriophorum angustifolium from a mine population (Parys Mountain) and a non-mine population (Skipwith Common)

Plants with and without Fe-plaques (see 6.2.3 for method) were transferred to 250 ml Pyrex beakers containing 0.1-strength Rorison solution (3.8 mg/l Fe as  $\text{FeSO}_4$ ) for 24 h equilibration period. Solutions were then replaced with 240 ml of the same nutrient solution containing  $^{32}\text{P}$  (carrier-free, Amersham International) with specific activity of 332 KBq/mg and 20 mg/l Fe ( $\text{FeSO}_4$ ). After a 24 h feeding period, plants were removed and transferred to beakers containing 0.1-strength Rorison solution for a 10 minute desorption followed by a 10 minute rinsing period in deionized water. Plants were blotted dry, divided into roots and shoots before sealing in pre-weighed foil envelopes for fresh weight determination. Root length measurements were also taken in order to calculate Fe-uptake per mm root.

#### 6.2.5 Preparation of samples for liquid scintillation spectrometry

Root and shoot samples were digested in 25 ml Pyrex test tubes containing 2 mls of nitric-perchloric ( $\text{HNO}_3/\text{HClO}_3$ ) digestion mixture (Appendix III) and made up to 10 mls. One

ml aliquots of each digested sample was added to disposable plastic scintillation vials containing 9 mls of scintillation fluid (Toluene: "Triton-X", 2:1 containing 4g 2,5-diphenyloxazole (PPO) and 0.1 g 1,4-di-2(5-phenyloxazolyl)-benzene (POPOP) per litre). Each sample was counted for 10 minutes using a Packard Tri-carb 300 CD Liquid Scintillation spectrometer. A quench curve was constructed by using  $^{59}\text{Fe}$  (see Appendix V) and  $^{32}\text{P}$  standards of known activity in 9 mls of Scintillation fluid in the presence of chloroform (0-1 ml; for  $^{59}\text{Fe}$ ) and root and shoot digests from 0-1 ml for  $^{32}\text{P}$ . As insufficient quenching of  $^{59}\text{Fe}$  was obtained with root and shoot digests, a more powerful quenching agent was used to obtain the quench curve. Quench correction was automatically performed for all samples based on the channels ratio method. Corrections for decay were applied manually. Total Fe uptake was calculated from measured radioactivity and the original specific activity of the feeding solution.

As it was difficult to distinguish between adsorbed and



absorbed Fe, Fe content of the shoot was taken as representing Fe absorption by the plant. Care must be taken when estimating total Fe translocation from roots to shoots based on  $^{59}\text{Fe}$ , since the  $^{59}\text{Fe}$  taken up may not have reached equilibrium with  $^{56}\text{Fe}$  within the pool from which translocation to the shoot occurs. However, it has been assumed that the status of  $^{59}\text{Fe}$  with respect to this pool was unaffected by the treatments applied.

#### 6.2.6 Data presentation

Concentrations of Fe in plant tissues were calculated based on the following formula,

$$\text{Total Fe concentration} = \frac{(\text{shoot Fe concn (ug/g)} \times \text{shoot weight (g)}) + (\text{root Fe concn (ug/g)} \times \text{root weight (g)})}{\text{shoot} + \text{root weight (g)}}$$

Note that total Fe uptake and total Fe concentration refer to two separate calculations (see section 6.2.5 and 6.2.6) and

are not the same.

Shoot, root and shoot root concentration ratios expressed in mg element/g fresh weight of plant are also presented. As well as concentration of Fe and P in plant tissues, uptake per mm root length was also calculated.

#### 6.2.7 Data analysis

The data were transformed using the Genstat statistical package. Analyses of variance (completely randomized design) were executed for all data sets. For ease of interpretation the values on the y-axis have been back-transformed. Unless otherwise stated significance was tested at the 5% level.

## 6.3 Results

### 6.3.1 Effect of iron concentration on iron uptake in Eriophorum angustifolium

Total plant Fe concentrations (Table 6.1) increased with an increase in external Fe supply ( $p < 0.001$ ). At 3.8 mg/l Fe, maximum total Fe concentrations were found after 24 hrs in the Parys population ( $p < 0.05$ ) and did not change significantly after 48 hrs. In contrast, maximum total Fe concentrations were found after 24 hrs in plants from the Skipwith site, after which there was a decline. At 100 mg/l Fe, total Fe concentrations increased with time of harvest in both populations ( $p < 0.001$ ).

Shoot Fe concentrations increased ( $p < 0.001$ ) over the 48 h period of study for both low and high Fe treatments, but at high Fe concentration (100 mg/l) more Fe was translocated to shoots ( $p < 0.001$ ). The trend was the same for both populations, but plants from the Skipwith site appeared to

Table 6.1 Total (a), shoot (b) and root (c) iron concentrations (mg/g) and shoot/root concentrations ratios (d) of plants of Eriophorum angustifolium from Parys Mountain and Skipwith Common grown in nutrient solution containing 1 and 100 mg/l Fe for 6, 24 and 48 hours. Values followed by the same letter are not significantly different ( $p < 0.05$ ). Comparisons made within treatments only

Time Site	Fe concentration (mg/l)									Significance of effect					
	1			100			1000			Harvest Sites		Harvest Sites		Harvest Sites	
	6h	24h	48h	6h	24h	48h	6h	24h	48h	X	X	X	X	X	X
(a) Total															
Parys	0.040 e	0.060 e	0.050 e	1.090 b	1.570 b	3.550 a									
Skipwith	0.070 c	0.130 e	0.020 c	1.050 b	1.580 b	2.440 d	***	**	***	ns	**	***	ns	**	***
(b) Shoot															
Parys	0.001 a	0.002 a	0.010 a	0.050 a	0.200 b	0.380 b									
Skipwith	0.010 a	0.002 a	0.004 a	0.150 ab	0.270 b	0.660 c	***	***	***	***	***	***	ns	ns	ns
(c) Root															
Parys	0.040 a	0.050 a	0.050 a	1.030 ab	1.310 b	2.880 bc									
Skipwith	0.070 a	0.130 a	0.020 a	0.770 ab	1.300 b	1.670 b	***	ns	***	ns	ns	ns	ns	ns	ns
(d) S/R															
Parys	1.040 a	1.070 a	1.140 a	1.050 a	1.240 a	1.320 a									
Skipwith	1.020 a	1.020 a	1.310 a	1.600 ab	1.240 a	1.660 ab	**	ns	**	ns	ns	ns	ns	ns	ns

take up more Fe over the period of study ( $p < 0.05$ ), particularly at 100 mg/l Fe.

Presence of Fe at 100 mg/l in feed solutions resulted in a marked increase in Fe absorption by roots compared with that when Fe was present at only 3.8 mg/l ( $p < 0.001$ ). At 3.8 mg/l Fe, Fe-absorption by roots of plants from the Parys and Skipwith sites were relatively constant with harvest but presence of Fe at 100 mg/l resulted in an increase in absorption over 48 hrs ( $p = 0.001$ ) in both populations. Absorption of Fe by plants from the Parys site was significantly greater after 6 hrs than for plants from the Skipwith site. Absorption of Fe by roots after 24 hrs was similar in both populations. After 48hrs, plants from the Parys site were absorbing more Fe than plants from the Skipwith.

More Fe was translocated to shoots at 100 mg/l Fe as shoot-root concentration ratios were greater in plants treated with 100 mg/l Fe compared with those when Fe was present at only 3.8 mg/l. There was almost a significant site difference

in shoot root concentration ratios ( $p < 0.1$ ); plants from the Skipwith site appeared to translocate more Fe to shoots at 100 mg/l Fe than plants from the Parys site.

### 6.3.2 Effect of pH on iron uptake by Eriophorum angustifolium

The effect of a range of pH from pH 3.0-6.0 on Fe uptake is shown in Table 6.2. In general, an increase in pH resulted in an increase in Fe uptake. Total Fe concentrations of plant tissues in both populations increased with an increase in pH. At pH 4.0 and 5.0 total Fe concentrations were similar for both populations.

Shoot and root Fe concentrations of plants from both populations increased with an increase in pH. Minimum Fe concentrations were observed in plants at pH 3.0. Intermediate Fe concentrations were found in plants at pH 4.0 and 5.0 while maximum concentrations were observed at pH 6.0. Roots absorbed more Fe than was translocated to shoots. Shoot root concentration ratios decreased with an increase in pH and

Table 6.2 Total (a), shoot (b) and root (c) iron concentrations and shoot/root concentrations ratios (d) of plants of Eriophorum angustifolium from Parys Mountain and Skipwith Common grown in nutrient solution at different pH with 20 mg/l Fe for 24 hours. Values followed by the same letter are not significantly different ( $p < 0.05$ ). Comparisons made within treatments only

Site	pH				Population	Treatment	Interaction	Significance of effect
	3.0	4.0	5.0	6.0				
<b>(a) Total</b>								
Parys	5.663 c	15.753 ab	12.305 b	24.656 a	ns	**		ns
Skipwith	3.647 c	21.955 ab	15.879 b	23.665 a				
<b>(b) Shoot</b>								
Parys	0.379 a	0.664 a	0.543 a	0.942 b	ns	***		ns
Skipwith	0.502 a	0.631 a	0.779 a	0.852 b				
<b>(c) Root</b>								
Parys	4.437 b	14.732 a	11.280 a	23.477 a	ns	***		ns
Skipwith	2.686 b	20.863 a	14.865 a	22.332 a				
<b>(d) S/R</b>								
Parys	1.559 a	1.073 b	1.101 b	1.051 b	ns	**		ns
Skipwith	1.639 a	1.055 b	1.071 b	1.062 b				

was similar for both populations, indicating that more Fe was present in shoots with respect to roots at low pH (only significant at pH 4.0). At high pH, high root Fe concentrations had a greater effect on shoot-root concentration ratios.

#### 6.3.3 Effect of iron plaque on iron uptake by Eriophorum angustifolium

Iron uptake by Eriophorum with and without Fe plaque was similar and was not significantly different between populations. Shoot and root Fe concentrations are presented only (Table 6.3). The presence of oxidized Fe-coatings on roots of plants does not therefore appear to form an effective barrier to Fe uptake.

#### 6.3.4 Effect of iron plaque on phosphorus uptake by Eriophorum angustifolium

Total P concentrations of plant tissue were greater in plants from both populations when plaqued (Table



Table 6.3 Shoot (a) and root (b) iron concentrations<sup>mg/g</sup> of plants of Eriophorum angustifolium from Parys Mountain and Skipwith Common with and without iron-plaque on the roots grown in nutrient solution with 100 mg/l Fe for 24 hours. Values followed by the same letter are not significantly different ( $p < 0.05$ ). Comparisons made within treatments only

Site	Treatment		Significance of effect		
	Unplaqued	Plaqued	Population	Treatment	Interaction
<hr/>					
(a) Shoot					
Parys	0.190 a	0.130 a	ns	ns	ns
Skipwith	0.177 a	0.181 a			
(b) Root					
Parys	3.49 a	6.17 a	ns	ns	ns
Skipwith	4.67 a	3.29 a			
<hr/>					

6.4). Shoot P concentrations were significantly different between treatments and populations; translocation of P to shoots in plants with plaque was dependent on origin of the population: it was significantly lower in plants without plaque from the Skipwith site than from the Parys site and was not affected by oxidized Fe coatings on roots. Translocation of P to shoots of plants from the Parys site was greater in plants without plaque and lower in plants with Fe plaque ( $p < 0.001$ ). An inverse relationship was apparent in root data; root P concentrations in plants without plaque were lower than P concentrations of roots with Fe plaque ( $p < 0.001$ ) and were significantly different between populations; roots with Fe plaque from the Parys population accumulated less P in comparison with those of plants from the Skipwith site.

Shoot root concentration ratios suggested that plants without plaque on roots accumulated more P in shoots in respect to roots than plants with plaque. Populations however, were not significantly different from one another. Root P concentrations expressed as uptake per mm root



suggested a depression in P uptake of roots with plaque from the mine population. P uptake by roots from the non-mine population expressed in the same way showed no difference in P uptake between plants with and plants without plaque.

## 6.4 Discussion

### 6.4.1 Effect of iron concentration on iron uptake by Eriophorum angustifolium

Iron uptake by Eriophorum angustifolium in the current study was dependent on concentration in solution and changed over a 48 hr period. In general, Fe uptake increased as Fe concentration increased in solution. Population response was found to differ in plants treated with 3.8 mg/l Fe. The decline in total Fe concentration after 48hrs in plants from the Skipwith site may suggest that this population has a greater sensitivity to Fe than plants from the Parys site. Lack of sensitivity of the mine-population to Fe may be a result of growing at greatly increased iron concentrations. Baker & Walker (1989), reported evidence to suggest that in metal-tolerant plants the metal concentrations which produce both stimulatory and inhibitory effects on plant growth and dry matter yield were higher than for normal (non-tolerant)

plants. The effect is manifested by an apparent "need" for otherwise toxic metals, but in reality the concentration required to cause a response in tolerant plants has been shifted up-scale towards higher treatment concentrations.

Cox & Hutchinson (1981) in a copper/nickel-tolerant population of Deschampsia caespitosa observed higher germination and survival rates on its native, contaminated soils than on a control medium. They concluded that adaptation of the tolerant population to higher metal levels had incidentally increased plant requirements for these metals. No evidence was available in the present work to suggest a similar requirement by Eriophorum from the Parys site.

Shoot and root Fe concentrations in Eriophorum increased with an increase in external iron concentration agreeing with some other studies (eg. Rediske & Biddulph (1953) for bean plants). In Eriophorum shoot Fe concentrations did not show a proportional relationship between amount accumulated and Fe

concentration in solution. Smirnoff (1981) examined Eriophorum grown in a maximum Fe concentration of 56 mg/l, and found that Fe concentrations in shoot tissues were generally a tenth of Fe-concentrations found in roots. At 3.8 mg/l translocation of Fe to shoots was similar in both populations; separation of populations was not apparent until plants were treated with 100 mg/l Fe. The non-mine population was found to translocate more Fe to shoots than the mine population, suggesting that plants from Skipwith were less able to restrict translocation of Fe to shoots. Plants from the Parys site appeared to limit accumulation of Fe to shoots. Similarly, Baker (1978a,b,c) observed in Silene maritima higher Zn concentrations in shoots of non- (Zn) tolerant plants than tolerant plants. Subsequent screening of a range of Zn tolerant populations of S. maritima confirmed that all tolerant races showed some degree of Zn exclusion from the shoot.

Fe-absorption by roots of mine and non-mine plants at 3.8 mg/l was similar. Population differences were only evident at

100 mg/l Fe. Mine plants from the Parys site accumulated significantly higher Fe concentrations over the study period, suggesting more Fe was immobilized by roots in this population. Although plants from the non-mine population accumulated less Fe in roots, more Fe was translocated to shoots. Baker (1978a) observed a similar response in roots of Zn-tolerant and non-tolerant S. maritima; tolerant plants accumulated higher Zn concentrations than non-tolerant plants. Shoot root concentration ratios of plants from the two populations confirm observations made individually for shoots and roots. Plants from the Skipwith site translocated more Fe to shoots with respect to roots.

#### 6.4.2 Effect of pH on iron uptake by Eriophorum angustifolium

Accumulation of Fe in Eriophorum was depressed at low pH (pH 3.0) and stimulated at pH 6.0 in the presence of a constant Fe supply of 20 mg/l. Uptake of Fe from nutrient solutions is considerably influenced by pH changes (Rediske & Biddulph 1953), caused by the influence of pH on the mobility



of Fe (Ponnamperuma 1972, Etherington 1982). The response of Eriophorum to decreasing pH did not agree however, with the findings of Rediske & Biddulph (1953). Increase in Fe uptake by bean plants observed by these authors was preceded by an increase in mobility of Fe and an increase in adsorption on root surfaces associated with a decrease in pH. However, Rediske & Biddulph used maximum Fe concentrations of 1 mg/l, so Fe concentrations were not in excess of plant requirements, and direct comparisons with the present work were not possible.

"Trauma" response by plants to sudden decrease in pH (all plants were initially grown at pH 4.0 and not given a period of re-adjustment to change in pH in this experiment) was considered, but damage caused by a reduction in pH to sensitive areas such as root membranes is likely to result in uncontrolled influx of Fe, thereby increasing Fe concentrations in roots. This was not found. It is possible that short-term effects of low pH may not immediately effect membranes but may be sufficient to disrupt general

physiological and biochemical functions such that over a 24 hr period Fe uptake is depressed. Increased absorption and translocation of Fe in Eriophorum at pH 6.0 suggests that an unknown factor may influence Fe-uptake. Fe-induced-deficiency enhancement of the release of phytosiderophores (non-proteinogenic amino-acids) mobilizes sparingly soluble inorganic Fe-III compounds by complexation of Fe-III and formation of Fe-phytosiderophores (Romheld & Marschner (1986), Marschner, Romheld & Kissel 1986). This mechanism of Fe-uptake (which occurs in the rhizosphere of soil with low Fe exchangeability), has only been found in the grasses (Marchner et al. 1986). Since the release of phytosiderophores is not affected by high substrate pH, this mechanism may affect Fe-solubility in this experiment, such that Fe is more mobile than would be predicted from pH measurements of the bulk culture solution. However, this does not explain the stimulation at pH 6.0 relative to pH 3.0 suggested in Table 6.2.

#### 6.4.3 Effect of iron plaques on iron uptake by Eriophorum angustifolium

Contrary to the results of work by Bartlett (1961), Howeler (1973) and Chen et al. (1980) on some other species, there was no evidence in this study that Fe plaque on the roots of Eriophorum angustifolium did not form a barrier to Fe uptake; roots with plaque accumulated similar amounts of Fe as roots of plants without plaque. However, shoot Fe concentrations suggested that translocation of Fe was unrestricted even in the presence of Fe plaque on roots.

In media where Fe supply is limiting, such as culture solutions, oxidation of Fe by roots of Eriophorum may be effective in immobilizing significant quantities of Fe thereby reducing concentration of Fe in solution. However, if culture solutions are renewed Fe-uptake is likely to continue unrestricted, as observed in this experiment. Capacity to restrict translocation to shoots may be limited by capacity of plants to immobilize Fe on the root surface (by deposition of Fe as Fe oxides). Once threshold capacities have been

exceeded Fe translocation to shoots may continue unrestricted. Such a response would be of limited importance in the field as Fe concentrations are less likely to be limiting. Thus the presence of plaques on roots of Eriophorum, does not appear to form an effective barrier to Fe absorption. Similarly, rhizosphere oxidation of Fe around the roots of Phragmites australis did not impede the uptake of Cu and Zn (St-Cyr and Crowder 1987). Whatever the extent of Fe-plaque on the roots of Phragmites plants, the total amount of metal inside and outside the root significantly correlated with the amount found in leaves. By contrast, Otte et al. (1987), found that plaque on roots of Spartina anglica and Aster tripolium reduced Zn uptake and was directly correlated with amount of Fe present on root surfaces. These authors also found that adsorption of Cu by roots of the same species was not influenced by amount of Fe present on the roots. Based on these observations it is suggested that Fe-plaques on plant roots are not universal barriers to heavy metal uptake, and may be specific to species

of metal.

#### 6.4.4 Effect of iron plaques on phosphorus uptake by Eriophorum angustifolium

Presence of high concentrations of oxidized Fe in the form of Fe plaques appears to have a direct effect on the amount of P adsorbed by roots in Eriophorum angustifolium. This accords with the work of Rediske & Biddulph (1953) for Phaseolus vulgaris. In Eriophorum, presence of Fe plaque on roots caused an increase in root P concentration, but translocation to shoots was not enhanced, suggesting that the bulk of P was immobilized and was not available for plant uptake. Jones (1975) similarly reported that ferric phosphate formed in the oxidized zone around roots was largely unavailable for absorption by roots of Carex flacca and Carex nigra. Translocation of P into leaves of P. vulgaris was reduced at low P concentrations corresponding to presence of heavy deposition of Fe on roots (Rediske & Biddulph 1953).

There was an inverse relationship between presence of Fe plaque on roots and translocation of Fe to shoots in Eriophorum (possible reasons have been described above). Plants from the Parys site translocated more P to shoots when Fe plaques were absent from roots than did plants from the Skipwith site. Presence of Fe plaques on roots resulted in a dramatic reduction of P translocation to shoots in this population. Fe plaques on roots of plants from the Skipwith population did not affect translocation of P to shoots, suggesting that Fe plaques on roots of plants from the Skipwith population did not interfere with P uptake. Differences in extent of Fe deposition on roots would have a significant effect on P adsorption and immobilization which may explain population differences in P translocation to shoots in plants with plaque; more extensive Fe deposits are likely to have a greater capacity to precipitate and immobilize P than lighter Fe deposits on roots, which would in turn limit the amount of P translocated to shoots. However, no direct evidence for population differentiation in

the capacity to form Fe plaques on roots was available from the data in this study. Alternatively, the lack of difference in P translocation observed between plants from Skipwith with and without plaque may reflect two concurrent processes; (1) a lower capacity to oxidize Fe by roots and therefore less precipitation and immobilization of P and; (2) the effect of elevated Fe concentration on the capacity of plants to take up P. (In Section 6.4.1, population differentiation to Fe tolerance was suggested. Greater sensitivity of the Skipwith population to elevated Fe concentrations (100 mg/l) was suspected. Fe toxicity at 20 mg/l Fe may reduce the capacity of plants from this population to translocate P, resulting in lower shoot P concentrations).

## 6.5 Conclusions

1) Iron uptake by Eriophorum angustifolium increased with an increase in Fe concentration in solution. Population differences in iron-uptake were apparent. At 3.8 mg/l Fe

supply, smaller total Fe concentrations were measured in plants from Skipwith than Parys, suggesting that the latter plants may have a higher Fe requirement. Adaptation of the Parys population to high Fe concentrations (relative to Skipwith) may have incidentally increased plant requirements for Fe. However, while lower sensitivity to high Fe concentrations may manifest as a "need" for Fe, in reality the concentration required to cause a response in the Parys population may have shifted upscale towards higher Fe concentrations.

Population differentiation in tolerance to Fe is suggested. The Parys population excluded more Fe (relative to the Skipwith population) from shoots at 100 mg/l Fe. Fe absorption by roots was significantly greater in the mine population after a 48h period, suggesting that more Fe was absorbed by roots since translocation to shoots was lower relative to the Skipwith population.

2) Iron uptake by Eriophorum was strongly influenced by pH.



At pH 3.0, Fe uptake was greatly reduced relative to pH 4.0, 5.0 and 6.0. At pH 4.0 and 5.0 intermediate values were observed, while at pH 6.0 there was a stimulation in Fe uptake. The cause of the "trauma" response observed with a decline in pH is far from obvious. It was suggested that disruption of an aspect of plant physiological or biochemical function caused by a decrease in pH may have resulted in depression of Fe uptake over the 24 h period of study. Proton extrusion may alter rhizosphere pH such that Fe is more soluble than would be predicted from pH measurements of the bulk solution, resulting in greater absorption of Fe at pH 6.0 than previously expected, but this does not satisfactorily explain the stimulation of Fe uptake observed at pH 6.0.

3) Iron uptake was not affected by the presence of Fe plaque on roots of Eriophorum. Fe uptake was similar in plants with and without plaque. Populations did not differ significantly in their response. Capacity to restrict Fe translocation to shoots may be limited by the capacity of

plants to immobilize Fe on roots in the form of Fe plaques, once a threshold concentration is exceeded Fe translocation to shoots continues unrestricted. Plants pretreated with 100 mg/l Fe are likely to absorb elevated amounts of Fe prior to treatment with a further application of 100 mg/l Fe which may effect the capacity of plants to exclude more Fe.

4) Presence of Iron plaques on roots of Eriophorum caused an increase in accumulation of P by roots. Despite this translocation to shoots was not enhanced suggesting that the bulk of P was immobile. Population differences in P uptake in plants with and without plaque were apparent. Plants from the Parys population without Fe plaque on roots translocated more P to shoots than did the plaque-less Skipwith plants. However, P translocation to shoots was severely effected in plaqued Parys plants while there was no apparent difference in P-uptake in relation to plaqueing on the Skipwith plants. This may be because the latter have less extensive Fe deposition on roots. Whereas the Parys plants may have more

extensive deposition of Fe, and correspondingly less in P translocation to shoots. Lower P-translocation in plaque-less Skipwith plants relative to the Parys plants may be a result of greater sensitivity to 20 mg/l Fe present in the nutrient solutions and a concomitant effect on P-translocation.

## Chapter 7

### General discussion

In the introduction to this work, two main aims were outlined. These were: i) to investigate the dynamics of selected metals in metal-rich wetlands with regard to concentration in the substrate and uptake by Eriophorum angustifolium and Phragmites australis; and (ii) to provide information on the resistance of these wetland plants to excess levels of heavy metals in the soil environment. The first objective was considered in Chapters 2 and 3, whilst Chapters 4, 5 and 6 provide information on the factors involved in the uptake of iron by Phragmites and Eriophorum. Chapter 4 also examined the response of Eriophorum to Mn and Cu. This final chapter is a synthesis of both field and laboratory work in the context of plant growth in and resistance to metal-rich environments.

The results will also be considered in a more general context, with regard to plant growth in, and adaptation to, anoxic conditions.

#### 7.1 Mechanisms of metal resistance in Eriophorum angustifolium and Phragmites australis.

There was some evidence of Fe toxicity in plants of both species treated with Fe concentrations in excess of 100 mg/l. The gross symptoms were root stunting and a decline in dry matter yield (Chapter 4). Eriophorum was found to tolerate 50 mg/l Mn and 1 mg/l Cu in solution culture; no information was available for the effect of Mn and Cu concentrations greater than the above. In the field, however, both species were able to grow successfully in heavy-metal-enriched conditions (5-900 mg/l Mn, 1-50 mg/l Cu) suggesting that both species possess a degree of metal resistance. In addition to plant resistance mechanisms other factors may prevent the entry of metals into plants. These are not mechanisms in the strict sense as they are not under

the control of the tolerant organism, but are of considerable ecological significance (Antonovics et al 1971). Two factors believed to be important have been studied in this work, these were pH and nutrient availability.

### 7.1.1 Factors affecting the availability of metals for uptake by Eriophorum angustifolium and Phragmites australis

#### 7.1.1.1 pH

The effect of pH over a 24 h period on iron absorption and translocation in Eriophorum was to depress Fe uptake at pH 3.0 and to stimulate Fe uptake at pH 6.0 (Chapter 6). These findings contradicted the work of previous researchers. It is well known that a decrease in pH causes an increase in the mobility of Fe (Ponnamperuma 1972, Etherington 1982) resulting in a corresponding increase in the amount of Fe adsorbed by roots (Rediske & Biddulph 1953), and that an increase in pH causes a reduction in Fe solubility (Rediske &

Biddulph 1953). "Trauma response" by Eriophorum was suspected, possibly due to the influence of pH on membrane integrity and function. Therefore few conclusions could be drawn from these data as to the effect of pH on Fe uptake in healthy plants. Stimulation of Fe absorption and translocation at pH 6.0 suggested that some unknown factor was affecting Fe uptake by Eriophorum. Fe-deficiency-induced release of phytosiderophores (non-proteinogenic amino-acids) mobilizes sparingly soluble inorganic Fe-III compounds by complexation of Fe-III and formation of Fe-phytosiderophores (Marshner, Romheld & Kissel 1986). This mechanism may effect Fe-solubility at high pH. The release of phytosiderophores is only slightly depressed by high substrate pH unlike the mechanism found in dicotyledonous plants and most monocots, where reductase activity and proton extrusion is severely impaired in substrates with high pH buffering capacity. This mechanism of Fe-uptake, which occurs in the rhizosphere of soils with low Fe-exchangeability, has only been found in grasses

(Marschner et al. 1986). Marschner et al. (1986) revealed that Fe-deficiency-induced release of phytosiderophores, has two advantages for grasses over the strategy used by dicotyledonous plants and most monocots namely the greater capacity to mobilize sparingly soluble inorganic Fe-III in the rhizosphere, and the lower sensitivity of the system to high substrate pH. This mechanism is probably more important when high pH affects the solubility of Fe such that exchangeable-Fe concentrations are low. At low pH for example pH 3.0, solubility of Fe is greater than it would be expected at pH 6.0, and Fe-excess rather than Fe-deficiency is likely to effect Fe-uptake by plants.

pH-mediated changes in the solubility of Fe, or indeed any potentially toxic metal, will determine the concentration available in soils and, together with redox potentials, will determine the concentration of Fe available for plant uptake.



#### 7.1.1.2 Nutrient availability

Translocation of Fe to shoots was reduced in Eriophorum supplemented with an additional supply of nutrients (Chapter 5). A decrease in translocation correlated with an increase in the accumulation of Fe on the roots and an increase in translocation of P in treated plants. Thus, the capacity to exclude Fe in Eriophorum appears to be correlated with the nutritional status of the plant. The "iron excluding power", as described by Tadano (1975), was lower in Eriophorum deficient in nutrients. An increase in Fe uptake by rice plants also correlated with a decrease in supply of K, P, Ca and Mg (Howeler 1973, Tadano 1975, Ottow et al. 1983, Benckiser et al. 1984). Thus, Fe uptake is likely to be determined by the physiological status of the plant (Tadano 1975), healthy plants being able to regulate Fe uptake better than unhealthy ones (Section 7.1.3).

The increase in P-translocation observed in plants

supplemented with additional nutrients suggested that adequate P-nutrition may be important in maintaining plant resistance to high Fe supply. Growth of loblolly pine (Pinus taeda L.) seedlings is reduced by flooded soil conditions and Fe-uptake is greatly increased. Foliage of such seedlings often appears deficient in P (McKelvin et al. 1987). However, if P is applied to the soil prior to flooding, growth is improved and Fe uptake appears to be reduced (Hook et al. 1983). Hence, application of P appears to alleviate some of the negative effects of flooding and P nutrition appears to be closely related to Fe concentration in young loblolly pine seedlings grown in flooded soil. The significance of an increase in availability of P may be two-fold, firstly, in maintaining metabolic processes such as energy transfer (Ottow et al. 1983) and ensuring adequate root growth and secondly, it may be part of the mechanism for immobilizing Fe in plant tissues (see section 7.2).

In the present work, it was suggested that the

availability of Fe was unaffected by changing Ca concentrations (Chapter 5). It was also concluded that Ca per se did not affect the resistance of Eriophorum to high Fe in the culture solution, but may be more important in combination with other nutrients in enhancing the resistance of Eriophorum to otherwise toxic concentrations of Fe.

The importance of K in affecting Fe-uptake in Eriophorum was not studied but its importance in the Fe-resistance of these plants cannot be dismissed. Rice plants deficient in K are most susceptible to Fe toxicity as a result of a reduction in the capacity to exclude Fe and restrict Fe translocation (Tadano 1975). A soil culture experiment by the latter author showed that rice plants grown without K were lower in K concentration, higher in Fe concentration and showed more severe symptoms of Fe toxicity than those grown with an adequate supply of K.

7.1.1.3. Oxidation of iron by plant roots- an exclusion mechanism?

External mechanisms of tolerance are those plant attributes which prevent entry of metal ions (Antonovics et al. 1971, Levitt 1980). The physical and chemical characteristics of a soil can thus be described. However, true external mechanisms of tolerance are in the control of the plant. External Fe precipitation effected by an increased oxidizing capacity of the root system has been postulated to play a role in metal resistance of wetland plants (Armstrong 1967, Tadano 1975, Green & Etherington 1977, Talbot & Etherington 1987) and has been referred to as an exclusion mechanism (Talbot & Etherington 1987). However, evidence provided by this study and by the latter authors has shown that plants growing in Fe-rich conditions cannot prevent Fe uptake but only restrict it and hence accumulate Fe in their tissues to varying degrees. In Eriophorum and Phragmites the degree of Fe accumulation was correlated with the extractability of metals in the soil.

In Chapter 6, oxidation of Fe by roots of Eriophorum was suggested to be important in lowering the overall concentration of Fe in solution. The formation of Fe plaque did not interfere with the accumulation of Fe by the plant once critical levels were exceeded. Both 'plaqued' and 'unplaqued' root systems absorbed similar amounts of Fe. Fe plaque is thus not an effective exclusion mechanism, but may be important at low ambient Fe concentration, in reducing Fe uptake by Eriophorum. Once critical levels are exceeded, there is resumed Fe uptake. Under field conditions, Fe plaque formation would not seem to be an effective mechanism in reducing Fe uptake by plants unless it functions in association with other resistance mechanisms. This is because Fe availability, unlike the conditions found in culture solutions, is not necessarily limiting, but is in a continuous state of flux. It seems likely that Fe oxidation by plant roots is part of a cross-resistance mechanism, since the primary function of rhizosphere oxidation is the supply of O<sub>2</sub> to root apices under conditions of low oxygen

tension.

i) Iron plaque and its significance in the exclusion of other heavy metals

No conclusion could be drawn from the field data in this study as to the effect of Fe-plaque in the exclusion of other heavy metals such as Cu, Zn and Pb, since no obvious relationship was found between Fe concentration and heavy metal concentrations in plant tissues of either species. However, Fe-plaque may be metal-species specific. Iron hydroxides are known to adsorb large amounts of cations, being the principle matrix binding Cu and Pb in soils (Lepp 1981). Otte et al. (1987) have shown that iron hydroxides, the principal component of Fe plaques on the roots of the salt marsh plant Aster tripolium, could both enhance and reduce Zn uptake, depending on the amount of Fe on the roots and the external Zn concentration. In contrast, St-Cyr and Crowder (1987), Crowder et al. (1987) concluded that Fe plaque is not a barrier to Cu

translocation to leaves of Phragmites.

7.2 Mechanisms of tolerance of Eriophorum angustifolium and Phragmites australis to toxic concentrations of iron and other heavy metals

In Chapter 1, tolerance was defined as the capacity of a plant to survive the effects of internal stress. Analysis of both field- and laboratory- grown plants showed that both species were able to tolerate high internal concentrations of heavy metals such as Fe, Mn and Cu (also Zn and Pb, field only). Tolerance in both species does not appear to be conferred by a single mechanism, rather a combination of internal and external mechanisms.

#### 7.2.1 The importance of the root

The significance of the root in restricting Fe uptake was demonstrated in chapter 5 (split-root experiment). Eriophorum, when provided with an adequate supply of nutrients, demonstrated restricted translocation of Fe to

shoots. Stimulation of root oxidation was observed in the split low Fe/high Fe treatments, as heavy deposits were present on roots treated in the half of the solution containing high Fe. It was suggested that adequate P supply was also important, possibly in affecting the capacity of the roots for internal precipitation of Fe through the formation of iron phosphates. As Fe plaque on the roots of Eriophorum was not found to restrict Fe uptake, internal precipitation and immobilization is likely to be a more significant mechanism in Fe tolerance. This requires further investigation because no distinction was made between adsorbed Fe and absorbed Fe.

Analysis of roots of Eriophorum grown at 100 mg/l Fe over 48 h (Chapter 6) showed a pronounced immobilization of Fe in the Skipwith and Parys populations, but more Fe was accumulated by plants from the Parys site, suggesting a possible difference in Fe tolerance in these populations and greater capacity of the roots of the more tolerant population to immobilize Fe. Other authors have



already highlighted the importance of the root in restricting metal transport to shoots (Baker 1978a, 1981, Talbot & Etherington 1987). The latter authors demonstrated the importance of the root in restricting Fe uptake in waterlogging tolerant *Salix cinerea* by removing the roots. This immediately caused a greater sensitivity to lower external concentrations of Fe, suggesting that immobilization of Fe by the root system is a part of the mechanism of avoiding potential Fe toxicity.

#### 7.2.1.1 Root uptake of other heavy metals

It seems likely that the root is also important in immobilizing other potentially toxic metals such as Cu, Zn and Pb. In the metal-rich Parys Mountain site, roots of both species were found to accumulate more of these metals than the shoots. In contrast, at low concentrations, such as the conditions found at Skipwith, there is for example, relatively unrestricted transport of Pb to shoots. In culture solution, roots of Eriophorum were found to

accumulate more Cu than the shoots. Again, much of the Cu on the root may be a product of adsorption. However, if shoot Cu concentrations represent the amount of Cu absorbed by plants, assuming linear uptake of Cu by Eriophorum, then some restriction in uptake is apparent. Baker (1978a) similarly reported that tolerant and non-tolerant populations of Silene maritima grown with increasing concentrations of Zn showed pronounced immobilization of Zn in both races. The root does not seem to be effective in regulating the uptake of Mn. Shoots tended to accumulate high concentrations of this metal. It is likely that tolerance to toxic concentrations of Mn involve mechanisms not located in the root.

#### 7.2.2 Compartmentation of heavy metals as a way of storing excessive concentrations

It has been demonstrated that the root is not a complete

barrier to uptake of Fe and other heavy metals. Once critical concentrations have been reached metals such as Fe, Cu, Zn and Pb are predominantly accumulated in senescing tissue (old and dead leaves). It is conceivable that metal tolerance could depend on the ability to store accumulated metals in organs or subcellular compartments where no sensitive metabolic activities take place (Verkleij & Schat 1989). Some plants are able to translocate excess metals into old leaves, for example the tree Fagus sylvatica (Denaeyer De-Smet 1973). Over 50% of the total absorbed Cu by this species was lost in a single growing season, the majority of this loss occurring by leaf abscission. In a perennial species, such a mechanism would be advantageous as a way of regulating internal concentrations.

In Phragmites, Fe, Mn, Zn and Pb concentrations in the rhizome were lower than in the rest of the plant and Fe and Mn did not appear to be accumulated over the growing season. A proportion of the essential elements in reed stems and leaves is moved below ground during the autumn for

overwinter storage in the rhizomes (Lawson 1985). Allen & Pearsall (1963) demonstrated that essential elements such as Mg are translocated while others like Ca, Fe and Mn are retained in senescent leaves. Non-essential elements, therefore, do not tend to be stored over winter but are released back into the soil on the fall and decomposition of the leaf and so are effectively removed from the plant. In contaminated soils, the nature of the 'detoxifying' mechanism outlined above may be merely an extension of the normal cycling of mineral nutrients by plants such as Phragmites.

#### 7.2.2.1 Shoot tolerance to high concentrations of manganese

Metal-sensitive metabolic processes are not necessarily limited to the shoot. Metal toxicity is often more conspicuously manifested in the root (Foy et al 1978). Moreover, many metal-resistant plants accumulate metals in the shoot (eg accumulation of Mn in the shoot of *Oryza sativa* (Vlamis & Williams 1967). Both Eriophorum and Phragmites (only field data evidence for the latter

species) were found to accumulate Mn in the shoot (Chapters 2,3 & 4). Tolerance mechanisms may thus be involved in Mn resistance. At all stages of development, leaves were found to accumulate similar amounts of Mn (Chapter 3), although there was some evidence that more Mn was accumulated in dead leaves (Chapter 2). The nature of Mn resistance is not known (Verkleij & Schat 1989), but it may be due to high cellular tolerance to Mn as suggested by the present work.

A degree of cellular tolerance to Fe is also implied in Eriophorum. Prolonged exposure to high concentrations of Fe caused gradual accumulation of the element in shoot tissues. Although there was a reduction in yield, plants were able to survive high internal concentrations. Similarly, Talbot & Etherington (1987) noted that although photosynthesis of rooted Salix cinerea was unaffected by 320 mg/l Fe, leaf Fe concentration was considerably more than that of the controls. They concluded that S. cinerea was less sensitive to excessive Fe uptake than the waterlogging (Fe) sensitive S. caprea.

### 7.2.3 Antagonism between iron and manganese uptake in plants and its possible effect on manganese toxicity

The results of single salt uptake studies seem especially irrelevant in studies of metal toxicity (Foy et al 1978) but more so when extrapolated to uptake of metals under field conditions. Under these conditions, more than one metal may be present which can influence plant function, growth and the pattern of uptake of other metals. These may occur frequently because of natural associations between contaminant metals (Burton, Morgan & Reig 1986). These interactions may be antagonistic, independent, additive, or synergistic in nature (Davis & Beckett 1978).

Antagonism between Fe and Mn uptake was apparent in Eriophorum. Plants from consistently 'Fe-rich' sites (Parys and Crymlyn) had lower leaf Mn contents (relative to Fe) than plants from sites with lower Fe. The importance of the ratio of Fe to Mn in plant tissue has been mentioned by several workers (Beauchamp & Rossi 1972, Ohki 1975, Foy et

al. 1978). The existence of a number of interactions between Fe and Mn was also demonstrated by Tanaka & Navasero (1966c). It was observed that an increase of Fe or Mn in the growth media would cause a respective decrease in the Mn or Fe concentration of rice plants. The change was most noticeable in the root, possibly indicating that these elements are competing for absorption sites. As both Fe and Mn are transition metals, uptake of both is presumably by similar pathways. An increase in Mn level causes an increase in Fe content of the culm and a decrease in the young leaves. Thus, the distribution pattern of Fe in the plant is changed by the concentration of Mn (Tanaka & Navasero 1966c). The converse effect of Fe on Mn is also true (Chapter 3). However, this is not a tolerance mechanism in the strict sense as it is not under control of the plant. As the wetlands investigated in this study particularly Skipwith, are rich in Fe and Mn this interaction may be very important in influencing the capacity of Eriophorum and Phragmites to tolerate high internal concentrations of Fe and Mn. Further

study is necessary before it is possible to assess the actual importance of this interaction in plant resistance to high concentrations of Fe and Mn under field conditions.

#### 7.2.4 Mineral deficiency and its effect on the resistance of Eriophorum angustifolium and Phragmites australis to iron and other heavy metals

Extreme macronutrient deficiency is a characteristic of mine wastes and metalliferous slags such as the conditions associated with lead-zinc mines (Baker 1978c). An extensive physico-chemical survey of various Fe-toxic soils in the tropics (Ottow et al., 1983), revealed that most of the sites concerned were characterized by low and deficient concentrations of P, K, Ca and Mg. A deficient/or unbalanced supply of P, K, Ca and Mg in Oryza sativa triggers an uncontrolled influx and uptake of Fe by an increase in low molecular weight metabolites, in combination with an enhanced permeability of the roots (Benckiser et al., 1984). The latter authors concluded that nutritional conditions,



exudation rate (a measure of metabolic root leakage), Fe-reducing activity of the rhizosphere and Fe uptake by wetland rice appear to be clearly related.

In Chapter 5, the effect of macronutrient deficiency and partial alleviation, on Fe tolerance in Eriophorum was examined. Of the two nutrient elements analyzed, P was found to be the most important in alleviating Fe toxicity in Eriophorum. There was an apparent inverse relationship between P concentration and Fe absorption in the plant. The decline in Fe uptake was not because only half of the roots were exposed to high Fe concentration (100 mg/l) as roots treated this way adsorbed more Fe, manifested as a stimulation in Fe oxidation by the root. Eriophorum and Phragmites grown in high Fe concentrations (100 and 1000 mg/l) (Chapter 4), were found to have lower tissue concentrations of P and Ca. Ottow et al. (1983) also found a similar response in rice. In addition to having excessive concentrations of Fe (290-1000 ug/g), and Mn (often >1000 ug/g), leaves suffering from Fe toxicity were deficient in

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K and P and sometimes Ca and Mg. Deficiency of these essential elements appears to increase root membrane permeability (Benkiser et al. 1984) and therefore Fe uptake.

nutrients in nutrient-poor substrates might result in deficiency.

It seems likely that at least one of the mechanisms involved in Fe tolerance in Eriophorum is sensitive to adequate nutrient supply as a means of maintaining effective root oxidizing power and thereby an Fe excluding mechanism. However, the role of P may lie in enhancing the plants capacity for immobilizing Fe in the roots.

A further conclusion can be drawn from these observations. The effect of excess Fe on plant growth and metabolism may be indirect, causing physiological disorder through nutritional stress rather than by high Fe supply per se (Benkiser et al. 1984).

7.3 Ecotypic differentiation in Eriophorum angustifolium and

Phragmites australis

Ecotypes are formed by response of the genotype to selective pressures exerted by the environment. Thus in similar but disjunct habitats, homologous ecotypes can be formed from one genetical parent material (Waisel & Rechav 1971). Therefore, populations subjected to heavy metal contamination may develop adaptive characteristics which enable them to survive successfully in such habitats.

Many "ecotypes" have been regarded by Van der Toorn (1972) as "biotypes" -plants that have a long term acclimation to specific environmental circumstances but without genetic differentiation. Data based on plants grown from seeds collected from the field (Chapter 4 and 6) may suggest that population differences have a genetic basis in Eriophorum, since the sensitivity of seedlings to high concentrations of Fe, Mn and Cu is partially dependant on the origin of the population.

### 7.3.1 Ecotypic differentiation in Eriophorum angustifolium

#### 7.3.1.1 Population response to iron

Total iron concentrations (material grown under controlled conditions) of plants from the Skipwith Common site were lower than plants from the Parys site, suggesting plants from the latter site had a higher Fe requirement (Chapter 6). The Parys population may be less sensitive to Fe through exposure to high soil Fe-concentrations relative to the Skipwith site, manifested as a "need" for Fe, but in reality the concentration required to cause a response in the Parys population has been shifted upscale towards higher treatment concentrations. At 100 mg/l Fe, plants from the Skipwith population were less able to restrict translocation of Fe to shoots. Plants from the Parys site appeared to limit accumulation of Fe to shoots. Baker (1978a, b, c) similarly observed in Silene maritima higher Zn concentrations in shoots of non-Zn-tolerant plants than in tolerant plants. Subsequent screening of a range of Zn tolerant populations of the same species confirmed that all tolerant races showed some

degree of Zn exclusion from the shoot. Mine plants from the Parys site accumulated significantly higher Fe concentrations over 48h, suggesting that more Fe was immobilized by roots in this population.

Population differentiation with regard to the control of Fe-uptake during waterlogging and Fe-efficiency has also been observed in clones of Dactylis glomerata from a well-drained heavily-grazed habitat and from an undergrazed poorly-drained soil. Plants from the latter habitat were able to control Fe-transport to shoots, while plants from the former habitat failed to control Fe-transport to shoots showing a 124 % increase in Fe content. (Etherington & Thomas 1986).

#### 7.3.1.2 Population response to iron and phosphorus

Plants from the Parys site translocated more P to shoots when Fe plaques were absent from the roots and shoot P concentrations were greater than were shoot P concentrations of plants similarly treated from the Skipwith site (Section 6.4.4). Presence of Fe plaques on roots correlated with a

considerable reduction in P transport to shoots in plants from the former site. P-transport to shoots of plants from the latter site was unaffected by presence of Fe plaques on roots. Differences in the extent of Fe plaque deposition on root surfaces is likely to have an effect on the amount of P adsorbed and immobilized by roots, in turn regulating P-transport to shoots. This may explain evidence for population differences in P-transport to shoots. There was no direct evidence of a difference between the two populations in root oxidizing capacity, although plants from the Parys site appeared to be more tolerant to high Fe concentrations. This may explain the lower transport of P in plants where the root systems were without Fe-plaque from the Skipwith site relative to plants from the Parys site. Greater sensitivity of plants from the Skipwith site to 20 mg/l Fe used in this study may cause a disruption in P-transport to shoots. Reduction in P-transport in plants from the Parys site appeared to be affected only at higher Fe concentrations.

### 7.3.1.3 Population response to manganese and copper

Irrespective of parent material, similar adaptive characteristics must dominate in plants subjected to similar ecological conditions (Waisel & Rechav 1971). It is conceivable therefore, that plants growing in waterlogged anaerobic conditions will be resistant to the high concentrations of available Fe and Mn (often characteristic of such conditions, (Armstrong 1982)) and found in this study (chapter 2). In the field, and in solution culture work, both populations were found to be tolerant to high tissue concentrations of these elements, although culture solution work revealed that the Parys population was more sensitive to increasing concentrations of Mn (Chapter 4; based on biomass data, a slight reduction in dry weight was observed in this population). Metalliferous environments are often contaminated by more than one metal in potentially toxic concentrations. Greater sensitivity to Mn, as suggested by the data presented in Chapter 4, may be a consequence of the characteristics, such as tolerance to Cu

enabling the Parys population to grow successfully in an environment contaminated by more than one metal. In contrast, populations growing in habitats with contrasting ecological conditions may show adaptive characteristics specific to that environment. Differential tolerance to increasing Cu supply was evident between the populations, and was dependent on whether the plants originated from a Cu-rich site or a low Cu site. Thus, plants originating from Parys were found to maintain relatively low tissue concentrations of Cu up to 1 mg/l, before showing an increase in uptake, in contrast to plants from the Skipwith population, where tissue concentrations of Cu were related directly to the concentration of Cu in solution.

### 7.3.2 Ecotypic differentiation in Phragmites australis

Ecotypes of Phragmites australis have been discussed by several authors (Bjork 1967, Waisel and Rechav 1971, Van der Toorn 1972) (Chapter 1). In this study no population



differentiation was evident with respect to resistance to Fe and Mn. Solution culture work indicated that both populations studied were resistant to potentially toxic concentrations of Fe (Chapter 4). As Fe and Mn are available in high concentrations in both sites, it is likely that both populations would show a similar resistance to Mn. Field data appear to confirm this prediction, as both populations were found to accumulate high tissue concentrations of this element. Differentiation between populations was apparent at the level of mineral nutrition. P and Ca concentrations in plant tissues in the Parys population were lower than for the Skipwith population, suggesting a lower requirement for both elements, possibly as a result of lower productivity of the former population. Clarkson (1967), in experiments controlling the rate of P supply to Agrostis species suggested that A. setacea had a low demand for P, by virtue of its inherently low growth rate enabling it to maintain growth at low P supply. Rorison (1968) examined seedlings of four ecologically-distinct species of grasses in

culture solution and also observed a relationship between growth rate and P absorption. Some of these grasses were able to survive conditions of low P because of their low growth rates. In the present study differences in size were observed between populations of Eriophorum angustifolium (mine plants from Parys appeared to have slower growth rates in solution culture work than non-mine plants from Skipwith Common) but no difference was observed in plants of Phragmites in solution culture. However, in the field, Parys Phragmites plants were visibly smaller than those growing at Skipwith. It is suggested that the main factor restricting plant size may be nutritional rather than due to the toxic action of high concentrations of metals. Selective pressure could then result in individuals with lower nutrient requirements.

In Chapter 4, it was suggested that the supply of nutrients used in pre-culturing Eriophorum plants was probably sufficient to maintain the higher nutrient requirements of the Skipwith population over the duration of

treatment with high Fe concentration. This may explain the relative lack of differentiation between Eriophorum and Phragmites populations under experimental conditions. It is suggested that at low nutrient availability and high Fe supply, the Parys plants may be superior to the Skipwith population in that plants may be adapted to these conditions by virtue of growing in a Fe-rich site suspected to have low nutrient availability. Clearly, further work is required to establish the significance of these suggestions. There is a need to examine more closely the chemical characteristics of the sites studied to establish whether plants from Parys are indeed subject to lower nutrient availability. There is also a need to establish any relationship between growth rate and ability to grow at low P and high Fe concentrations.

One further point must be made, that of the problem of interpreting the results of laboratory work in relation to the response of individuals in the field. The lack of conclusive evidence in the response of the two populations in terms of Fe

tolerance (and adaptation to low nutrient availability) may be easier to understand if an interactive basis for tolerance is considered. The Parys population (of possibly both species) may be more tolerant to conditions with a combination of metals such as Fe and Cu and low nutrient availability.

In conclusion, the lack of differential tolerance to Fe (and Mn) in populations of Phragmites suggests that these plants may be constitutionally tolerant to high concentrations of these elements by virtue of their growth in waterlogged environments. In addition, selective processes may have resulted in individuals able to tolerate low nutrient availability. Reciprocal transplants of Phragmites plants taken from Skipwith and Parys appear to confirm this suggestion. Plants transferred from the Skipwith site in April 1986 although stunted, were found to survive exposure to the conditions characteristic of the Parys Mountain site (Appendix VI).

Little evidence was available for ecotypic differentiation in resistance to Fe in Eriophorum from the

two study sites (Chapter 4), but Fe-uptake studies (Chapter 6) suggested that plants from the Parys site may be more Fe-tolerant relative to the Skipwith population. The suspected lower productivity of the Parys site may have resulted in individuals able to survive conditions of very low nutrient availability. As there were relatively few differential physiological characteristics observed between the two populations under the growth conditions of this study, both populations of Eriophorum and possibly Phragmites may be at the early stages of ecotypic differentiation. Ecological and physiological traits of Phragmites populations from glycophytic and halophytic populations were investigated by Waisel & Rechav (1971). Plants from the two populations grown under non-saline conditions showed similar growth rates, rates of Na-uptake and ion distribution in various organs. Under saline conditions growth of the halophytic population was found to be superior. Higher germination percentages under saline conditions, as well as faster growth rates of seedlings seemed to be distinctive characteristics of adaptive

value of the halophytic ecotype. As no morphological differences were found and since very few differential physiological characteristics were encountered, the two populations were thought to be at the very early stages of ecotypic differentiation.

#### 7.4 Interpretation of the work in relation to the types of tolerance shown in Eriophorum angustifolium and Phragmites australis

Eriophorum angustifolium may show constitutional tolerance to Fe (Chapter 4), but other data (Chapter 6) suggest that Eriophorum from the Parys population was more Fe-tolerant relative to the Skipwith population. Interpretation of the work in relation to the 'types' of tolerance shown by this species is complicated by these observations. For a species to be considered constitutionally tolerant to a metal, no difference in tolerance should be observed between control material and plants collected from

the contaminated site. Although there may be constitutional tolerance to Fe in Eriophorum, the Parys population may be in the early stages of differentiation in relation to Fe-tolerance.

The response of both populations of *Phragmites* to Fe (and Mn) in excess was suggested to be an intrinsic feature of plants from waterlogged environments. McNaughton et al. (1974) and Taylor & Crowder (1984), demonstrated that Typha latifolia possessed an inherent or constitutional tolerance to heavy metals. Comparisons were made between clones of T. latifolia from a site contaminated with Pb, Zn and Cd (McNaughton et al. 1974) and Cu and Ni (Taylor & Crowder 1984), with clones from an *uncontaminated site*. No evidence was found suggesting that evolution of metal tolerance had occurred in populations of T. latifolia growing on contaminated sites. Growth of clones from both locations was inhibited under conditions of metal-stress but differences in growth between populations from the two sites could not be detected, contrary to the bulk of evidence presented by

Antonovics et al. (1971) for other plant species. The majority of experimental work into the tolerance of plants to heavy metals confirms the belief that populations surviving in metal-rich soils are differentiated from other populations of the same species growing in non-contaminated sites by the possession of genetically-based tolerances. As this work suggests this may not always be the case.

The differentiation of populations cannot explain the apparent success of some species to metal-rich environments where little or no difference in the control population is observed. Evolution of metal tolerance in a species means that initially, the physiological trait to deal with toxic concentrations of metal is absent or only present in a reduced capacity. However, some species may be fortuitously tolerant as a coincidence of adaptation to other stress factors, or in the case of plants growing in waterlogged conditions of low redox potential, are tolerant because of adaptation to the characteristic conditions of these habitats.

The Parys sediments contain a number of metals in



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potentially toxic concentrations, such as Fe, Mn and Cu. Eriophorum in this study appears to be tolerant to all three of these elements and multiple tolerance is suggested. Phragmites plants from the same habitat may also show similar tolerances, and by virtue of tolerance to both Fe and Mn, is tolerant to more than one metal.

#### 7.5 Interpretation of the work in relation to plant resistance to anoxic conditions

The ability of plants to survive and grow in waterlogged conditions has been explained in terms of: low O<sub>2</sub> requirement, active translocation of O<sub>2</sub> from aerial plant parts to sites of active O<sub>2</sub> demand (roots), ability to exclude or tolerate soil-borne toxins and the provision of air-space tissue (Chapter 1).

Roots of Eriophorum and Phragmites from both field and laboratory conditions revealed red-brown deposits of ferric compounds along their length. Bartlett (1961), found that iron oxide coatings were common on the roots of

hydrophytes and showed that root oxidizing activity was specifically correlated to the ability to tolerate waterlogged soil.

The low redox, waterlogged conditions in this study are evidence of low availability of O<sub>2</sub> and are associated with high availability of soluble, divalent forms of Fe and Mn. Both species studied were able to tolerate high concentrations of Fe and Mn in the field and the laboratory. The ability to exclude or tolerate high concentrations of Fe and Mn has been shown to correlate with waterlogging tolerance (Martin 1968, Talbot & Etherington 1987, Waldren, Davies & Etherington 1987, Waldren et al. 1987). The distribution of the two heathers, Erica cinerea and E. tetralix has been attributed to a differential response of plants to Fe-toxicity (Jones & Etherington 1970). On waterlogging pot-cultured plants, E. cinerea died quickly, following the development of a characteristic waterlogging syndrome, which included leaf discoloration and massive leaf water loss. On analysis, the former plants were found to have taken up more

Fe than plants of E. tetralix which were unaffected by waterlogging.

Studies of the nature of the work presented here involve a complex of conditions which are difficult to separate. Resistance to Fe for example, probably involves a cross-resistance mechanism, combining oxidation of the rhizosphere with the exclusion of Fe through precipitation and immobilization on plant roots, which in turn is usually determined by the presence of air-space tissue (Smirnoff & Crawford 1983), as well as tolerance to high cellular concentrations of Fe. Whatever the exact mechanisms of tolerance, it is clear that the capacity to exclude reduced toxins from root uptake is not a sufficient explanation for the adaptation of at least two wetland plants to their waterlogged metal-rich environment.

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APPENDIX I

Composition of Rorison Culture solution (Hewitt 1966)

Element	Concentn mg/l	Source	Stock Solution/l	Nutrient Solution/l	Strength Full 0.1
Ca	80	Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O	94.48g	5	0.5
N	56				
Mg	24	MgSO <sub>4</sub> ·7H <sub>2</sub> O	246.5	1	0.1
K/P	78/31	K <sub>2</sub> HPO <sub>4</sub>	174.0	1	0.1
Fe	3	Fe-EDTA	25.0	1	0.1
(Mn	0.5	MnSO <sub>4</sub> ·4H <sub>2</sub> O	2.028 )		
(B	0.5	H <sub>3</sub> BO <sub>3</sub>	2.863 )		
(Mo	0.1	(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O	0.184 )	1	0.1
(Zn	0.1	ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.440 )		
(Cu	0.1	CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.393 )		

pH (see methods of chapters)

Variables

Fe	1, 10, 100, 1000	FeSO <sub>4</sub> ·7H <sub>2</sub> O	24.82	0.2, 2, 20, 200
Ca	0.08, 0.8, 8, 80	CaCl <sub>2</sub>	220	0.02, 0.2, 2, 20 per 20 l
N	56	NaNO <sub>3</sub>	340	1
Mn	0.5, 5, 25, 50	Mn(SO <sub>4</sub> )·4H <sub>2</sub> O	4.1	0.5, 5, 25, 50
Cu	0.1, 0.25, 0.5, 1	Cu(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O	0.018	13.3, 33.3, 66.6, 133.3

pH adjusted by addition of 1M HCl or NaOH

## APPENDIX II

### A) Propagation of Eriophorum angustifolium

#### a) Seedlings

##### i) Seed storage and method of cleaning

Seed was collected from Parys Mountain and Skipwith Common in June-July 1986 and 1987 and stored at room temperature in unsealed envelopes. Seeds were cleaned by first removing the pappus and then rubbing against a 2 mm sieve to separate the seed from the chaff. Cleaned seed was then stored in waxed envelopes under the above conditions.

##### ii) Germination

Seeds were germinated on moist filter paper in sealed petri-dishes at 25°C in a controlled environment room.

Germination of seeds began nine days after imbibition. Seedlings were watered daily with 0.1 strength Rorison solution adjusted to pH 4.0. Relative humidity was maintained at approximately 100% for a further 7 days, after



which the lid of the petri dish was removed. Fourteen days after germination seedlings were removed from the filter paper by hand and transferred to 'Styrofoam' floats on which they were supported by non-wetting cotton wool in 0.1 strength Rorison culture solution at pH 4.0. Growing conditions were 16h (25°C) day/8h (23°C) night, with 70% relative humidity. Due to the low growth rate of E. angustifolium collected from the above sites, it was necessary to grow the seedlings for twelve weeks before they had reached a size suitable for experimentation (>10 cm length of shoots).

b) Mature plants

Vegetative plants of approximately constant size were collected from Parys Mountain and Skipwith Common in April 1986 and 1987. Dead leaves were removed from the plants and discarded. In order to remove surface contaminants such as particulate iron and soil, plants were washed three times in tap water and twice in distilled water. Plants were then transferred to 'Styrofoam' floats on which they were

supported by non-wetting cotton wool in 0.1-strength Rorison solution at pH 4.0. Growing conditions were identical to the above. Culture solutions were changed every three days.

B) Propagation of Phragmites australis

Plants were propagated from subsidiary shoots collected from Parys Mountain and Skipwith Common. All scale leaves were removed and discarded. In order to ensure clean material plants were washed as in section b above. Growing conditions were the same as those used for Eriophorum.

## APPENDIX III

### Chemical analysis

a) Nitric/Perchloric digestion method (not suitable for nitrogen analysis)

i) Digestion mixture

Nitric acid (Analytical reagent, A.R.) 350 ml

Perchloric acid (A.R.) 70 ml

Sulphuric acid (A.R.) 35 ml

The acids are mixed carefully in the order given and stored in a cool place.

#### Procedure

A known weight of sample (<0.5 g) was placed in 25 ml Pyrex test-tubes and 2 ml of digestion mixture was added to each test-tube. All samples were then transferred to a fume cupboard. Test tubes were heated initially to 60° C in a

digestion block (Grant Block Thermostat model BT5-16) until brown fumes of nitric acid were produced and the sample completely digested (excessive heating at this stage causes a plug of sample to rise up the tube which then does not fully digest). The temperature was then raised to 80° C until all the brown fumes of nitric acid have been driven off. After this the temperature was raised in stages to 210° C. The digestion was complete when white fumes were formed within the digestion tube and the mixture was clear. After digestion the samples were transferred to 10 ml volumetric flasks (test-tubes were washed out with a jet of deionized water to ensure all of the sample was removed) and made up to 10ml with deionized water.

b) Colorimetric determination of P (modified from Allen et al. 1974)

i) Reagents

4.8 g of ammonium molybdate-antimony-tartrate was dissolved

in 2M  $H_2SO_4$ . 0.1 g of sodium antimony tartrate was also dissolved in 2M  $H_2SO_4$ , added to the former and made up to 500 mls with more 2 M  $H_2SO_4$ .

ii) Phenolphthalein indicator

0.5 g of phenolphthalein was dissolved in 50 ml of 95% ethanol and 50 ml of deionized water.

iii) Procedure

Two mls of sample was pipetted in a 50 ml volumetric flask and drops of phenolphthalein indicator were added to the flask. Drops of 10M NaOH were then added until the solution turned pink in colour (shake carefully). This colour was then removed by slowly adding drops of M HCl (shake carefully to ensure the solution is mixed adequately). This procedure was necessary to neutralize the acidic sample before adding the reagents (ammonium-molybdate-antimony-tartrate and ascorbic acid). Five mls of the former reagent was then added, followed by two mls of 0.1 M ascorbic acid (2 g in 100mls deionized water)

iv) Spectrophotometer

The spectrophotometer was set to 882 nm wavelength and allowed to warm up for 30 mins.

v) Phosphate standard (not from the above reference)

21.9354 g of  $\text{KH}_2\text{PO}_4$  was dissolved in a small volume of deionised water. Two and a half mls of  $\text{H}_2\text{SO}_4$  and a few drops of  $\text{CHCl}_3$  as a preservative, was then added to the solution and made up to 1 litre. This produced a solution containing 5000 ug/ml P. This was further diluted to produce a stock solution containing 50 ug/ml P. A calibration curve was constructed with known concentrations of P. Absorbance was found to be linear to 0.5 ug/ml P, it was therefore decided to calibrate the curve to a maximum of 0.5 ug/ml P.

vi) Typical calibration curve

P (ug/ml)	absorbance
0.00	0.000
0.05	0.037
0.1	0.095

0.25

0.185

0.5

0.347

This calibration curve was found to be reproducible with little variation.

APPENDIX IV

Seasonal Fluctuations in Element Composition in Plant Tissues- Additional Data (Chapter 3)

1(a) Iron concentration in tissues of Phragmites australis. Mean and standard error of means of Fe concentrations of plant parts harvested in April, June and September 1986 from Parys Mountain, Crymlyn Bog and Skipwith Common. Unbracketed data presented as natural logarithms, data in brackets are back-transformed means of the former.

Sites	Psect	Middle Leaf	Lower leaf	Rhizome	Root	SED
Parys1	Harv					
	April	7.475 (1763)	7.966 (2881)	5.452 (233)	8.149 (3204)	0.514
	June	7.185 (1319)	7.792 (2421)	6.654 (776)	7.698 (2204)	"
Parys2	Septr	7.233 (1384)	7.588 (1974)	5.307 (202)	9.227 (10168)	"
	April	8.010 (3011)	8.020 (3041)	6.569 (713)	10.601 (40175)	"
	June	7.086 (1195)	7.384 (1610)	6.817 (913)	9.602 (14794)	"
Parys4	Septr	6.049 (424)	5.772 (321)	5.813 (335)	8.791 (6575)	"
	April	6.271 (529)	6.251 (519)	5.876 (356)	8.163 (3509)	"
	June	5.314 (203)	5.509 (247)	5.013 (150)	9.344 (11430)	"
Skipwith	Septr	5.572 (263)	5.338 (208)	5.544 (256)	8.241 (3793)	"
	April	5.201 (181)	5.126 (168)	5.305 (201)	6.478 (651)	"
	June	5.044 (155)	4.613 (101)	4.494 (89)	6.022 (412)	"
Crymlyn1	Septr	4.460 (86)	4.743 (115)	4.518 (92)	6.210 (498)	"
	April	6.437 (625)	6.664 (684)	7.266 (1431)	9.111 (9054)	"
	June	4.845 (127)	5.392 (220)	4.580 (98)	9.522 (13657)	"
Crymlyn2	Septr	5.858 (350)	5.767 (320)	5.369 (215)	9.600 (14765)	"
	April	6.482 (653)	7.015 (1113)	5.259 (192)	9.275 (10668)	"
	June	4.049 (57)	4.760 (116)	4.773 (118)	9.038 (6417)	"
	Septr	5.909 (368)	6.263 (525)	5.043 (155)	7.851 (2568)	"



(b) Manganese concentration in tissues of Phragmites australis. Mean and standard error of means of Mn concentrations of plant parts harvested in April, June and September 1986 from Parys Mountain, Crymlyn Bog and Skipwith Common. Unbracketed data presented as natural logarithms, data in brackets are back-transformed means of the former.

Sites	Psect	Middle Leaf	Lower leaf	Culm	Culm base	Rhizome	Root	SED
	Harv							
Parys1	April	4.424 (83)	4.559 (95)	4.535 (93)	4.792 (121)	4.491 (89)	4.999 (148)	0.712
	June	3.997 (54)	4.402 (82)	4.414 (83)	3.688 (40)	5.686 (295)	4.653 (67)	"
	Sept	4.424 (83)	4.244 (67)	4.579 (97)	4.705 (111)	4.210 (67)	5.354 (211)	"
Parys2	April	3.666 (39)	3.636 (38)	3.497 (33)	5.642 (282)	3.787 (44)	6.483 (654)	"
	June	2.875 (18)	3.008 (29)	3.300 (27)	3.631 (38)	6.203 (494)	5.495 (243)	"
	Sept	3.279 (27)	3.089 (22)	3.617 (37)	4.325 (76)	4.144 (63)	5.896 (364)	"
Parys4	April	4.517 (92)	4.562 (69)	3.671 (39)	4.947 (141)	4.441 (85)	5.484 (241)	"
	June	4.077 (59)	4.279 (72)	3.768 (43)	4.094 (60)	4.201 (67)	4.776 (112)	"
	Sept	4.391 (81)	4.280 (72)	4.445 (85)	4.268 (72)	4.776 (119)	5.396 (221)	"
Skip	April	4.512 (91)	5.020 (151)	3.716 (41)	4.437 (85)	4.269 (71)	4.429 (84)	"
	June	3.576 (36)	4.752 (116)	3.421 (31)	3.362 (29)	3.999 (55)	3.491 (33)	"
	Sept	4.917 (137)	4.758 (117)	3.412 (30)	4.442 (85)	3.847 (47)	4.610 (100)	"
Crym1	April	4.527 (92)	4.947 (141)	3.868 (48)	4.636 (103)	4.044 (57)	5.585 (266)	"
	June	3.580 (35)	4.262 (71)	3.734 (42)	3.790 (44)	4.322 (75)	4.361 (78)	"
	Sept	4.638 (59)	4.957 (70)	3.076 (95)	4.550 (38)	4.061 (58)	4.958 (142)	"
Crym2	April	4.638 (103)	4.957 (142)	3.076 (22)	4.550 (95)	3.495 (33)	5.488 (242)	"
	June	4.238 (69)	4.807 (122)	3.589 (36)	4.652 (105)	4.068 (58)	4.550 (95)	"
	Sept	5.379 (217)	5.450 (233)	5.140 (171)	5.138 (170)	4.287 (73)	5.520 (250)	"

(c) Copper concentration in tissues of *Phragmites australis*. Mean and standard error of means of Cu concentrations of plant parts harvested in April, June and September 1986 from Parys Mountain, Crymlyn Bog and Skipwith Common. Unbracketed data presented as natural logarithms, data in brackets are back-transformed means of the former.

Sites	Psect	Middle leaf	Lower leaf	Dead leaf	Culm	Culm base	SED
Parys1	Harv						
	April	3.542 (35)	3.481 (32)	3.339 (28)	4.434 (84)	4.370 (79)	0.58
	June	2.941 (19)	3.016 (20)	3.524 (34)	3.916 (50)	3.062 (21)	"
	Sept	3.939 (51)	3.722 (41)	3.096 (22)	3.872 (48)	4.179 (65)	"
Parys2	April	3.386 (30)	3.435 (31)	2.913 (18)	4.380 (80)	5.683 (294)	"
	June	2.585 (13)	2.875 (18)	2.804 (17)	3.578 (36)	3.392 (30)	"
	Sept	3.527 (36)	3.335 (28)	3.014 (20)	3.948 (52)	4.392 (81)	"
Parys4	April	3.379 (29)	3.487 (33)	2.776 (16)	4.906 (135)	3.941 (51)	"
	June	2.453 (12)	2.627 (19)	2.527 (8)	3.703 (47)	3.405 (50)	"
	Sept	3.150 (23)	3.192 (24)	3.114 (23)	3.612 (37)	4.122 (62)	"
Skipwith	April	2.818 (17)	2.965 (19)	2.068 (8)	3.859 (47)	3.914 (50)	"
	June	2.014 (7)	2.435 (11)	2.040 (13)	3.348 (10)	2.710 (40)	"
	Sept	3.137 (11)	3.012 (13)	2.962 (10)	3.368 (40)	5.587 (34)	"
Crymlyn1	April	2.378 (10)	2.578 (14)	2.279 (15)	3.700 (24)	3.515 (22)	"
	June	2.319 (10)	2.616 (14)	2.701 (15)	3.168 (24)	3.072 (22)	"
	Sept	2.868 (18)	2.973 (20)	2.803 (16)	3.557 (35)	4.114 (61)	"
Crymlyn2	April	2.353 (11)	2.299 (10)	1.869 (6)	3.753 (43)	3.210 (25)	"
	June	2.153 (9)	2.179 (9)	2.054 (8)	3.098 (22)	2.962 (19)	"
	Septem	3.247 (26)	3.122 (23)	2.731 (15)	3.446 (31)	3.722 (41)	"

(d) Calcium concentration in tissues of Phragmites australis. Mean and standard error of means of Ca concentrations of plant parts harvested in April, June and September 1986 from Parys Mountain, Crymlyn Bog and Skipwith Common. Unbracketed data presented as natural logarithms, data in brackets are back-transformed means of the former.

Sites	Psect	Middle leaf	Lower leaf	Culm	Culm base	Rhizome	SED
Parys1	Harv						
	April	5.953 (385)	6.383 (592)	5.154 (173)	6.515 (675)	4.947 (141)	0.93
	June	5.872 (355)	6.627 (755)	5.280 (196)	5.038 (226)	8.507 (908)	"
	Sept	6.547 (697)	6.723 (831)	5.277 (196)	5.421 (226)	6.811 (908)	"
Parys2	April	6.317 (554)	6.346 (570)	5.572 (263)	7.116 (1232)	4.574 (97)	"
	June	6.796 (894)	6.951 (1044)	5.220 (185)	5.428 (228)	6.646 (770)	"
	Sept	6.685 (800)	6.575 (717)	5.285 (197)	6.172 (479)	5.632 (279)	"
Parys4	April	7.303 (1485)	7.158 (1284)	6.578 (719)	7.099 (1211)	7.483 (1778)	"
	June	7.377 (1599)	7.353 (1556)	6.198 (492)	6.231 (508)	7.619 (2037)	"
	Sept	7.026 (1126)	6.758 (861)	5.230 (187)	5.946 (382)	6.600 (735)	"
Skipwith	April	7.925 (2766)	8.549 (5162)	7.545 (1891)	6.400 (602)	6.908 (1000)	"
	June	7.715 (2242)	8.682 (5896)	7.444 (1710)	7.149 (1273)	6.912 (1004)	"
	Sept	8.616 (5519)	8.896 (7303)	5.273 (195)	5.812 (334)	7.034 (1135)	"
Crymlyn1	April	8.074 (3210)	8.538 (5105)	7.695 (2197)	7.536 (1874)	6.427 (618)	"
	June	6.643 (767)	7.541 (1884)	5.300 (200)	5.431 (228)	7.223 (1370)	"
	Sept	7.607 (2012)	7.634 (2067)	5.598 (270)	5.080 (161)	6.452 (634)	"
Crymlyn2	April	8.233 (3763)	7.858 (2586)	7.144 (1266)	6.390 (596)	6.145 (466)	"
	June	8.131 (3398)	8.124 (3374)	5.758 (317)	5.906 (367)	7.847 (2558)	"
	Sept	8.314 (4081)	7.568 (1935)	4.987 (147)	5.048 (156)	6.553 (701)	"

2(a) Fe concentration in tissues of Eriophorum angustifolium. Mean and standard error of means of Fe concentrations of plant parts harvested in April, June and September 1986 from Parys Mountain, Crymlyn Bog and Skipwith Common. Unbracketed data presented as natural logarithms, data in brackets are back-transformed means of the former.

Sites	Psect	Upper leaf	Dead leaf	Stem base	Root	SED
Parys1	Harv					
	April	6.274 (531)	7.135 (1255)	6.100 (446)	5.373 (216)	1.14
	June	7.081 (1188)	7.781 (2395)	8.188 (3548)	10.490 (35954)	"
	Sept	6.807 (904)	7.620 (2039)	7.971 (2896)	8.993 (8047)	"
Parys2	April	6.135 (462)	7.851 (2568)	9.494 (13280)	6.257 (522)	"
	June	6.069 (432)	9.285 (10775)	7.506 (1819)	8.572 (5282)	"
	Sept	5.135 (1926)	8.504 (2855)	7.134 (1254)	8.803 (6654)	"
Parys3	April	7.563 (1926)	7.957 (2855)	6.755 (858)	6.226 (506)	"
	June	5.879 (357)	8.801 (6641)	7.809 (2463)	6.732 (856)	"
	Sept	5.403 (222)	8.305 (4044)	7.118 (1234)	6.992 (1088)	"
Skipwith	April	4.055 (58)	7.404 (1643)	6.173 (480)	7.720 (2253)	"
	June	4.050 (57)	6.625 (754)	6.334 (480)	5.757 (316)	"
	Sept	4.174 (65)	6.121 (455)	5.201 (181)	5.289 (198)	"
Crymlyn1	April	6.194 (490)	8.431 (4587)	8.109 (3324)	6.545 (696)	"
	June	6.160 (453)	7.715 (2242)	7.315 (1503)	7.557 (1914)	"
	Sept	5.288 (198)	7.854 (2576)	6.547 (697)	7.243 (1398)	"

2(b) Mn concentration in tissues of Eriophorum angustifolium. Mean and standard error of means of Mn concentrations of plant parts harvested in April, June and September 1986 from Parys Mountain, Crymlyn Bog and Skipwith Common. Unbracketed data presented as natural logarithms, data in brackets are back-transformed means of the former.

Sites	Psect	Upper leaf	Middle leaf	Lower leaf	Dead leaf	Stem base	Root	SED
	Harv							
Parys1	April	5.489 (242)	5.405 (223)	5.573 (263)	4.908 (135)	5.392 (220)	4.866 (130)	0.75
	June	5.061 (158)	5.105 (165)	4.719 (112)	5.468 (237)	5.476 (239)	4.911 (136)	"
	Sept	4.841 (127)	4.431 (84)	4.447 (85)	4.750 (116)	5.128 (169)	4.185 (66)	"
Parys2	April	4.903 (135)	4.573 (97)	4.923 (137)	4.351 (78)	5.480 (240)	4.299 (74)	"
	June	4.206 (67)	3.573 (36)	3.587 (36)	4.182 (66)	4.226 (68)	3.601 (37)	"
	Sept	3.465 (32)	3.217 (25)	3.636 (38)	4.079 (59)	4.013 (55)	3.851 (47)	"
Parys3	April	5.153 (173)	4.407 (82)	7.020 (1119)	4.550 (959)	6.097 (445)	5.325 (205)	"
	June	4.880 (132)	4.928 (138)	4.976 (148)	4.859 (129)	6.435 (623)	4.470 (87)	"
	Sept	4.385 (80)	4.167 (65)	4.184 (66)	4.399 (81)	4.450 (86)	3.532 (34)	"
Skipwth	April	5.861 (351)	5.674 (291)	6.000 (403)	5.061 (158)	4.718 (112)	6.224 (505)	"
	June	5.158 (174)	5.336 (208)	5.089 (162)	5.680 (293)	4.736 (114)	4.635 (103)	"
	Sept	5.475 (239)	5.742 (312)	5.322 (205)	5.271 (195)	5.573 (263)	4.547 (94)	"
Crym1	April	5.771 (321)	6.776 (877)	6.722 (830)	5.084 (161)	6.414 (610)	6.429 (620)	"
	June	5.055 (157)	5.315 (203)	5.155 (173)	5.543 (255)	5.549 (257)	5.126 (168)	"
	Sept	5.244 (189)	5.621 (276)	5.541 (255)	6.047 (423)	5.815 (335)	5.167 (175)	"

2(c) Cu concentration in tissues of Eriophorum angustifolium. Mean and standard error of means of Cu concentrations of plant parts harvested in April, June and September 1986 from Parys Mountain, Crymlyn Bog and Skipwith Common. Unbracketed data presented as natural logarithms, data in brackets are back-transformed means of the former.

Sites	Psect	Middle leaf	Lower leaf	Dead leaf	Stem base	Root	SED
Parys1	Harv						
	April	5.369 (215)	5.950 (384)	5.031 (153)	5.237 (188)	4.569 (96)	0.70
	June	5.009 (150)	4.429 (84)	5.354 (211)	5.232 (187)	5.309 (202)	"
	Sept	4.593 (89)	4.370 (79)	4.823 (124)	5.447 (232)	4.557 (128)	"
Parys2	April	4.199 (67)	5.375 (216)	4.949 (141)	6.129 (459)	4.870 (130)	"
	June	3.485 (33)	3.332 (28)	4.811 (123)	4.540 (94)	4.726 (113)	"
	Sept	3.691 (40)	4.118 (61)	4.988 (147)	4.870 (130)	4.522 (92)	"
Parys3	April	5.335 (207)	7.664 (2130)	5.294 (199)	5.273 (195)	4.801 (122)	"
	June	4.080 (59)	4.850 (128)	5.409 (223)	5.233 (187)	4.458 (86)	"
	Sept	3.662 (39)	4.685 (108)	5.119 (167)	4.587 (98)	4.344 (77)	"
Skipwith	April	1.882 (7)	3.425 (31)	3.885 (49)	2.625 (14)	5.089 (162)	"
	June	2.077 (8)	2.461 (12)	3.057 (21)	2.682 (15)	3.341 (28)	"
	Sept	3.227 (25)	3.297 (27)	3.759 (43)	3.797 (45)	3.682 (40)	"
Crym1	April	2.148 (9)	4.083 (59)	4.308 (74)	3.113 (22)	3.511 (33)	"
	June	2.416 (11)	2.567 (13)	3.336 (28)	4.361 (78)	3.167 (24)	"
	Sept	3.228 (25)	3.156 (23)	3.955 (23)	4.278 (72)	3.856 (47)	"

2(d) Ca concentration in tissues of Eriophorum angustifolium. Mean and standard error of means of Ca concentrations of plant parts harvested in April, June and September 1986 from Parys Mountain, Crymlyn Bog and Skipwith Common. Unbracketed data presented as natural logarithms, data in brackets are back-transformed means of the former.

Sites	Psect	Upper leaf	Middle leaf	Stem base	Root	SED
	Harv					
Parys1	April	6.350 (572)	6.905 (997)	7.232 (1383)	6.111 (451)	1.47
	June	6.980 (1075)	6.950 (1043)	7.580 (1959)	6.580 (721)	"
	Sept	5.942 (381)	6.002 (429)	7.173 (1304)	5.251 (191)	"
Parys2	April	6.268 (527)	6.150 (469)	7.478 (1769)	6.052 (425)	"
	June	6.238 (512)	6.038 (419)	6.831 (926)	6.287 (538)	"
	Sept	5.596 (269)	5.429 (228)	6.015 (410)	5.911 (369)	"
Parys3	April	7.374 (1594)	7.170 (1300)	7.717 (2246)	7.008 (1105)	"
	June	6.603 (737)	6.803 (901)	8.049 (3131)	6.404 (604)	"
	Sept	5.559 (260)	5.580 (265)	6.217 (501)	4.917 (137)	"
Skipwth	April	8.162 (3505)	8.300 (4024)	8.182 (3576)	7.858 (2586)	"
	June	7.736 (2289)	7.898 (2692)	8.009 (3008)	7.589 (1976)	"
	Sept	7.461 (1739)	7.740 (2298)	8.153 (3474)	7.148 (1272)	"
Crym1	April	7.995 (2966)	8.292 (3992)	7.391 (1621)	8.121 (3364)	"
	June	7.726 (2267)	7.936 (2796)	8.379 (4355)	7.811 (2468)	"
	Sept	6.663 (783)	7.117 (1233)	8.079 (3226)	6.521 (679)	"

## APPENDIX V

### Construction of a Quench Curve for Counting $^{59}\text{Fe}$

#### 1) Method

An aqueous solution was prepared containing a known activity of  $^{59}\text{Fe}$ , which was 10,817 dpm. Five scintillation phials were set up, each containing 9 ml of scintillant (Toluene, PPO and POPOP with Triton X-100 (see Chapter 6)) and 1 ml of radioactive solution. 0, 0.05, 0.10, 0.20, 0.50 and 1.00 ml of quenching agent, chloroform was added to these phials. These quenched samples were then used to produce an efficiency curve for  $^{59}\text{Fe}$  counting. This was programmed into an allocated programme channel number in the Tri-Carb 3000 LSS Spectrometer. The curve was stored in the system memory and data from subsequent (unknown) sample measurements were reduced to dpm automatically by interpolation via an algorithm. Below is an example of an efficiency correlation



curve constructed for counting  $^{59}\text{Fe}$  with appropriate program settings. A similar quench curve was constructed for counting  $^{32}\text{P}$ .

```

PROGRAM #: 15
REGION A: LL-UL= 0- 273 LCR= 0 BKG= 0 % 2 SIGMA= .2
REGION B: LL-UL= 5- 273 LCR= 0 BKG= 0 % 2 SIGMA= .2
REGION C: LL-UL= 0- 0 LCR= 0 BKG= 0 % 2 SIGMA= .2
NUCLIDE 1 = 10817
TIME= 2.00 QIP= SIE SCR= B/A K=1.000

```

PI	PH	S#	TIME	CPMA/K DPH1/K	%DEV	CFMB/K DFM2/K	%DEV	CPMC/K	%DEV	QIP	FLAGS	SCR	MIN
15	15	1	2.00	9394.00	1.46	8764.50	1.51	.00	.00	476.	L	.933	3
15	15	2	2.00	8756.00	1.51	7521.50	1.63	.00	.00	297.	L	.859	7
15	15	3	2.00	8389.50	1.54	6634.50	1.74	.00	.00	232.	L	.791	10
15	15	4	2.00	6834.00	1.71	3819.50	2.29	.00	.00	164.	L	.559	13
15	15	5	2.00	5510.00	1.91	2245.00	2.98	.00	.00	142.	L	.407	15
15	15	6	2.00	5395.00	1.93	2083.00	3.10	.00	.00	139.	L	.386	18

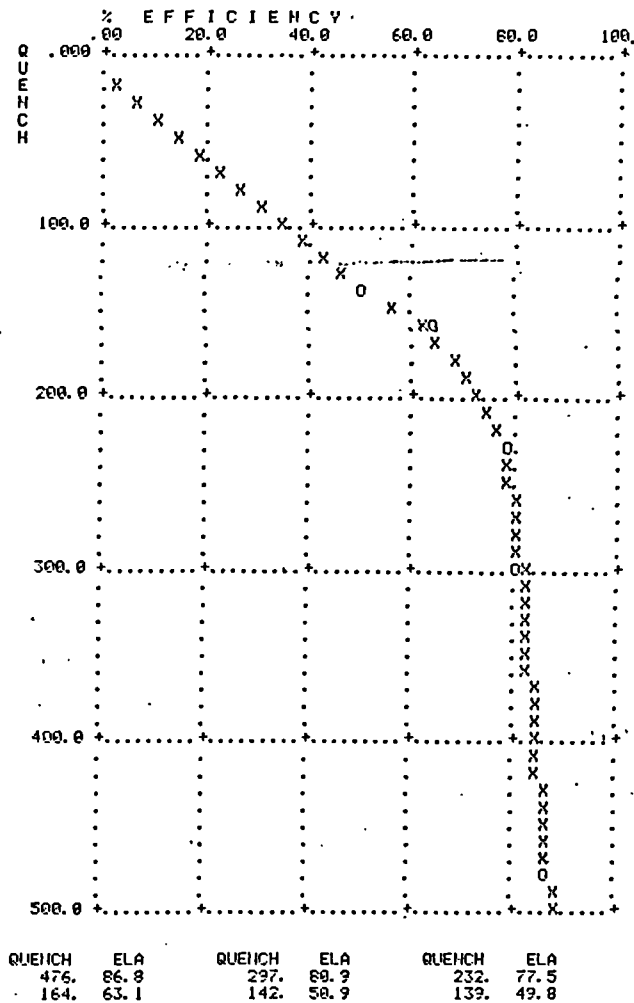


Figure A.1 Counting efficiency curve for  $^{59}\text{Fe}$ .

## APPENDIX VI

### Reciprocal transplant study using Phragmites australis

Blocks of soil (30x30x30cm) containing rhizome and dormant shoot material of Phragmites were transferred in April 1986 between Parys Mountain and Skipwith Common. Establishment and growth of the plants were then monitored over a three year period. Skipwith plants were found to be stunted while leaves were discoloured, but continued to survive over the above period. Plants from Parys Mountain transferred to Skipwith Common survived for one season only, but appeared to have been shaded by the more vigorous growth of the Skipwith vegetation.